The Genetic Predictors of Severe Outcome in Patients with Anterior Uveitis

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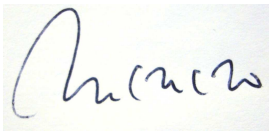
Declaration

I Victor Menezo, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in this thesis.



Personal contribution

This thesis reports the candidate's clinical and laboratory research over the period 2001 to 2004. There is clearly a large volume of work involving diverse aspects of clinical and laboratory research, which is beyond the capacity of a single investigator working in isolation. The candidate has worked as a member of a team of researchers.

The candidate has clinically assessed, diagnosed and managed nearly all the patients reported in this thesis. His supervisor has assisted in assessing the patients and supervised the laboratory work. The candidate phenotyped all the patients and participated in the genotyping of the majority of them. He critically reviewed and analysed the clinical and laboratory data. The candidate contributed to all aspects of the research studies in this thesis and has been involved in the clinical and laboratory aspects of each project, the analysis of the results and the writing of the papers.

The candidate has been uniquely placed to be the pivotal member of the research team. He has developed the clinical acumen to assess the patients and the laboratory skills to determine the direction and significance of the basic research.

The molecular work in chapters 4, 5 and 6 was carried out by:

[1] Sarah K Bond: Primer design and optimization of IL-1RN +2018, IL-6 -174, IL-10 -1082, TNF -238, -308.

[2, 3] Ni-Wen Kuo and [2] Penny A Lympany: Statistical advice, primer design, optimization and genotyping of *TNF* -1031, -863, -857, -308, -238, *LTA* +720, +365, +249; *TNFRSF1A* -201, -230, -845, -839, -1135; *TNFRSF1B* +1663, +1668, +1690, +676.

[2, 4] Tun Kuan Yeo: Identification and genotyping of 15 SNPs of the 2 chemokine genes and 2 chemokine receptor genes as shown in table 6.1

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Within the candidate's research group authorship is given on the strength of one's contribution. The principal authors are those with the major input and are usually the first three authors.

Abstract:

Uveitis is a generic term for a wide variety of different types of intraocular inflammation with different clinical phenotypes and visual outcomes. The explanation for why some patients develop chronic anterior disease whereas others do not is unknown. It seems likely that host factors such as the cytokine milieu of the aqueous humor may be an important factor in determining outcome. In turn, their secretion is genetically determined and cytokine gene polymorphisms have been associated with high or low level production whatever the stimulus.

Purpose:

The aim of this study was to identify key cytokine and chemokine polymorphisms associated with disease susceptibility, clinical phenotype, and development of visually significant complications in patients with anterior uveitis.

Methods:

PCR amplification was used to genotype a number of biallelic SNPs in several cytokine genes. This genetic data was then compared between patients and healthy controls, and within the patient group itself for association with clinical disease outcomes.

Results:

Our results show that a significant difference in the frequency of TNF-857T allele in patients with idiopathic anterior uveitis. We found a significant association between TNF-308 allele G and patients with anterior uveitis who were HLA-B27 positive. Patients with HLA-B27 associated anterior uveitis who developed visually threatening complications were more likely to carry the TNFRSF1A-201T or TNFRSF1A-1135T alleles. In addition, the frequency of IL-1ra allele T was found to be significantly associated with chronicity of the disease. The frequency of MCP-1 (-2076T) allele was found to be significantly higher in healthy individuals when compared to patients with acute idiopathic anterior uveitis.

Conclusions:

These results suggest that genetic variations in proinflammatory mediators may influence the susceptibility and severity of the inflammatory response in eyes of patients with anterior uveitis.

This knowledge may be useful in identifying prognosis and responsiveness to anti-TNF blockade in patients with anterior uveitis.

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List of abbreviations

AAU	Acute anterior uveitis
AU	Anterior uveitis
AqH	Aqueous humor
APC	Antigen presenting cell
AS	Ankylosing spondylitis
BAL	Broncoalveolar lavage
CAU	Chronic anterior uveitis
CMO	Cystoid macular oedema
EAU	Experimental autoimmune uveitis
EIU	Endotoxin-induced uveitis
EMIU	Experimental melanin-induced uveitis
FHC	Fuchs' Heterochromic Cyclitis
HLA	Human leukocyte antigen
IAU	Idiopathic anterior uveitis
IBD	Inflammatory bowel disease
IL	Interleukin
IL1-ra	Interleukin 1 receptor antagonist
JIA	Juvenile idiopathic arthritis
Kd	Kilodaltons
KP	Keratic precipitates
LD	Linkage disequilibrium
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
MW	Molecular weight
NK	Natural killer
PRRs	Pattern-recognition receptors
PCR	Polymerase chain reaction
PMN	Polymorphonuclear leukocyte
PRR	Pattern-recognition receptor
PS	Posterior synechiae
PsA	Psoriatic arthritis
RhA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
TCR	T cell receptor

TLRs	Toll-like receptors
TNF	Tumour necrosis factor
Treg	Regulatory T cells

CHAPTER ONE

1. INTRODUCTION

1.1. GENERAL IMMUNOLOGY

The immune system coordinates the immune response through two major arms that detect and defend against microbial challenges and discriminate between self and non-self antigens. The first is the innate or non-specific immune system, which constitutes a first line of host defences responsible for the immediate and rapid immune response against microbes. Its main components are the physical and chemical barriers, such as epithelia and antimicrobial substances produced at the site of epithelial surfaces, blood proteins including members of the complement system and a cellular milieu than includes neutrophils or polymorphonuclear cells (PMN), monocytes, macrophages, natural killer (NK) cells, and immature dendritic cells (Medzhitov et al. 2000).

The other is the adaptive or specific immune system which is a highly evolved defence mechanism, is antigen-specific, is mediated by antigen receptors on B and T lymphocytes, requires clonal expansion and is characterized by immunologic memory (Table 1.1).

Table 1.1. Cardinal features of innate and adaptive immunity (Medzhitov & Janeway 2000).

	Innate immune response	**Adaptive immune response**
Onset	Immediate	Delayed
Components	Neutrophils, monocytes, macrophages, mast cells, basophils, eosinophils, NK cells, APCs, complement system, acute phase proteins, cytokines and chemokines	T lymphocytes, B lymphocytes, cytokine and chemokine system
Immune recognition mechanism	Germ-line encoded Pattern-recognition receptors Recognise PAMPs Not clonally expressed	Somatically generated Antigen-specific receptors Recognise antigen epitopes Clonal expression, selection , and expansion
Number of receptor specificities	In the hundreds Specifically of each receptor is genetically predetermined	Between 10^{14} to 10^{18} Unique gene recombination and rearrangements to generate the enormous diversity of antigen receptors
Immunologic memory	Absent	Present

1.1.1. Innate immunity

Neutrophils or PMN are the most common immune cells in peripheral blood and represent an important component of the innate immune system since they are the first cells to arrive at sites of acute inflammation thus constituting the first line of defence against microbial organisms. Once at the site of inflammation they produce pro-inflammatory cytokines and chemokines that recruit and activate other immune cells, such as monocytes, macrophages, NK cells and immature dendritic cells (Joffre et al. 2009; Yamashiro et al. 2001).

Monocytes are an incompletely differentiated cell type that circulates in the peripheral blood after leaving the bone marrow. They belong to the human mononuclear phagocyte system and play an important role in the innate immune response via their role in cytokine production, phagocytosis and chemotaxis. CD14 is a major cell surface receptor of monocytes and $CD14^+$ monocytes in peripheral blood migrate into inflamed tissue where they undergo differentiation into myeloid dendritic cells or tissue macrophages dependent upon the cytokine environment, and thereby contributing to the development of adaptive immunity (Ziegler-Heitbrock 2007).

Dendritic cells are terminally differentiated bone marrow cells whose main function consists of presenting antigens to naïve T cells. Following the capture of antigens at the site of inflammation they migrate to lymphatic tissues, such as the spleen and regional lymph nodes where they present the processed antigen to the resident lymphocytes via MHC class II molecules. In addition to this dendritic cells are potent stimulators of B and T cells lymphocytes and can modulate the development of T-cell mediated immune responses into Th1 or Th2 type.

1.1.1.1. Toll-like receptors

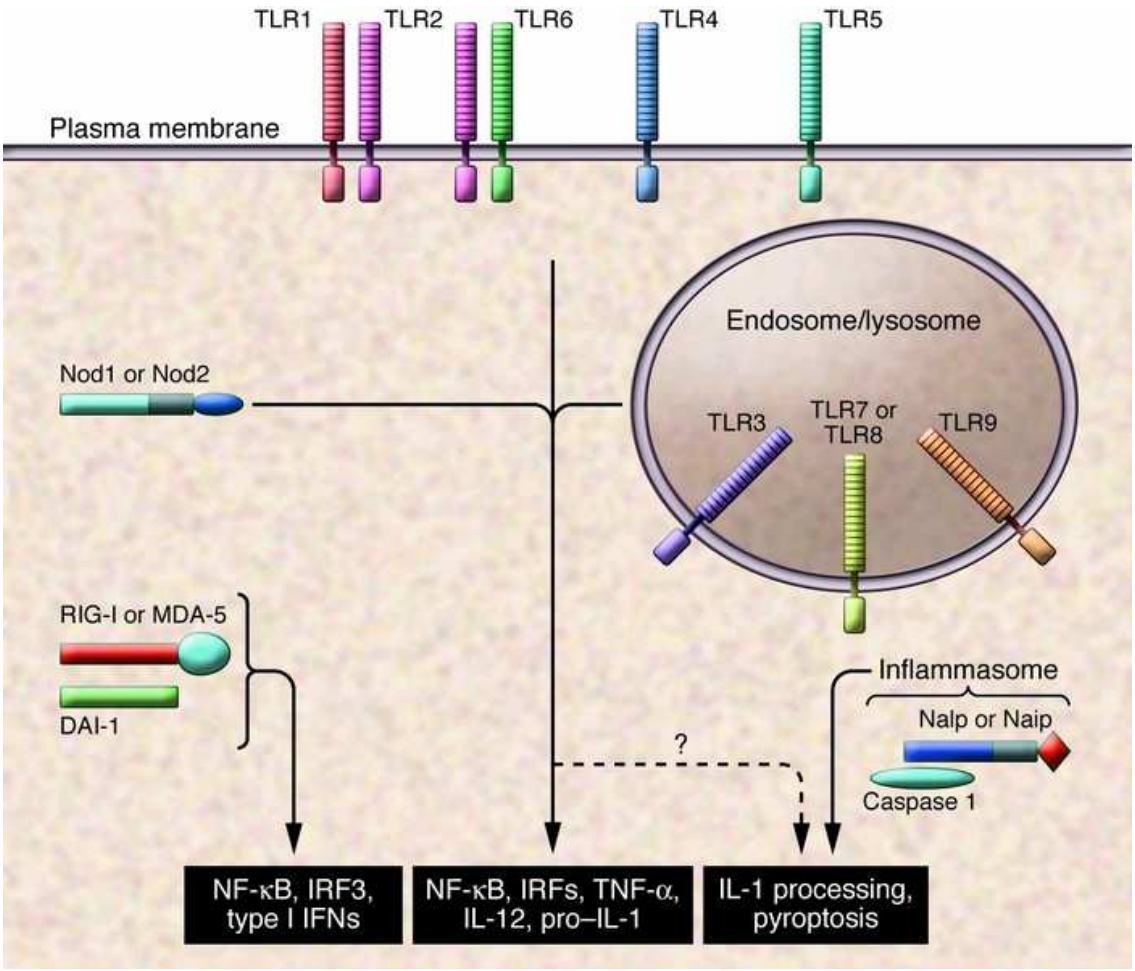
There has been an overwhelming increase in the total number of known receptors involved in innate immune recognition in recent years; moreover, pathogens can be extremely heterogeneous and mutate at much higher rate than any of their hosts. Therefore the innate immune system recognises only highly preserved structures called pathogen-associated molecular patterns, with bacterial lipopolysaccharide (LPS) being one of the best-known examples. The receptors of the innate immune system that recognize them are known as pattern-recognition receptors (PRRs).

Structurally, PRRs belong to several families of proteins that are expressed in many effector cells of the innate immune system; however, functionally they can be divided in three classes, secreted, endocytic and signaling.

Signaling PRRs, such as NOD2 recognize pathogen-associated molecular patterns and activate signal transduction pathways that induce the expression of a variety of immune

response genes, including pro-inflammatory cytokines (Du et al. 2009; Medzhitov & Janeway 2000) (Figure 1.1).

Figure 1.1. Schematic representation of the PRR families within the innate system. From Barton GM (Barton 2008).



Toll-like receptors (TLRs) are a family of PRRs that recognize the above mentioned pathogen-associated molecular patterns. They are expressed on immune cells that are most likely to encounter microbes, such as neutrophils, macrophages and dendritic cells, and each TLR has a unique specificity for ligands (Takeda et al. 2005). The activation of TLRs by their ligands results in the production of pro-inflammatory cytokines, chemokines and activation of immune cells (Medzhitov et al. 2002) initiating a rapid response characterised by the recruitment of leukocytes to the site of infection in order to eliminate the pathogen (Takeda & Akira 2005) and activation of specific adaptive immune responses.

Ten human TLRs have been identified to date and each one of them recognizes a pathogen-associated molecular pattern from a single class of micro-organism (Table 1.2).

The cytoplasmic portion of TLRs shows high similarity to the IL-1receptor family, and it is termed Toll/IL-1 receptor (TIR) domain, however the extracellular portions are structurally and functionally unrelated. Whereas TLR2 recognises peptidoglycans from gram-positive bacteria, TLR4 recognises LPS of gram-negative bacteria (Takeda & Akira 2005). Microbial recognition of TLRs facilitates dimerisation of TLRs, and TLR2 is shown to from a heterodimer with TLR1 or TLR6, which triggers the activation of signalling pathways and ultimately the activation of the transcription factor NF-κβ, which is essential for the induction of inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and IL-12 (Takeda & Akira 2005) (Figure 1.2). Therefore, activation of specific TLRs on different antigen presenting cells (APCs) leads to different pathways of cytokine gene expression (Hirschfeld et al. 2001). At the same time these APCs can activate naïve $CD4^{+}$T cells and induce their differentiation into Th1, Th2, or Th17 cells, providing a link between innate immune recognition and the triggering of the adaptive immune response.

They have been implicated in the pathogenesis of a number of chronic inflammatory autoimmune disorders, including AS and IBD which interestingly can be associated with intraocular inflammation (Cario et al. 2000; Yang et al. 2007).

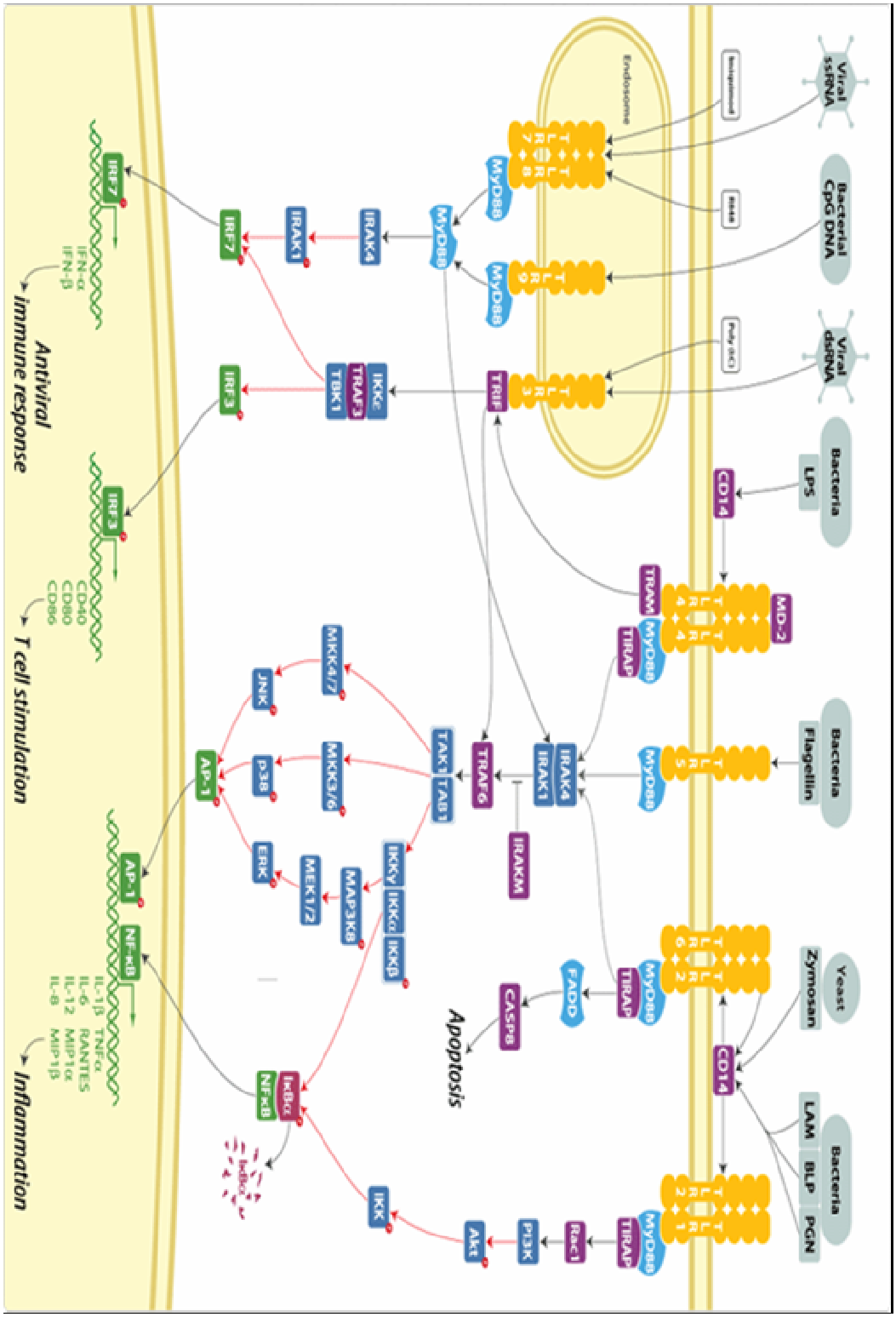
TLRs, in particular TLR4 have been shown to be expressed in the human eye (Song et al. 2001), and although the human iris endothelium does not appear to express TLR4 in vivo, cultured human iris endothelial cells have been found to express TLR4 mRNA and respond to LPS stimulation with the production of pro-inflammatory cytokines (Brito et al. 2004). Furthermore, Chang et al. demonstrated the TLR4 functional expression by resident APCs in the normal uvea (Chang et al. 2004), as well as changes in the expression and function of TLR4 and TLR2 in patients with active AAU (Chang et al. 2007). These findings support another potential pathogenic role of PRRs in the development of anterior uveitis (AU).

Table 1.2. Summary of human TLRs and their known ligands. Courtesy of Dr Kelly Mai, University of New South Wales, Sydney.

TLRS	**Ligands**	**Location**
*TLR1**	lipoproteins/lipopeptides (bacteria)	Cell surface
*TLR2**	lipoproteins/lipopeptides (bacteria) peptidoglycan and lipoteichoic acid (bacteria) zymosan (fungi) *HSP70 (host cells)*	Cell surface
TLR3	dsRNA	Cell compartment
TLR4	LPS (bacteria) *HSPs (bacteria and host cells)* *hyaluronic acid fragments (host cells)* viral proteins	Cell surface
TLR5	flagellin (bacteria)	Cell surface
*TLR6**	diacyl lipopeptides (bacteria)	Cell surface
TLR7	imidazolequinoline antiviral drug ssRNA	Cell compartment
TLR8	ssRNA (viral)	Cell compartment
TLR9	unmethylated CpG DNA (bacteria)	Cell compartment
TLR10	Unknown	Unknown

*TLR2 forms heterodimers with TLR1 and TLR6

Figure 1.2. TLR signaling and ligand specificities of TLRs. Courtesy of Dr Kelly Mai, University of New South Wales, Sydney.



1.1.2. Adaptive immunity

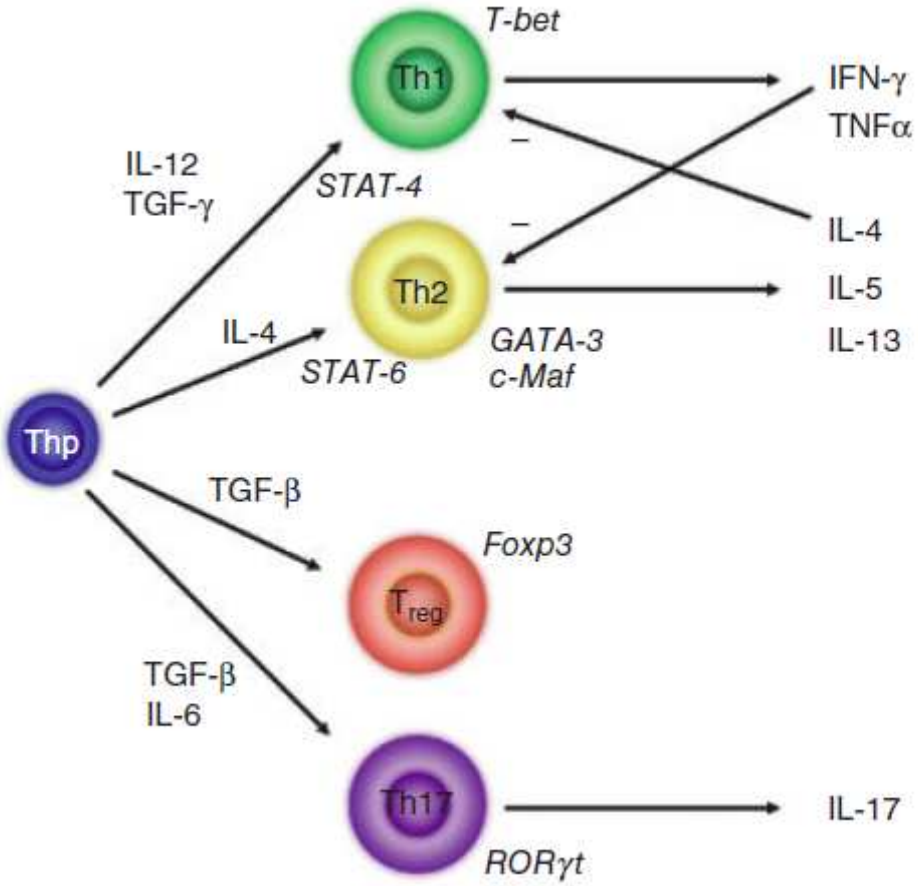
The adaptive immune response involves the proliferation of antigen-specific B and T lymphocytes. B cells develop in the bone marrow and circulate in the lymphatic network. They secrete immunoglobulins or antigen-specific antibodies that are responsible for eliminating extracellular pathogens.

T cells originate in the bone marrow and migrate to the thymus where they undergo a process of proliferation during their first encounter with an antigen. This primary immune response generates both effector T and B cells, as well as memory T and B cells. The presence of T cell receptors (TCR) enables the identification of the appropriate antigens. The main functions of T cells are to regulate the immune responses to protein antigens and to serve as effector cells for the elimination of intracellular pathogens. Cytokine production and cytotoxicity are critical effector cell functions, which can be attributed to two main separate T cell subpopulations $CD4^+$ T cells or cytokine-secreting cells (Th) and cytotoxic cells (Tc) or $CD8^+$T cells.

The Th1 cell and Th2 cell paradigm proposed by Mosmann in the late 80s' to explain different adaptive immune responses against different pathogens correlates well with their pattern of cytokine production (Mosmann et al. 1986). Whereas Th1 cells are defined by the production of IL-2 and IFN-gamma and are primarily responsible for cell-mediated inflammatory reactions (Sacks et al. 2002), Th2 cells are involved in humoral immunity (Anthony et al. 2007) and produce IL-4, IL-5, IL-10, and IL-13.

Another characteristic of Th1 and Th2 cells is that T cell subsets produce cytokines that serve as own autocrine growth factor and promote differentiation of naïve T cells to that subset. Furthermore, cytokines produced by each subset cross-regulate others development and activity; however this simplified classification of T cell effector functions into cell-mediated and humoral immunity has been a source of debate and controversy, and efforts to resolve these issues in recent years have resulted in the discovery of a new subset of T-cells that exhibit different functions from Th1 and Th2 cells and are characterized as the main producers of IL-17A, IL-17F, IL-21, and IL-22 (Weaver et al. 2006) (Figure 1.3).

Figure 1.3. Th cell lineage. From Afzali et al. (Afzali et al. 2007).



While it is known that the Th1 cytokine IFN-gamma regulates cell-mediated immunity and Th2 cytokines regulate humoral responses, the function of IL-17 is still not well understood, although recent published data indicates that IL-17 is a pro-inflammatory mediator that acts in synergy with TNF to regulate inflammatory gene expression (Ruddy et al. 2004).
Th17 cells are characterized by being preferential producers of IL-17, IL-22, and IL-21, and thus inducing variable tissue reaction in relation to the distribution of their receptors. As with Th1 or Th2 cells there is no single surface marker specific for Th17, although the absence of CCR5 in the presence of CCR2 seems to define the Th17 phenotype (Sato et al. 2007), These receptors are widely expressed in epithelial and endothelial cells and can therefore act as regulators of different immune responses. There is a mutual antagonism between Th17 and Th1 cells, which derives from the induction of reciprocal T-cell developmental pathways by IL-23 and IL-27 (Weaver et al. 2006).
In chronically inflamed lesions in contrast to in vitro differentiated peripheral blood-derived Th17 cells, which are reported to produce both IFN-γ and IL-17, many infiltrating Th17 cells only produce low levels of IFN-γ, IL-4, or other Th2 associated cytokines, but high levels of IL-17 and IL-22 are produced at much higher levels (Pene et al. 2008). These findings corroborate earlier reports showing that the pro-inflammatory IL-22 is produced by activated Th17 cells in both animal and human models of inflammatory disease (Annunziato et al. 2007; Liang et al. 2006); and that Th17 clone cells isolated from psoriatic lesions induced the expression of psoriasin, an inflammatory protein in a IL-22 dependent manner (Pene et al. 2008).
Similarly, in EAU INF-γ secreting Th1 cells were thought to be the main inflammatory driver, given the elevated levels of INF- γ in the retina during the acute phase of uveitis; however IL-12 downregulates EAU and antibodies to IFN- γ appear to exacerbate the disease, suggesting a paradoxical role of Th1 cells conferring protection against chronic uveitis (Tarrant et al. 1999).

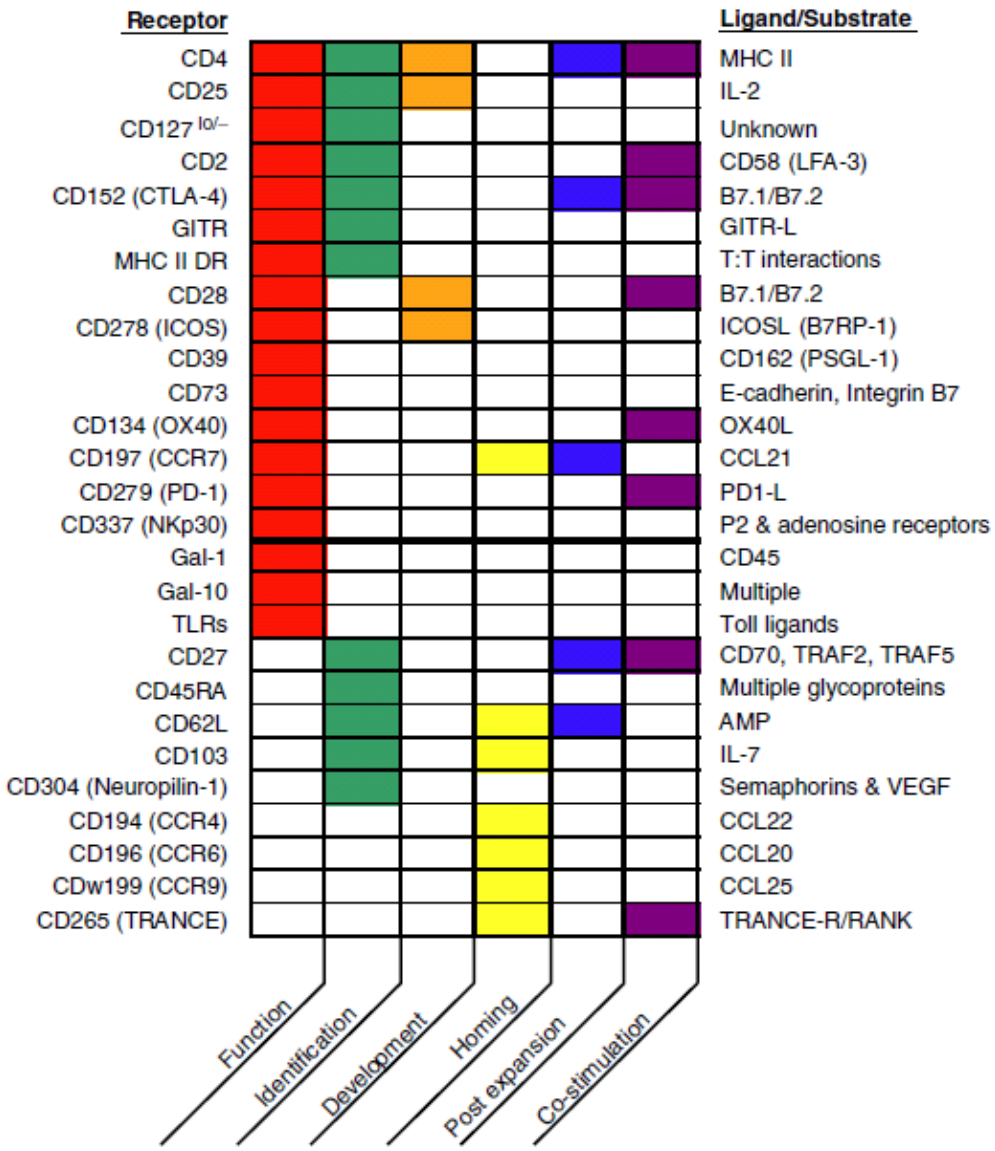
Th17 cells have been found in peripheral blood mononuclear cells (PBMC) of patients with active uveitis, and their expression was upregulated following stimulation with IL-12. In addition, IL-17 expression was inhibited by IFN- γ (Amadi-Obi et al. 2007). In the same study, IL-27 was unexpectedly found to be ubiquitous in retinal cells, upregulated by IFN- γ and inhibited Th17 proliferation, suggesting that Th1 cells may have a protective role in uveitis by antagonizing Th17 cells through IFN- γ mediated induction of IL-27 (Amadi-Obi et al. 2007).

Another subtype of T cell with immunoregulatory capacity has been identified and shown to play an important role in modulating T cell function. They are also defined by their cell

surface markers, mechanism of action and tissue of origin, and the expression profile and functional significance of some of these markers is summarized in figure 1.4 (Figure 1.4). The exact mechanism by which these regulatory T cells (Treg) exert their regulatory functions remains poorly understood, and although it is thought that they operate via a cell-to-cell contact, both IL-10 and TGF-β production have also been implicated (Shevach et al. 2008). Specifically, Treg have the capacity to actively block immune responses, inflammation, and tissue destruction by suppressing the functions of a wide array of cell types, including CD4+ helper T cells, CD8+ cytotoxic T cell, and APCs among others (Brusko et al. 2009). Among the different subtypes, the naturally occurring $CD4^+$ $CD25^+$ Treg cells are essential for the active suppression of autoimmunity and it has been shown that in the human and murine $CD4^+$ CD25 knock-out models severe autoimmune disease develops (Wildin et al. 2001). Several studies have demonstrated alterations in the $CD4^+$ $CD25^+$ population in patients with uveitis (Chen et al. 2008; Hamzaoui et al. 2006; Nanke et al. 2008).

One of the markers that the $CD4^+$ $CD25^+$ Treg population expresses is the forkhead-winged-helix transcription factor Foxp3, responsible for their development and function (Fontenot et al. 2003; Sather et al. 2007). It also appears to be the most specific nuclear transcription factor for Treg, and differences observed between the percentage of $CD4^+$ $Fox3p^+$ lymphocytes in patients with active and inactive uveitis suggests that a deficiency of Treg populations may play a role in some uveitis entities (Yeh et al. 2009), which would correlate with the observation that often autoimmune disorders manifest with relapsing episodes of inflammation and periods of quiescence, supporting the hypothesis of a skewed Th cell profile towards pro-inflammatory lineage such as Th1 and Th17, or a loss of Treg lineage during these periods of acute flare-ups.

Figure 1.4. Important markers in the immunobiology of Tregs. From Brusko et al. (Brusko & Bluestone 2009).



1.2. OCULAR IMMUNOLOGY

1.2.1. Ocular immune privilege

Since the first experimental description of ocular immune privilege in the 1940s' (Medawar 1948) further research has called attention to the existence of two types of immune privilege: immune privilege sites and tissues. Immune privileged sites, such as the eye can be defined as places where foreign tissue can experience extended survival, whereas in other sites they are rejected and they differ from conventional ones in that they can survive when placed at conventional sites, whereas non-privileged tissues are promptly rejected (Streilein 2003). Hence immune privilege is an evolutionary process that allows immune effectors to provide local protection without disrupting the local tissue or specialized organs and is achieved by dynamic interactions between the immune system and specialized tissues.

Several features unique to the eye contribute to its immune privilege.

The first one is the integrity of the blood ocular barrier, which restricts inflow of blood borne pathogens into the eye. Secondly, the absence of lymphatics combined with the major outflow of aqueous humor (AqH) through the trabecular meshwork into the venous circulation, makes the spleen rather than lymph nodes its primary lymphoid organ. Thirdly, the existence of soluble immunomodulatory factors in the AqH, such as neuropeptides and cytokines such as TGF-β, that can suppress immune mediated inflammation and complement activation (Streilein et al. 1992). In addition to this, the expression of immunomodulatory ligands, such as FasL on the surface of parenchymal cells induces apoptosis of ingressing T cells, and elevated levels in AqH during episodes of active uveitis suggest they have a regulatory role in uveitis (Sugita et al. 2000). And finally, endogenous tolerance promoting APCs that are strategically located in the iris, ciliary body and retina. The result is that antigenic material placed in or arising from the anterior chamber elicits a different form of systemic immune response, which includes T and B cells that eliminate pathogens in the absence of inflammation, as well as Treg cells that suppress induction and expression of immunogenic inflammation secondary to Th1 and Th2 cells, and excludes effector $CD4^+$ T cells that eliminate pathogens via immunogenic inflammation. This response is known as anterior chamber-associated immune deviation (ACAID). Now know that this process is mediated by $F4/80^+$ macrophages that present the ocular inoculated antigen to a cluster of B cells, NK cells, CD4, and CD8 T cells in the spleen (Al-Mansour et al.).

1.2.1.1. Experimental uveitis

It is difficult to obtain sufficient human ocular tissue for research purposes. It is rarely possible to obtain ocular tissue for histology via biopsies, hence animal experimental models of uveitis have been developed to provide a better knowledge of the immunobiology of the

uvea and surrounding ocular structures during uveitis, and to test new therapeutic strategies (Table 1.3).

1.2.1.1.1. Experimental autoimmune uveitis

Experimental autoimmune uveitis (EAU) may be induced in several mammalian species by immunizing them with a soluble bovine retinal extract. EAU consists of an immune-mediated response against soluble retinal antigens. It is a $CD4^+$ Th1 cell mediated disease, in which the T-cell infiltrate, triggers a cytokine cascade and a non-specific cellular infiltrate, that are responsible for cell damage. Histologically this cellular milieu consists mostly of mononuclear cells. In EAU the inflammation affects mainly the posterior segment, but often has an anterior segment inflammatory component with deposition of fibrin, as it is often seen in humans in severe acute presentations of uveitis.

1.2.1.1.2. Endotoxin induced uveitis

Endotoxin-induced uveitis (EIU) is an animal model for acute anterior uveitis (AAU) in the human, but unlike human disease, it does not induce long lasting tissue damage. Systemic LPS injection in Lewis rats induces the adherence of monocytes to retinal blood vessels, followed by invasion into the retina. The popularity of EIU in research is partly due to the recognized association between AAU and gram negative bacterial infection. Depending on the animal used the inflammatory response has a different anatomical predilection. In Lewis rats the uveitis will develop mostly in the anterior chamber, whereas in the mouse, the inflammatory infiltrate will accumulate in the vitreous and around retinal vessels. Histological findings include an early infiltrate of PMN leucocytes, followed by a mononuclear component.

Interestingly, not all mouse strains injected with LPS develop EIU. In the C3H/HeJ mice a point mutation within the coding region of the TLR4 gene results in a functional disruption of TLR4 signaling rendering these mice resistant to LPS induced uveitis, yet highly susceptible to gram negative infection (Poltorak et al. 1998).

1.2.1.1.3. Experimental melanin-induced uveitis

Experimental melanin-induced uveitis (EMIU) is a recurrent uveitis observed when Lewis rats are immunized with bovine choroidal melanin. Like EAU is a T cell-mediated ocular inflammation. Histological inflammation appears as early as clinical signs are present. It consists mostly of an accumulation of macrophages and T cells in the uvea, but as in EAU PMN are also frequently seen. Whereas in EIU neutrophil infiltrate predominate, in EMIU mononuclear cells are the predominant type, especially in the ciliary body and they later migrate to the iris. Clinically is characterized by bilateral, recurrent subacute uveitis.

Although the clinical signs appear to resolve spontaneously, the time taken for resolution is longer than in the EIU model. EMIU appears to be the model more closely related to human disease.

1.2.1.1.4. Experimental autoimmune encephalitis associated anterior uveitis

Experimental autoimmune encephalitis associated AU (EAE/AU) is a model for recurrent AU, which is associated with inflammation of the spinal cord. Similar to the other models of autoimmune uveitis, CD4+ T cells of Th1 phenotype mediate the inflammatory response in response to peptides that posses both the encephalitogenic and uveiticogenic properties. Following immunization of Lewis rats with myelin basic protein (MBP) EAE and AU appear to manifest simultaneously, but AU has a longer clinical course and often persists after clinical signs of EAE have subside. Interestingly after the initial episode of EAE, rats develop a total resistance to further attempts to induce active EAE but are susceptible to further episodes of uveitis. The inflammatory cells in the proximity of nerve bundles and iris vessels consist mainly of monocytes.

Table 1.3. Comparison of animal models for autoimmune uveitis.

Model	**Antigen**	**Target**	**Clinical relevance**
EAU	S-Antigen IRBP Rhodopsin Recoverin Phosducin	Retinal photoreceptors	Posterior uveitis
EIU	LPS	Anterior uveal epithelium	Recurrent anterior uveitis Posterior uveitis
EMIU	Melanin	Uveal melanocytes	Anterior uveitis Posterior uveitis
EAE/AU	MBP	Myelinated iris nerves and spinal cord	Anterior uveitis MS

IRBP: Interphotoreceptor Retinoid Binding Protein; MS: Multple Sclerosis

1.3. CYTOKINES

Cytokines are regulatory, soluble proteins of a relative small size (10-45 Kd) produced by cells in different organs in response to a variety of inducing stimuli. They constitute a diverse group of molecules with a wide range of effects, including pro-inflammatory, anti-inflammatory, and chemoattractant functions. They play an important role in controlling cell proliferation and cell differentiation phenotype. They are involved in regulating the immune response to foreign antigens and other forms of defence against infection by viruses and other pathogens. They are also required for cellular renewal, wound healing, development of cellular, humoral immunity, and inflammatory responses. Cytokines transduce signals from the outside of the cell by binding to specific cell-surface receptors, activating a series of intracellular protein kinases which control gene and protein expression via factors such as NFκβ.

The combination of cytokines and their concentrations at a specific site may differ, and their action on a given tissue or cell can be influenced by the microenvironment of the target cell. These pleiotropic effects make cytokines able to induce many different types of responses, often on different cell types.
Due to the overlap in their biological effects and mechanisms of action classifying these molecules has been difficult; in addition to this, cytokine effects are typically redundant as different cytokines may induce the same biological effect. Hence, although cytokines are generally classified according to the type of receptor they bind, their nomenclature attempts to reflect their main function and order of their discovery.
Functionally, cytokines can be divided into pro-inflammatory e.g.: TNF-α, anti-inflammatory e.g.: IL-10, proliferative e.g.: CSF3 (GCSF), regulatory e.g.: TGF-β, and chemokines, and the balance between them has been shown to determine the degree of inflammatory response. Several studies have shown cytokine and chemokine release during intraocular inflammation (Ahn et al. 2006b; Hill et al. 2005; Takase et al. 2006).

1.3.1. Molecular basis of cytokine action

Cytokines work in a network where the response of an individual cell will depend upon the pattern of cytokines it is subjected to, and to the set of cytokine receptors that it expresses. Many cytokines work by causing aggregation of receptors at the cell surface, leading to the activation of second messenger systems. These effects mostly occur in the same cell that produces them in an autocrine manner or in cells that are close by in a paracrine fashion.

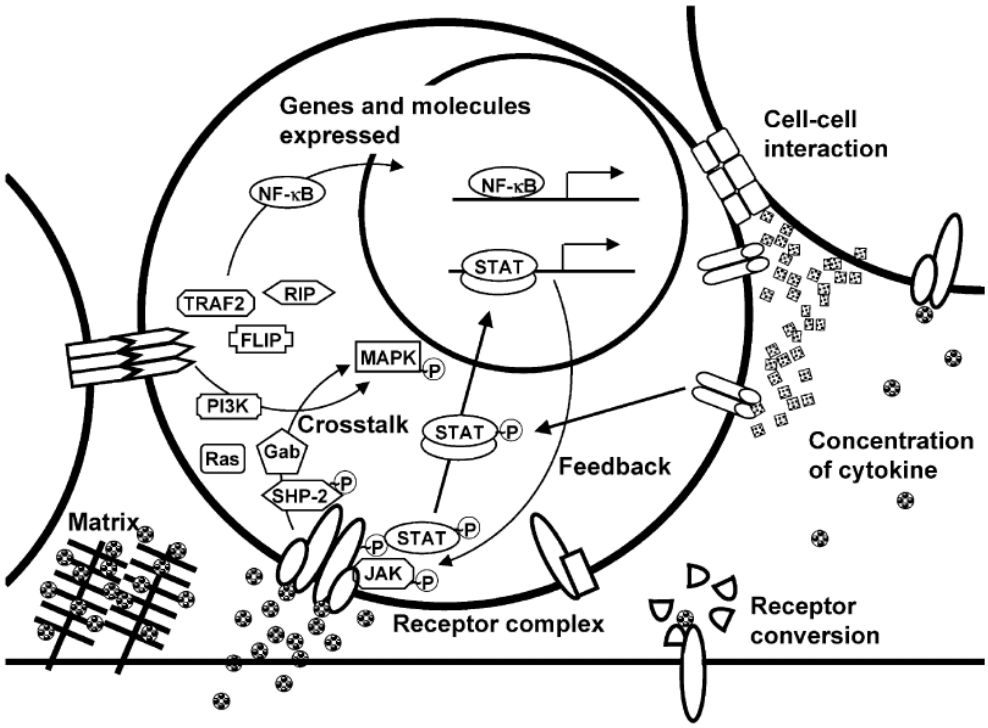
Cytokine action can be regulated at an extracellular level and is determined by the amount and duration of availability of the cytokine produced, as well as the strength and distance

between the target cell and the cytokine producing cell. The cellular milieu that may respond to the cytokine signal is in turn determined by the expression of the cytokine receptors on the cell surface (Ishihara et al. 2002b).

Inside the cell, cytokine signalling is regulated at the cell membrane in the cytoplasma and in the nucleus. Following cytokine binding to its specific receptor, a specific transduction pathway is activated and regulated by different adaptor molecules. The set of genes transcribed by these transduction pathways will help to determine the final biological output (Ishihara & Hirano 2002b), and although these signaling pathways are well characterized most of the studies are limited to a single pathway, which fails to demonstrate the complexity of the inflammatory responses.

Important mediators for the main cytokine signal-transduction pathway are the Janus kinases (JAKs) and signal transducers and activator of transcription (STATs). Cytokine receptors in general lack intrinsic tyrosine kinase activity and hence, need to liaise with receptor-associated kinases in order to initiate a phosphorylation cascade. Ligand binding to a cytokine receptor induces the homo- or heterodimerization of the receptor subunits and activates the JAK family tyrosine kinases, which induce the tyrosine phosphorylation of the receptor's cytoplasmic tail. Some of these phosphorylated tyrosines serve as docking site for STATs. Once bound to the receptor, STATs are then phosphorylated, resulting in the acquisition of high-affinity DNA-binding activity, which facilitates their action as nuclear transcription factors. Seven STATs arranged across three different chromosomes have been described to date in mammalian cells. They differ by a SH2 domain that contributes to their selective response to cytokines (Schindler et al. 2007). Among them, STAT3 is required for the regulation of naïve T cells towards the development of Th17 cell lineage and recent data has suggested that targeted deletion of STAT3 in $CD4^+$ cells may prevent the development of EAU (Liu et al. 2008). Moreover, most of our knowledge of pro-inflammatory signaling pathways comes from members of the TNF, IL-1, IL-6 cytokine families and more recently TLRs, where this cooperative induction of gene expression is dependent on the presence of binding sites for STAT and NFκβ (Yoshida et al. 2004). NFκβ transcription factors not only regulate genes which are essential for both the innate and adaptive immune responses (Brown et al. 2008) , but also recent research has focused in the development of anti-inflammatory therapies targeting NFκβ (Karin et al. 2004), as well as demonstrated the role of NFκβ in the resolution of inflammation (Fong et al. 2008; Lawrence et al.) (Figure 1.5).

Figure 1.5. Factors involved in the determination of cell specificity of a cytokine. From Ishihara et al. (Ishihara & Hirano 2002b).



1.3.2. Cytokine genes polymorphisms

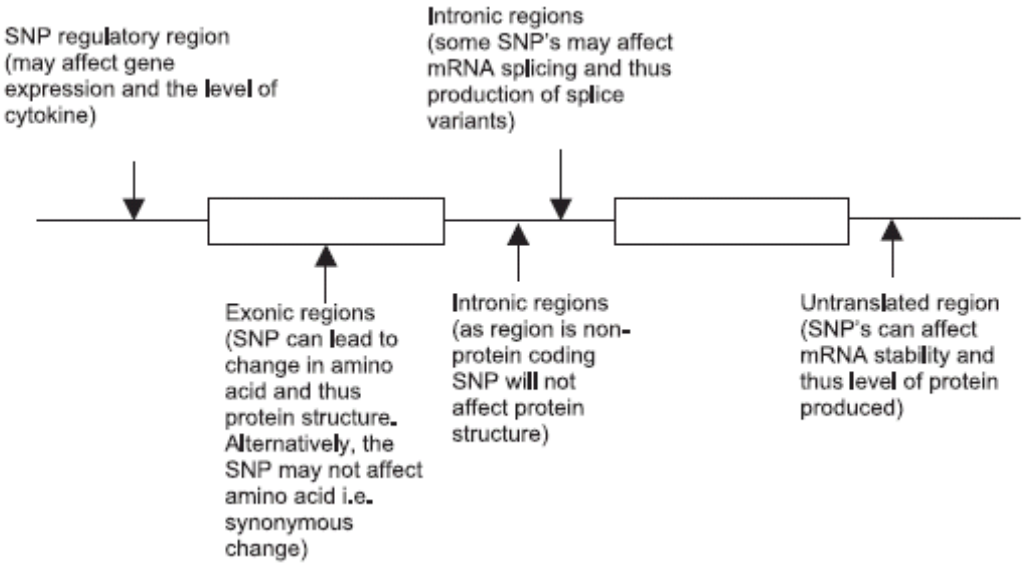
Cytokines are key players in the regulatory mechanisms of the immune system, and it is clear that variations in their levels (quantitative) or changes in their structure (qualitative) have been associated with disease susceptibility and different clinical outcomes (Smith et al. 2009). One of the approaches to investigate their role is through the study of candidate cytokine gene polymorphisms using case-control association studies, and the genetic markers used in most of these population-based studies are single nucleotide polymorphisms (SNPs).

A SNP is an inherited change of a single nucleotide in the DNA sequence, and is distinguished from rare variations in that the less frequent allele is required to have a frequency of 1% or more. Biallelic SNPs are highly abundant across the genome and are often used in association studies, and the frequencies of polymorphic alleles vary between different populations (Cross et al. 2010; Huang et al. 2007).

Thus, multiple SNPs can often be found within the whole length of a gene but only those located in exons of cytokine genes can alter amino acid sequences that subsequently lead to a change in a protein function (Figure 1.6). Although cytokine expression is typically modulated by polymorphisms within the promoter regions of the cytokine genes (Lu et al. 2008b), SNPs that are not in protein-coding regions have been found to influence transcription factor binding (Schena et al. 2006).

A large number of polymorphisms within the coding and non-coding region have been identified and thousands of disease-association studies have been carried out using these variants. In this literature review I have summarized those cytokines and chemokines that have been the centre of the different studies of this thesis, which in turn have been reported in the literature to be associated directly with intraocular inflammation and related autoimmune inflammatory disorders.

Figure 1.6. Cytokine gene SNPs and their possible consequences. From Ollier WER. (Ollier 2004).



1.3.2.1. TNF-α

The prominent role of TNF in inflammation has been convincingly demonstrated by many in vitro and animal studies, such as those that show that transgenic mice expressing human TNF coupled to the 3' untranslated region of β globin developed severe arthritis. Furthermore, the evidence for TNF involvement in the pathogenesis of human autoimmune disease is highlighted by the efficacy of TNF-blockage in the management of a wide spectrum of autoimmune disorders such as Crohn's disease and rheumatoid arthritis (RhA) (Lin et al. 2008).

However the biological functions of TNF are complex and on occasion contradictory. While during the acute phases of inflammation local production of TNF appears to be beneficial, as it increases the expression of adhesion molecules on the vascular endothelium allowing for neutrophils and macrophages to recruit locally at the site of inflammation, prolonged exposure to TNF has been associated with severe toxic shock induced by bacterial endotoxins. TNF is a pro-inflammatory cytokine with immunomodulatory activity. There are two forms of TNF, a 26 Kd membrane bound form and a mature secreted 17 Kd form which is released following enzymatic cleavage by a metalloproteinase disintegrin called TNF-α converting enzyme (TACE), which also controls the amount of circulating soluble form of TNFR2 (Peschon et al. 1998).

In humans TNF- α is produced mainly by monocytes/macrophages, although T and B lymphocytes, fibroblasts, and epithelial cells also produce significant amounts (Vassalli 1992; Wilson et al. 1997). Abnormally high levels of TNF-α are produced mainly by macrophages and T cells (Brinkman et al. 1997), in particular after the stimulation of macrophages by LPS following incubation of macrophages with IFN-γ (Wilson et al. 1997), gram-positive and gram-negative bacteria, mycobacterium tuberculosis, red blood cells infected with Plasmodium falciparum, different viruses, as well as major histocompatibility complex (MHC) class ligands such as bacterial superantigen toxic shock syndrome toxin 1 and staphylococcal enterotoxin B (Underhill et al. 1999). Other cytokines such as IL-2 or granulocyte-macrophage colony-stimulating factor may also induce TNF release from macrophages (Vassalli 1992).

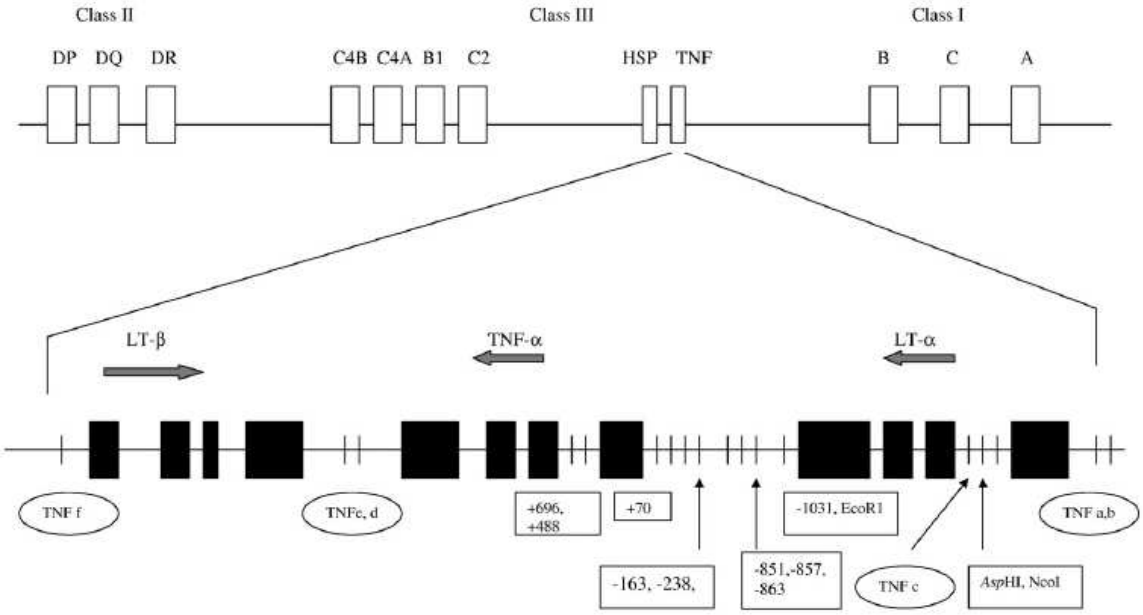
The TNF-α gene is located in the HLA class region of the MHC on the chromosome 6p21,3 approximately 250 kilobases (kb) centromeric of the HLA-B locus and 850 kb telomeric of the HLA-DR in both humans and mice (Spies et al. 1989; Wilson et al. 1997). The 5' flanking region of the TNF gene contains multiple regulatory sites structurally similar to these found in immunoglobulins and cytokine regulatory elements (Spriggs et al. 1992). The 3' untranslated region, on the other hand also plays an important role in the transcription of the gene through clearance of mRNA transcripts.

Most of the activities of TNF are mediated through two receptors TNFR1 and TNFR2. They belong to a family of receptors with repeating cystein-rich extracellular motifs. Both of them are present in almost all types of cells, but whereas TNFR1 has a ubiquitous distribution, TNFR2 is mostly confined to cells of the haematopoietic lineage (Armitage 1994; Ryffel et al. 1993).
The TNFR1 gene (TNFSF1A) is located on chromosome 12p13.2 and controls the induction of apoptosis (Bradley et al. 2001), cell activation (Vandevoorde et al. 1997), and B cell trafficking (Tkachuk et al. 1998). The TNFR2 gene is located on chromosome 1p36.2 and plays an important role in thymocyte and peripheral T cell proliferation, NK cell activation, apoptosis of activated T cell and neutrophil activation (Pantelidis et al. 1999). Binding of the trimeric ligands of TNF- α to the TNF receptor induces "molecular switching" of the receptor, which then induces the signals required for immune function (Bazzoni et al. 1996).

The TNF cluster is located within the class III region of the MHC on chromosome 6p21, where genetic alterations in the TNF-α are involved in high TNF production (Tsukamoto et al. 1998). TNF mediates its functions by binding to specific TNF receptors. Signalling through these receptors influences T cell proliferation and pro-inflammatory responses, and inappropriate signalling may trigger severe inflammatory reactions (Hajeer et al. 2000). This is based on in vitro observations that individual differences in cytokine levels after in vitro culture may be attributed to SNPs or microsatellite polymorphisms within the TNF gene. Eight DNA variants or SNPs have been described within the TNF promoter at positions -1031 T/C, -863 C/A, -857 C/T, -575 G/A, -376 G/A, -308 G/A, -244 G/A, -238 G/A relative to the transcription start site (Bayley et al. 2004) (Figure 1.7).
Among them, the two most commonly studies are -308 G/A polymorphism (rs1800629) and the -338 G/A polymorphism (rs361525). It is the presence of the less common A allele in the -308 nucleotide position that is associated with enhanced TNF-α production (Mira et al. 1999). However; there still remains controversy with regards to the functional role of the TNF -308 SNP. Whereas initial studies in the late 90s' found the allele A to be responsible for a higher inducible levels of transcription than the allele G, suggesting that the -308 polymorphism in the context of TNF-α gene can effect transcription and induce elevated levels of TNF (Kroeger et al. 1997; Wilson et al. 1997), later reports have failed to demonstrate functionality for the -308 SNP (Kaijzel et al. 2001; Kroeger et al. 2000). Similarly, another putative functional promoter polymorphisms such as -238 G/A (rs361525) and -857 C/T (rs1799724) have also shown contradicting functionality (Bayley et al. 2001; Hohjoh et al. 2001; Kaluza et al. 2000), highlighting the complexity of the effect of SNPs on TNF in vitro. In summary, while the -308 G/A polymorphism appears not to affect TNF functionality, the function of

others remains controversial and haplotype analysis of the candidate gene may prove to be a more helpful approach (Bayley et al. 2004).

Figure 1.7. TNF complex in chromosome 6. From Elahi et al. (Elahi et al. 2009).



1.3.2.1.1. Influence of TNF-α gene polymorphisms on autoimmune disorders

Despite the controversy on TNF gene polymorphisms on TNF production, it remains clear that TNF-α is a potent immune mediator and pro-inflammatory cytokine that is involved in the pathogenesis of many inflammatory disorders, and several studies have analysed the potential contribution of the above mentioned polymorphisms to susceptibility or severity of a wide range of conditions, in particular autoimmune diseases. Most autoimmune disorders are associated with specific HLA genotypes, and some of the associations between TNF-α polymorphisms could be explained by linkage disequilibrium (LD) with HLA and vice versa. There is a genetic contribution to RhA from several alleles of the HLA-DRB1 gene located in the MHC class II region (6p21) explained by the "shared epitope" theory (Michou et al. 2006), however the important physiological role of the TNF-α gene within the MHC has lead to speculation about its role in RhA.

A study where the data was stratified according to shared epitopes subtypes (HLA-DR1 and HLA-DR4) the TNF -308 A and TNF -857 T alleles were found to be associated with RhA in patients carrying the SE, furthermore the -308 A allele was significantly associated with RhA susceptibility in individuals heterozygous for the SE carrying HLA-DR4, while the -857 T allele was significant in individuals homozygous for the SE (Waldron-Lynch et al. 2001). Similarly, Date et al. demonstrated that the -857 T allele may increase the effect of the DRB1*0405/DQB1*0401 in predisposing to the development of systemic juvenile RhA (Date et al. 1999). In two different ethnic populations Ozen et al. did not find any associations between the TNF -238 G/A polymorphism and disease severity in juvenile idiopathic arthritis (Zheng et al.), but a worse outcome of disease in a Turkish children carrying the TNF -308 A allele (Ozen et al. 2002), which correlates with a more recent study showing a protective role of the TNF -308 G allele (Schmeling et al. 2006)

TNF-α has a prominent role in the inflammatory process and bone resorption in patients with ankylosing spondylitis (AS). However, opinion remains divided as to whether TNF-α polymorphisms play a role in the pathogenesis of this disorder.

Early studies by Fraile et al. and Verjans et al. failed to demonstrate any association between -308 or -238 polymorphisms in a Spanish and Dutch populations (Fraile et al. 1998; Verjans et al. 1991). In another similar study in Dutch patients Kaijzel et al. found that although the frequency of the TNF -238 A allele was increased in HLA-B27 positive patients, this was not different from their HLA-B27 positive controls and therefore the authors concluded that the association between TNF -238 G allele and AS was secondary to the HLA-B27 gene (Kaijzel et al. 1999).

In contrast, one study showed a significant association between the -238 A allele in HLA-B27 negative patients and AS compared to the B27 negative control (Gonzalez et al. 2001).

Rudwaleit et al. found that HLA-B27 positive patients had lower numbers of TNF-α positive T cells in peripheral blood compared with controls, and heterozygous patients for the -308 polymorphism were associated with a higher percentage of TNF-α positive T cells than homozygous patients for the allele G (Rudwaleit et al. 2001). Another two studies by McGarry et al. and Vargas-Alarcon in different populations demonstrated that TNF-α -308 polymorphisms (allele G, and allele A, respectively) were involved in susceptibility to AS, but not with outcome (McGarry et al. 1999; Vargas-Alarcon et al. 2006). A more recent study in a Chinese population revealed a significant association between HLA-A333-B58-Cw10 haplotypes and TNF-α -308 G/A polymorphisms independently, playing a possible role in the pathogenesis of AS (Lu et al. 2008a).
A recent meta-analysis of the TNF-α -308 and -238 polymorphisms and AS susceptibility published recently showed no significant association in the overall population sample, nor did it reveal any significant association between the B27 positive patients and the matched B27 positive controls (Lee et al. 2009).
The frequencies of the TNF-α -308 GA and -238 GA genotypes were lower in patients with HLA-B27 positive associated AU than in patients who were HLA-B27 negative. In addition to this, when compared to healthy HLA-B27 positive individuals the frequency of the TNF-α -238GA genotype was also found to be lower among patients, suggesting that the presence of allele A at nucleotide -238 to be a risk factor for the development of intraocular inflammation among individuals who are HLA-B27 positive (El-Shabrawi et al. 2006).

Several polymorphisms implicated in susceptibility to sarcoidosis as well as disease prognosis lie within the MHC class I and class II genes (Sharma et al. 2003), which are closely linked with the genes for TNF-α and LT-α. TNF-α not only regulates granuloma formation (Baughman et al. 2003) but its levels fluctuate with disease activity (Prasse et al. 2000). Similarly to AS, there is some heterogeneity in the TNF allelic association in different populations.
A series of studies looking at the -308 and -238 polymorphisms in African American patients did not found any associations with sarcoidosis (Pandey et al. 2002; Rybicki et al. 2004). Although Yamaguchi et al. did not find any association with TNF-α polymorphisms in a Japanese cohort, the TNF-β allele 1 (LTA_Ncol_G) was found to be a marker for prolonged clinical course in Japanese patients with sarcoidosis (Yamaguchi et al. 2001).
In an Indian population however, both TNF -308 G/A and TNF -238 G/A polymorphism were found to influence soluble levels of TNF-α and sACE. Additionally the haplotype GTCCGG was identified as a major risk factor for disease susceptibility, and increased levels of sACE in the patient population (Sharma et al. 2008). Grutters et al. found an increased of the rarer TNF -857 T allele in two different Caucasian populations from the UK and the Netherlands and a

subgroup analysis showed a significant increase in the less common TNF -308 A allele in patients with Löfgren's syndrome, which correlates with Swider et al. findings in German patients (Grutters et al. 2002; Swider et al. 1999).

TNF-α genes, in particular the -308 A/G polymorphism have been inconsistently associated with SLE where there is also a strong association with the HLA-DR antigen. A meta-analysis of 21 studies of this polymorphism concluded that the -308 G/A polymorphism may confer susceptibility to the disease in Europeans but not in Asian-derived population (Lee et al. 2006).

1.3.2.1.2. TNF-α therapy

TNF-α has been detected in human eyes with a variety of inflammatory conditions (Ahn et al. 2006b; Santos Lacomba et al. 2001b) and plays a key role in the regulation of the inflammatory process seen in EAU (Dick et al. 1998). Neutralising TNF in mice with EAU suppress the proliferative response seen to retinal antigens (Sartani et al. 1996). It is believed thought that activated T cells cannot cross the blood retinal barrier directly unless TNF-mediated changes to the vessel endothelium occur (Xu et al. 2005). In the EAU model a constant feature is increased TNF expression and TNF modulates leukocyte migration. Its central role in ocular inflammation makes TNF-α an attractive target for immune therapy, although there remains some controversy as to whether TNF-blockage can suppress permanent histological damage (Robertson et al. 2003).

Inhibition of TNF-α results in a decrease in adhesion molecules, pro-inflammatory cytokines as well as regulation of chemokines (Taylor et al. 2000).

Accumulating evidence suggests that not only soluble TNF-α but also transmembrane TNF-α is involved in the inflammatory response (Horiuchi et al. 2010) and extensive clinical experience has shown a significant therapeutic effect from TNF inhibitors in both systemic and organ specific inflammatory diseases (Mitoma et al. 2008), in particular in those associated with inflammatory eye disease such as seronegative spondyloarthropathies, JIA, Crohn's disease, and RhA (Theodossiadis et al. 2007).

The use of anti-TNF-α antibodies has shown to be effective in the management of panuveitis in patients with Behçet's disease (Sfikakis et al. 2001), and most recently the chimeric anti-TNF-α monoclonal antibody infliximab has been shown to be safe and effective in the short and long-term treatment of refractory posterior uveitis (Benitez-del-Castillo et al. 2005; Joseph et al. 2003). Unfortunately, despite the clinical efficacy in treating joint disease in patients with coexisting arthritis and uveitis, the management of intraocular inflammation has not always been effective. This may be explained by the presence of unique cell adhesion

molecules, subsets of antigen-presenting cells, subsets of lymphocytes and cytokine profile in autoimmune diseases occurring in different organs (Smith et al. 2001).
There are two monoclonal antibodies and a soluble receptor available for use clinically, which bind to soluble and membrane form of TNF-α and can neutralise the pathological effects of TNF-α.

1.3.2.1.2.1. Etanercept

Etanercept is a genetically engineered recombinant fusion protein that consists of two identical chains of the extra cellular human TNF-receptor p75 monomer fused to the Fc domain of human IgG1 (Mohler et al. 1993). This dimeric protein binds to circulating TNF and lymphotoxin-α preventing cell surface interaction and cytokine activation. It is normally given by subcutaneous injection at a dose of 25mg twice weekly, although studies have shown that 50mg once a week has equal effect to twice-weekly injections in patients with RhA.

JIA is the most common systemic chronic autoimmune disease of childhood with about 30% of patients developing uveitis in the course of the disease, in particular those with oligoarticular or seronegative polyarticular disease (Schmeling et al. 2005). Given the significant clinical response seen with the use of etanercept in about 80% of patients with polyarticular JIA (Lovell et al. 2000), different groups have evaluated the efficacy and safety of etanercept in children with chronic uveitis associated with JIA that did not respond to other forms of immunosuppression. Reiff et al. observed that etanercept at a dosage of 0.4mg/kg was safe and effective in reducing intraocular inflammation in 63% of patients after 6 months (Reiff et al. 2001), and this effect was maintained for up to 2 years (Reiff 2003). A more marked difference between systemic and ocular outcomes were also demonstrated by Schmeling et al. who noted a significant or complete response of arthritis in 87% of patients with uveitis, whereas the frequency of uveitis flares seemed to be higher during the course of treatment with etanercept than during the pre-treatment period. In addition to this, two patients out of 229 developed uveitis for the first time while being treated with etanercept (Schmeling & Horneff 2005), suggesting that inhibition of TNF-α might contribute to the induction of exacerbating uveitis in certain circumstances. This paradoxical effect remains unexplained however, a “dechallenge-rechallenge” phenomena has been observed in EIU mice that were treated with either repeated injections of TNF-α or with a single injection of antibody against it. Although both treatments conferred protection against LPS, TNF-resistant mice and those treated with anti-TNF-α antibody demonstrated an exacerbation of EIU when compared with control mice, suggesting that this resistance to TNF involves an complex network of cytokines and unlike the systemic effect of LPS, TNF-α may not be the only

molecule involved in the pathogenesis of EIU and may even have a protective role in this disease (Kasner et al. 1993).

1.3.2.1.2.2. Infliximab

Infliximab is a chimeric IgG monoclonal antibody composed of human constant region and murine variable regions. It has a great affinity for TNF-α receptors and inhibits its activity (ten Hove et al. 2002). Differently to etanercept, it does not bind to lymphotoxin, and is administered by an intravenous infusion at baseline, 2 weeks, 6 weeks, and thereafter eight weekly. The dose varies from 3mg/kg in RhA to 5mg/kg in AS or psoriatic arthropathy, but higher doses of up to 20mg/kg have been used in a cohort of 17 children with chronic uveitis, with few side effects (Kahn et al. 2006).

A great experience in the use of infliximab has been gained from its use in patients with AS, where uveitis is the most common extrarticular manifestation (Hamideh et al. 2001). A meta-analysis by Braun et al. looking at the outcomes of 4 placebo controlled studies and 3 open labelled studies, showed a significant difference between the incidence of AU flares during placebo treatment and the incidence during treatment with anti-TNF-α agents. Although a subset analysis suggested that episodes of AU were less frequent in those on infliximab than on etanercept, this did not reached a statistically significant difference (Braun et al. 2005). A more recent retrospective study comparing the efficacies of anti-TNF-α antibody and the soluble TNF-α receptor treatments in reducing uveitis flares in patients with spondyloarthropathies showed a significant reduction in the number of uveitis flares per patient-years. However, subdata analysis showed that the soluble TNF-α receptor was not efficacious in reducing the occurrence of uveitis flares in these patients (Guignard et al. 2006). In addition to this, there were two patients without any history of uveitis prior to anti-TNF-α treatment that developed uveitis after being treated with etanercept.
A single infusion of intravenous infliximab was highly effective in suppressing inflammation in a cohort of seven patients with HLA-B27 positive AAU, without any other adjuvant treatment other than topical cycloplegia (El-Shabrawi et al. 2002). Markomichelakis et al. demonstrated the effectiveness of a single dose of infliximab in reducing cystoid macular oedema (CMO) and improving vision in a small series of patients with refractory uveitis, including one patient with HLA-B27 positive AU (Markomichelakis et al. 2004). This data suggests that infliximab is an effective and relatively safe agent in the treatment of acute HLA-B27 uveitis, in particular in the management of very severe cases.
Although it appears that in patients with JIA etanercept may be more efficacious and be better tolerated than infliximab (Lahdenne et al. 2003), their efficacy in controlling the course of uveitis is not clear, and a few small case series reporting the use of infliximab in the

management of refractory uveitis associated with JIA suggest a better efficacy in controlling ocular inflammation, in particular as an adjuvant agent to simultaneous immunosuppressive therapy (Murphy et al. 2004; Richards et al. 2005).

1.3.2.1.2.3. Adalimumab

Adalimumab is a fully humanised IgG1 monoclonal antibody that binds with high affinity and specificity to TNF and neutralises the biological activities of this cytokine by blocking its interaction with the p55 and the p75 cell surface receptors (Kempeni 1999; Kempeni 2000). Similarly to etanercept, it is given by subcutaneous administration at a usual dose of 40mg at two weekly intervals. It has shown to be efficacious in children with chronic uveitis associated with arthritis who did not respond to previous anti-TNF therapy, especially in younger patients and these with shorter duration of JIA (Tynjala et al. 2008). In a mixed cohort of children with JIA associated uveitis and of idiopathic origin, adalimumab showed an improvement or stabilization of the uveitis in 94% of eyes (Vazquez-Cobian et al. 2006). Furthermore, as opposed to the results seen in children with CAU associated to arthritis adalimumab seem to be much more effective in controlling intraocular inflammation that it is against the arthritis (Biester et al. 2007).

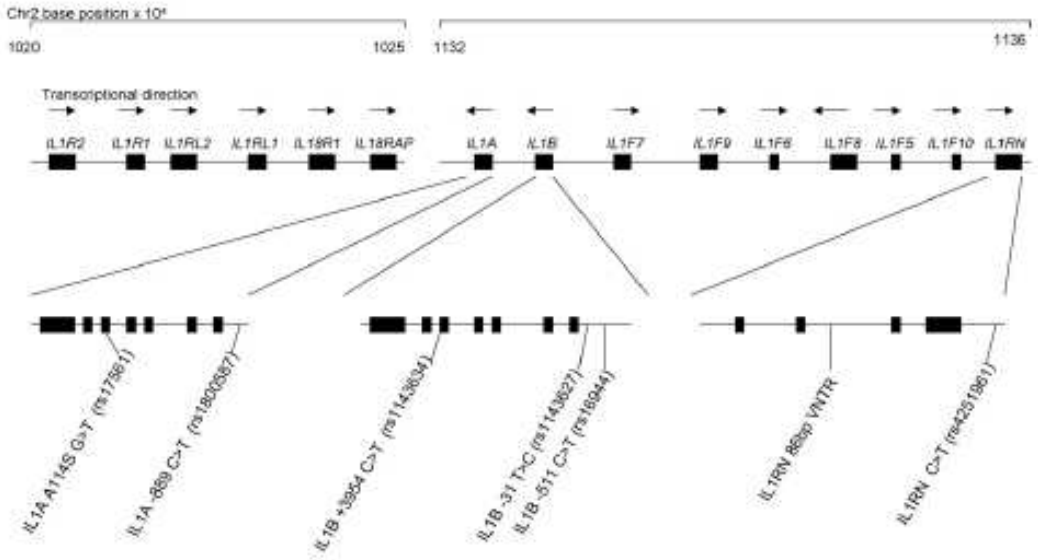
Although treatment with TNF-blockers is generally safe and initial studies did not show a significant increase in adverse side effects, Kavanaugh et al. and Weinblatt et al. reported a number of infections in patients with RhA treated with etanercept (Kavanaugh et al. 2000; Weinblatt et al. 1999), and therefore TNF inhibition should be avoided in patients with acute, recurrent or chronic infections. Another side effect of anti-TNF therapy is the development of anti-double stranded DNA antibodies. This was only seen in the group of patients treated with infliximab and was not necessarily associated with the development of lupus-like disease. Interestingly, one of the side effects reported by Smith et al. was the development of intraocular inflammation for the first time in patients taking etenarcept (Smith et al. 2001). Special caution has to be paid when treating pregnant women, since the presence of TNF in mouse embryos suggests the role of TNF in embryogenesis and may have implications in anti-TNF therapy during pregnancy (Vassalli 1992).

1.3.2.2. Interleukin-1

The complexity of the cytokine network is epitomised by the IL-1 family. IL-1 is located in a 430 kb region in the human chromosome 2q14 region and contains three related genes that encode the pro-inflammatory cytokines IL-1α, IL-1β, and IL-1RN (Nicklin et al. 2002) (Figure 1.8).

The product of IL-1RN is the endogenous receptor antagonist or IL-1ra (Eisenberg et al. 1990; Seckinger et al. 1987). There are two known specific receptors for IL-1. The biologically active type I receptor (IL-1RI) and an inactive type II (IL-1RII), which binds IL-1 preventing from interacting with the functional IL-1RI (Colotta et al. 1994).
IL-1 has been implicated in the modulation of autoimmune disorders including uveitis (Dinarello et al. 1993), and it is upregulated in eyes with EAU and uveitis (El-Shabrawi et al. 2000).

Figure 1.8. Map of the IL-1 gene cluster on chromosome 2. From Smith et al. (Smith & Humphries 2009).



1.3.2.2.1. Interleukin-1 receptor antagonist

IL-1ra family includes one secreted isoform (sIL-1ra) and three intracellular isoforms (icIL-ra 1, 2, and 3). The secreted isoform variety binds to IL-1 receptors (type I and type II) located on the plasma membrane of many different cells with similar avidity than IL-1α and IL-1β but without inducing intracellular responses (Hannum et al. 1990). The intracellular isoforms on the other hand bind to IL-1 receptors and inhibit the stimulatory effects of IL-1, making IL-1ra the natural antagonist of IL-1 by competitive binding to a specific receptor (Merhi-Soussi et al. 2005).

Imbalances in the IL-1 and IL-1ra system have been shown to be important in the development and outcome of inflammatory diseases (Arend 2002; Mikuniya et al. 2000). More recent research has confirmed downregulation of pro-inflammatory cytokines such as IFN-γ and IL-17 in the IL-1 knockout mice, while IFN-γ, IL-17, and TNF-α were upregulated in the IL-1ra knockout mice, confirming that the IL-1/IL-1ra system is crucial for auto-antigen-specific T cell induction and contributes for example to the development of EAE (Matsuki et al. 2006).

IL-1ra is released in vivo during experimentally induced inflammation and is thought to be part of a naturally occurring feedback mechanism to limit the extent of IL-1 activity (Dinarello 1991). In experimental and animal models of arthritis neutralizing antibodies to IL-1ra exacerbated the LPS-induced arthritis in rabbits, and the administration of IL-1ra suppressed inflammation and tissue damage (Zheng et al. 2006). Similar effects have been observed in other inflammatory disorders such as IBD (Ludwiczek et al. 2004).

1.3.2.2.1.1. IL-1ra allele polymorphisms and disease

The IL1RN gene contains an 86bp variable-number tandem repeat (VNTR) or IL1RN*2 in intron 2, which is in LD with other polymorphisms in IL1RN, among them one at position +2018 within exon 2 (Cox et al. 1998; Tarlow et al. 1993). Although the frequency of different alleles varies depending on the studied population, the frequency of allele 1 (IL-1RN*1) in every population studied to date is more common than the allele 2 (IL-1RN*2) (Mwantembe et al. 2001).

IL-1RN*2 has been associated with an increased production of IL-1ra in vitro upon monocytes stimulation with several cytokines including TNF-α (Danis et al. 1995). This findings have been replicated by Hurme et al. who found increased plasma levels of IL-1ra in healthy blood donors who carried the IL-1RN allele 2 (Hurme et al. 1998). However, IL-1RN*2 allele has also been linked to decreased IL-1ra levels when compared to other alleles, most likely due to the heterogeneity of disorders and ethnic groups studied and in a recent study by Rafiqet al. 5 out of 7 genotyped IL-1RN SNPs were associated with plasma IL-1ra

with varying degree of significance (Rafiq et al. 2007). The authors found several SNPs to be in LD, in particular the IL1RN (rs579543) variant allele, which is known to influence circulating levels of IL-1ra with highest production in carriers of the homozygous TT genotype (Graziano et al. 2009).

Several studies have shown an association between the IL-1RN*2 allele and a variety of different autoimmune or inflammatory diseases and the majority of these cases have been associated with severity rather than a predisposition to acquire the disease. Different authors have reported an association between IBD and IL-1RN*2. Andus et al. found that patients with IL-1ra genotype 2 had significantly reduced concentrations of IL-1ra in inflamed mucosa of patients with IBD, particularly these with Crohn's disease (Andus et al. 1997). Patients who carried the IL-1RN*2 allele but not the IL-1β allele 2 were found to have an increased susceptibility to ulcerative colitis and Crohn's disease. Since non-carriers of IL-1β allele 2 were more often present in the subgroup of patients carrying the IL-1RN*2, and no association of these alleles was detected in the group of healthy controls, the authors concluded that the IL-1β/IL-1RN allelic cluster may participate in the susceptibility to chronic IBDs (Bioque et al. 1995). Given that both ulcerative colitis and Crohn's disease are associated with SpA, polymorphisms in IL-1RN have also been investigated and two separate studies in two different European populations found that the allele IL-1RN*2 was significantly increased in AS, compared to healthy individuals, independently from other polymorphisms in the IL-1β gene (McGarry et al. 2001; van der Paardt et al. 2002). Increased susceptibility but no disease activity or severity were reported in patients with JIA who had an increase carriage of IL-1RN*2 compared to a control group (Vencovsky et al. 2001).

1.3.2.2.1.1.1. IL-1ra therapy

Gene therapy with IL-1ra was tried with success in the animal model for the treatment of arthritis (Pan et al. 2000), and Bresnihan et al. confirmed both the efficacy and safety of the treatment with subcutaneous recombinant human IL-1ra in patients with severe and active RhA (Bresnihan et al. 1998). Similar studies have followed, and treatment with IL-1ra showed a retarded radiographic disease progression in patients with RhA (Jiang et al. 2000). Human recombinant IL-1ra has shown to inhibit the inflammatory response triggered by IL-1 when injected into the anterior chamber or intravitreally as shown in different studies (Bhattacherjee et al. 1987; Rosenbaum et al. 1987). Rosenbaum et al. found that IL-1ra decreased the cellular infiltration and protein extravasation after the administration of intravitreal IL-1α.when given within two hours after the initial administration of IL-1α (Rosenbaum et al. 1991). Interestingly, intravitreal injection of IL-1ra inhibited IL-1 induced inflammation in the animal model, but failed to reduce the inflammation secondary to the

intravitreal administration of endotoxin possibly due to the presence of other pro-inflammatory cytokines or that the effects of IL-1 were primarily intracellular and therefore resistant to the activity of exogenous IL-1ra (Rosenbaum et al. 1992).
Conversely, the results of another experiment showed that human IL-1ra injected in the anterior chamber decreased the postoperative inflammation after intraocular lens implant in the rabbit eye (Nishi et al. 1994), and more recently Lim et al. showed a significant decrease in the cellular immune response and in the expression of cytokines such as IFN-gamma, TNF, IL-1β, IL-1α, and IL-6, supporting the role of IL-1ra in immune responses of immune-mediated ocular inflammation (Lim et al. 2005).
Current research has also shown an increase in corneal allograft survival rate following topical administration of IL-1ra eye drops through an inhibitory effect on CD_1-positive cells in high risk corneal transplantation (Jie et al. 2004).

1.3.2.2.1.1.1.1. Anakinra

Anakinra is the commercially available antagonist to IL-1 receptor. It is a recombinant version of the human IL-1ra (rHuIL-1Ra) (Hannum et al. 1990). IL-1ra is released in vivo during experimentally induced inflammation as part of a naturally feedback mechanism (Dinarello 2005), and blocks IL-1 action by binding to its receptor (Hannum et al. 1990), but does not result in signal transduction (Dripps et al. 1991). It was initially used in patients with RhA showing significant improvement on clinical, laboratory, and radiological parameters (Bresnihan et al. 1998; Jiang et al. 2000). Similarly IL-1 has been implicated in the mediation of uveitis and is up-regulated in eyes with EAU (El-Shabrawi et al. 2000; Foxman et al. 2002).
Not all forms of chronic inflammatory arthritis depend on TNF. Pascual et al. found an increase in expression of IL-1b by circulating mononuclear cells, and a dysregulation of IL-1 production in patients with systemic onset JIA (Pascual et al. 2005). They administered subcutaneous, recombinant IL-1ra to a small group of patients with systemic onset JIA, who did not respond to other forms of immunosuppression including anti-TNF agents. All patients had a significant improvement in their clinical symptoms and laboratory findings. With a similar rational Teoh et al. showed a successful outcome of the use of subcutaneous anakinra in a patient with CINCA syndrome and panuveitis who had showed resistance to previous treatment with etanercept (Teoh et al. 2007). Previous to this report, Matsubayashi et al. had already demonstrated the benefits of anakinra in the use of CINCA associated ocular complications (Matsubayashi et al. 2006).

1.3.2.3. Interleukin-6

IL-6 is a multifunctional cytokine with a molecular weight (MW) ranging from 21 to 28 Kd produced by a wide variety of cells of the innate immune system, such as dendritic cells, monocytes, macrophages, lymphocytes and PMNs, but also by other type cells which include fibroblasts, epithelial cells, endothelial cells and smooth muscle cells, regulating the immune response, hematopoiesis, the acute phase response, and inflammation (Ishihara et al. 2002a). IL-6 signaling is regulated by negative feedback of suppressors of cytokine signaling and the protein inhibitors of activated STATs. IL-6/IL-6 receptor interaction leads to activation of STAT3, which in turn targets the suppressors of cytokine signaling (Kishimoto 2006). IL-6 can also elicit the development of specific cellular and humoral immune responses, including end-stage B cell differentiation, and T cell activation, suggesting the importance of IL-6 in modulating the transition between acute and chronic inflammation (Kaplanski et al. 2003), and overproduction of IL-6 leads to inflammation and several autoimmune disorders (Ishihara & Hirano 2002a).

1.3.2.3.1. Association studies of IL-6 with disease

Since the first report indicating that high levels of IL-6 in synovial fluid from the joints of patients with RhA were responsible for the possible involvement of IL-6 in RhA (Hirano et al. 1988) a vast number of studies have demonstrated the involvement of IL-6 in the pathophysiology of other arthritic diseases (Ishihara & Hirano 2002a). A more recent study looking at the gene expression of IL-6 in children with autoimmune arthritis confirmed the circulating monocytes as the main cellular type expressing IL-6 in children with active systemic JIA (Ogilvie et al. 2007). Concentrations of IL-6 in synovial fluids correlated positively with the amount of infiltrative cells in synovial tissue in patients with RhA (Matsumoto et al. 2006). Serum levels of IL-6 are found to be elevated in patients with Crohn's disease and they correlate with disease activity, and treatment response (Reinisch et al. 1999). Analysis of inflamed intestinal mucosa in patients with IBD revealed higher amounts of IL-6 than in intestinal mucosa with inactive disease and local infiltrating T cells, macrophages and B cells as being the cellular source of localised IL-6 production in this patients (Mitsuyama et al. 1991; Stevens et al. 1992). IL-6 transcripts are elevated only in patients with active IBD, confirming not only the role of IL-6 in mediating inflammation in the intestinal mucosa but also its involvement in the pathogenesis of IBD (Matsuda et al. 2009).

IL-6 can induce T cell expression of IL-2 receptor, which in turn would activate the proliferation of T cells in the presence of IL-2 and consequently enhance a positive feedback caused by the spontaneous release of IL-2 from T cells in sarcoidosis (Lotz et al. 1988), where

augmented expression of the IL-6 gene has shown to correspond with increases in IL-6 levels in BAL fluid, alveolar macrophages and alveolar fibroblasts (Balamugesh et al. 2006).

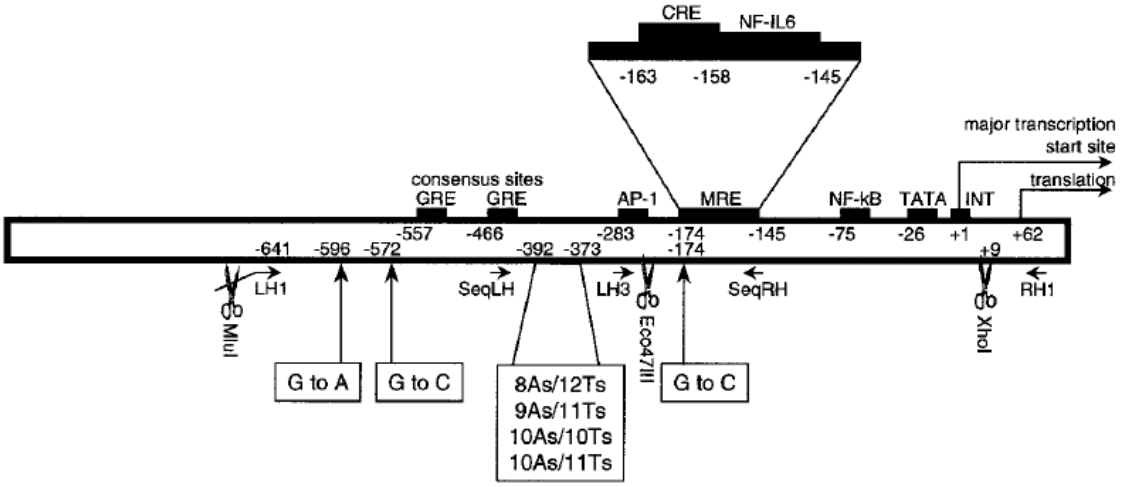
1.3.2.3.1.1. IL-6 and uveitis

Studies from two different groups have demonstrated a high concentration of IL-6 among other pro-inflammatory cytokine and chemokines AqH of patients with idiopathic intraocular inflammation (Ooi et al. 2006; Takase et al. 2006) and more interestingly, the level of IL-6 correlates with the number of neutrophils present in the cellular milieu in the AqH (Curnow et al. 2005). Serial sampling of AqH of a murine model of EAU demonstrated an increased local production of IL-6 at the onset of the diseases followed by a decrease as the inflammation settled (Ohta et al. 2000). In agreement with this finding serum levels of IL-6 in a small group of heterogeneous types of uveitis were found to be significantly elevated durin ghte acute phase of the disease, and decreased during remission (Kramer et al. 2007). Furthermore a comparison between AqH and serum levels of IL-6 from patients with uveitis showed significant higher levels in AqH than in serum (Santos Lacomba et al. 2001a). These findings suggest the pathogenic role of this cytokine in uveitis.

1.3.2.3.2. IL-6 gene and polymorphic sites

The human IL-6 gene is located in the 7p21 chromosome; it is approximately 5 kb in length and consists of five exons and four introns. The human IL-6 is synthesized as a precursor protein of 212 amino acids. Whereas the first 28 amino acids can be removed without affecting significantly the biological activity of IL-6, the removal of only 4 amino acids from the C-terminal of the IL-6 molecule may result in a complete loss of its biological activity (Kruttgen et al. 1990). The IL-6 gene does not contain any common polymorphism within coding regions, and thus most of the research has focused on promoter polymorphism. There are four described genetic polymorphisms in the IL-6 promoter (-597G/A, -572G/C, -373A/T, and -174G/C) (Terry et al. 2000), which are naturally occurring haplotypes. The -174 G/C (rs1800795) polymorphism in the 5’ flanking region appears to affect IL-6 transcription (Figure 1.9).

Figure 1.9. Schematic representation of the 5'flanking region of the IL-6 gene, showing the four polymorphic sites, transcription factor binding sites, primer binding sites, and restriction enzyme cutting sites used for cloning. From Terry et al. (Terry et al. 2000).



Fishman et al. characterized this SNP in a Caucasian population from North London, by creating a luciferase reporter construct of the 5'flanking region (-550 to +61bp). After transfection into HeLa cells a reduction of the -174 C allele was observed, whereas after stimulation with LPS, expression of the -174 C allele did not change, but the construct containing the -174 G allele showed a significant increase (Fishman et al. 1998). Terry et al. designed a construct that contained the haplotypic variants of the proximal promoter (Terry et al. 2000); when using the smallest construct (-221 to +13bp) they demonstrated an increase in the construct containing the -174 C allele following IL-1β stimulation, although this was not statistically significant. Using the larger construct (-641 to +13bp), they found an increase in IL-1β-stimulated HeLa cells for all haplotypes, and following treatment with dexamethasone all haplotypes except AG28/12G showed a reduction in expression. The AG28/12G haplotype is a lower producer than the AG28/12C suggesting that the -174 C allele is involved in greater IL-1 stimulation. The authors concluded that IL-6 promoter polymorphisms may have an influence on transcription through complex interactions determined by the haplotype type, and that alone they are not functional.
A meta-analysis of the -174 G/C and levels of IL-6 has been unable to show any correlation after looking at a total of 5659 subjects, adding further evidence to the existence of additional loci that may have a role in IL-6 expression (Huth et al. 2009).

Nevertheless, higher levels of IL-6 have been associated with the presence of allele C and CC genotype in patients with chronic inflammation (Foster et al. 2000). The acute inflammatory response seen after coronary artery bypass surgery is also associated with the release of IL-6, specially in these patients who carried at least one C allele of either the -174 or -572 polymorphisms compared to those who were homozygous for the allele G (Brull et al. 2001). Homozygous patients for allele G have been related to increased plasma levels in patients with primary Sjogren's syndrome, although in this study the G/C base exchange polymorphism at position -174 did not predispose patients to developing the disease (Hulkkonen et al. 2001b). In type I diabetics of Caucasian origin, a significant increase in the frequency of the G allele compared to normal controls and a corresponding decrease of the C allele frequency was found. There was also a higher frequency of homozygous for the allele G in the diabetic groups compared with normal controls (Jahromi et al. 2000). There is evidence of increased production of IL-6 among other pro-inflammatory cytokines within inflammatory cells in Kaposi sarcoma lesions promoting the development of Kaposi sarcoma in HIV infected subjects. In this group of patients, those who were homozygous for allele G were associated with increased IL-6 production and were over represented (Brockmeyer et al. 1999). On the other hand, two different studies concluded that the IL-6 G/C promoter polymorphism did not contribute to susceptibility to develop SLE in Caucasian patients

(Linker-Israeli et al. 1999; Schotte et al. 2001). Similarly, no significant differences were found in the allele, genotype, or carrier frequencies of this functional promoter polymorphism in patients with IBD (Klein et al. 2001).

1.3.2.4. Interleukin-10

IL-10 is a 40 Kd regulatory cytokine, whose main role is to inhibit the production of pro-inflammatory cytokines. Constitutively produced endogenous IL-10 ameliorates the development of EAU by suppressing the de-novo priming of antigen-specific T cells and inhibiting leukocyte recruitment (Agarwal et al. 2008) as an example of the inhibitory functions of IL-10. NADPH oxidase 1 (Nox1) has been recently identified as multicompetent enzyme upregulated by TNF-α in colon epithelial cells (Kuwano et al. 2008), which in turn is down-regulated by IL-10 in colon epithelial cells (Kamizato et al. 2009).

The gene encoding IL-10 is located in chromosome 1 at 1q31-32 and is expressed by $CD4^+$ T cells, $CD8^+$ T cells, monocytes, macrophages, keratinocytes, and activated B cells (Kim et al. 1992; Powrie et al. 1997). Human IL-10 complementary DNA (cDNA) exhibits a high degree of nucleotide homology with mouse IL-10; the only difference being the insertion of a human Alu repetitive sequence element in the 3' untranslated region of the human IL-10 cDNA clone (Moore et al. 2001).

The 5'-flanking region of the human IL-10 gene contains two dinucleotide repeats, IL-10G and IL-10R located at 1.1 Kb and 4.0 kb respectively upstream of the transcription initiation site (Eskdale et al. 1995; Eskdale et al. 1996), indicating that innate differences in IL-10 production are locus-dependent.

The IL-10 proximal promoter region contains several SNPs including -1082 G/A (rs1800896), -819 C/T (rs1800871), and -592 C/A (rs1800872) which produce three haplotypes in Caucasians (GCG, ATA, ACC) (Smith & Humphries 2009). Rees et al. showed that the IL-10 -1082 G/A polymorphism occurs within an ETS-like transcription factor binding site (Rees et al. 2002), and is in LD with the -819 C/T and -592 C/A polymorphisms.

1.3.2.4.1. Association studies of IL-10 polymorphisms with disease

Crawley et al. examined the functional significance of the IL-10 gene in the -1137 to +25 region from individuals homozygous for the IL-10 haplotypes mentioned above (Crawley et al. 1999). The ATA construct conferred weaker transcriptional activity than GCC, and IL-10 levels measured from LPS-stimulated whole blood were found to be lower in those subjects who were homozygous for the ATA haplotype. These results have been reproduced by Suarez et al. in a Caucasian population from Northern Spain using RT-PCR quantified extracted mRNA. Levels of mRNA expression were significantly increased in individuals carrying the

GCC/GCC genotype, compared to those carrying the ATA/ACC genotype (Suarez et al. 2003). When they analyzed the genotypes at -1082 separately, they found a significant difference between the -1082 genotypes independent of the -819/-592 genotype, suggesting that -1082 may be the most important functional SNP. They also conclude that although not significant, there was a trend for higher IL-10 levels among individuals who were homozygous for the -1082 G genotype. More recently, the same group of authors examined the influence of these polymorphisms in patients with IBD (Castro-Santos et al. 2006). Stratified analysis demonstrated a significant association between the -1082 AA genotype (low IL-10 producers) and severe disease, in agreement with previous findings from Tagore et al. who showed a decreased IL-10 G allele in patients with IBD (Tagore et al. 1999). Looking at patients with rheumatoid disease, Huizinga et al. demonstrated more severe organ damage in patients with RhA with the -1082 AA genotype compared to those with the -1082 GG genotype (Huizinga et al. 2000).

1.3.2.5. Th 17 cytokines

One of the essential characteristics of the recently described Th17 lineage of CD4+T cells is their ability to produce IL-17A. Recent studies have shown that these cells also express IL-17F, IL-22, and IL-21 and although their existence had already been reported, they were thought to originate from a different T cell type. Differentiation of these Th17 cells is partly driven by TGF-β, with contributions by IL-1, TNF-α, but mostly by IL-6 through a complex process that involves activation of different transducer and activator of transcription factors such as retinoid-related orphan receptor-γt and STAT3 (Kimura et al. 2007; Leppkes et al. 2009).

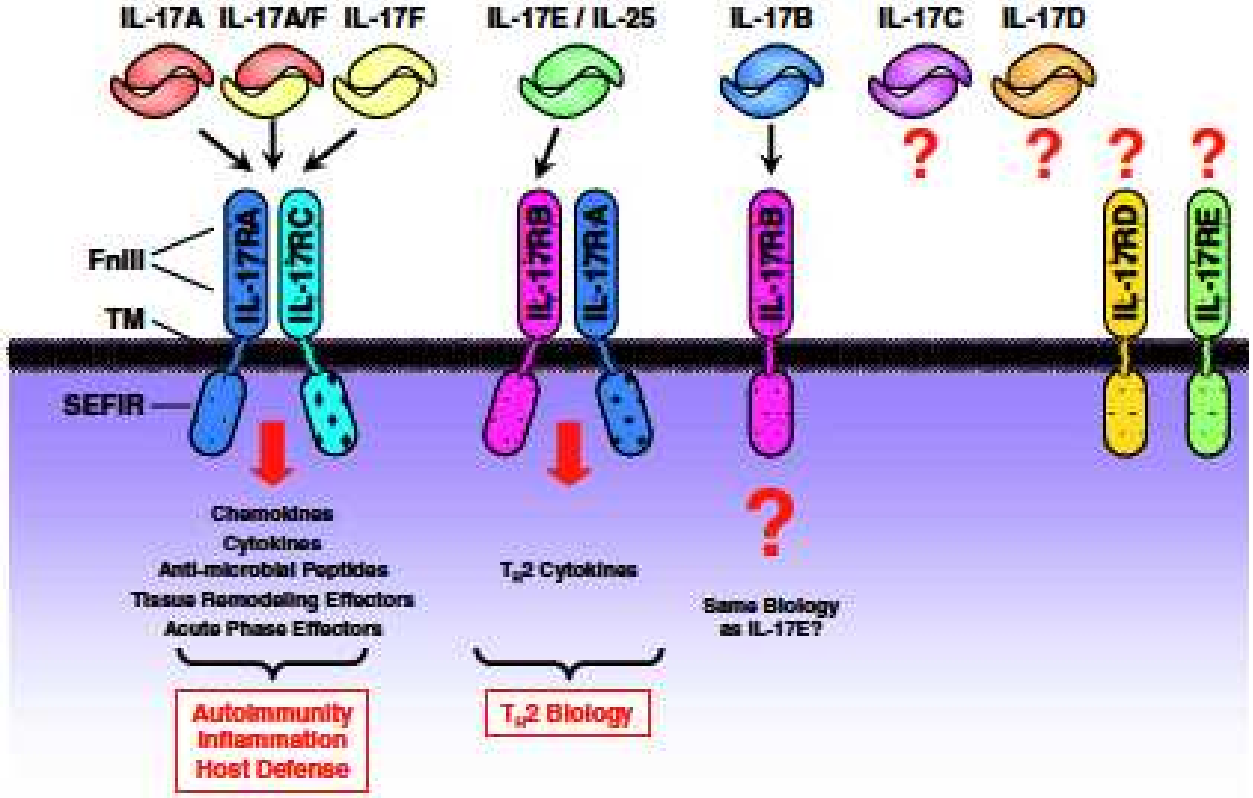
1.3.2.5.1. Interleukin-17

The IL-17 family of cytokines includes IL-17A also called IL-17, IL-17B, IL-17C, IL-17D, and IL-17F. IL-17E (IL-25) is not produced by Th17 cells, but by Th2 cells, and might be involved in Th2-type allergic responses. IL-17A and IL-17F occupy the same location on chromosome 6p12 (Kawaguchi et al. 2004), and can form a hetrodimeric cytokine, which mediates biological activities, through shared receptors (Figure 1.10).
Upregulated Th17 responses or increased IL-17 production has now been linked with chronic inflammation. Most cells expressing IL-17 receptors can produce pro-inflammatory factors such as IL-1, IL-6, TNF, or IL-18 among many others that are involved in the pathogenesis of chronic inflammatory conditions like RhA, psoriasis, IBD, or MS. In RhA, the production of TNF, IL-1, and IL-17 by synovial cells is predictive of joint damage (Kirkham et al. 2006). IL-17 together with IL-6 is highly expressed in brain lesions of patients with MS. Furthermore in these patients where the lesions are restricted to the optic nerve and spinal

cord, the levels of IL-17 and IL-8 in serum and CSF are found to be significantly higher when compared to patients with conventional MS (Ishizu et al. 2005). IL-17 expression is also increased in the intestinal mucosa and serum of patients with exacerbations of IBD (Fujino et al. 2003), and interestingly blockade of IL-23 in the IL-10 deficient mice has shown to ameliorate colitis through the inhibition of IL-17 and IL-6 (Yen et al. 2006). IL-17 is also elevated in patients with Behçet's disease (Hamzaoui et al. 2002) and it is implicated in the trafficking of leukocytes in AU (Saruhan-Direskeneli et al. 2003) highlighting the importance of Th17 cells in inflammatory eye diseases.

Jang et al. have recently investigated the influence of IL17 polymorphisms on disease susceptibility in a Korean population (Jang et al. 2008). Analysis for the IL-17 gene polymorphism identified three sites with gene variants of IL-17F on exon 3 (126A/G, 155G/A, and 161A/G). Haplotype frequencies showed that the AG haplotype was more frequently seen in Behçet's patients compared to their control group, suggesting that the GG haplotype of the IL-17 gene plays a protective role to the development of the disease, whereas those individuals carrying the AG haplotype would have a higher risk for developing the disease.

Figure 1.10. Overview of IL-17 family ligands and IL-17 receptor family and known functions. From Pappu et al. (Pappu et al. 2010).



1.3.2.5.2. Interleukin-22

IL-22 is one of the IL-10 family cytokines, and it was first identified as an IL-10-related T cell-derived inducible factor from a lymphoma cell line (Dumoutier et al. 2001). IL-22 is produced by fully differentiated Th17 cells and it appears to be produced by Th17 cells in response to IL-23 (McGeachy et al. 2007). The main target cells of IL-22 are cells of the skin, digestive tract, lung and kidney, and it has been found to be upregulated in psoriatic skin (Wolk et al. 2004). The preferential production of IL-22 by T cells (Th1, Th17) suggests that IL-22 plays a role in the pathogenesis of T cell mediated diseases.

In vitro, high concentrations of IL-22 increased significantly the production of CCL2 and subsequent proliferation of synovial fibroblasts derived from patients with RhA, and in RhA IL-22 expression in synovial tissues was found to be increased (Ikeuchi et al. 2005). More interestingly there is new evidence that it can target human RPE cells and Li et al. found several genes which included IL-22 to be highly expressed in patients with a heterogeneous group of uveitis compared to a control group. In addition IL-22 had an apoptotic effect on RPE cells which had a detrimental effect on the blood retinal barrier integrity (Li et al. 2008). These results more importantly, are consistent with those found by another group demonstrating the presence of high levels of Th17 cells in peripheral blood mononuclear cells of patients with active scleritis or uveitis (Amadi-Obi et al. 2007), confirming the role of Th17 cells in intraocular autoimmune inflammation.

1.3.2.5.3. Interleukin-21

IL-21 is a novel pro-inflammatory cytokine involved in the immune response of the Th17 cell pathway (Korn et al. 2007). Its receptor shares the common γ chain and signals through the Jak/STAT pathway. Additionally IL-21 is produced by activated CD4+ T cells (Ozaki et al. 2000; Parrish-Novak et al. 2000). IL-21 has been shown to induce proliferation and differentiation of T and B lymphoid cells as well as increase the activity of CD8+T and NK cells (Leonard et al. 2008). It has been implicated in the pathogenesis of EAE (Korn et al. 2007), and experimental colitis (Fantini et al. 2007) by modulating the balance between Treg and Th17 cells.

In a recent report Liu et al. (Liu et al. 2009) demonstrated a significant increase in expression of IL-21 mRNA and IL-21R during the acute phase of the disease of EAU in mice immunized with human IRBP peptide 161-180 compared to the recovery phase and normal controls, suggesting the involvement of IL-21 in the development of uveitis. Furthermore, following stimulated cell culture with recombinant IL-21 combined with TGF-β, IL-17 levels in the supernatant of mice with EAU were markedly increased, adding further evidence of the role of IL-21 in the development acquired immunity and association with the production of autoimmune disease.

1.4. CHEMOKINES

Chemokines or chemotactic cytokines are group of small secreted proteins with a MW between 8- to 12Kd (Rollins 1997). The molecular hallmark of the chemokine family is the presence of four cysteine residues. To date 53 human chemokines and 23 receptors have been cloned and characterized (Colobran et al. 2007). This superfamily of chemokines is now subclassified into four groups according to their arrangement of their cysteine residues located in the N-terminal region, as designated C, CC, CXC, and, CX3C, in which C represents the number of cysteine residue in the N-terminal and X represents the number of intervening amino acids in between the two first cysteines (Murphy et al. 2000).

Chemokines are not stored within cells, and their production is stimulated by a great range of stimuli. Although their cellular sources can vary, the majority of CXC and CC chemokines are produced by stimulated monocytes, macrophages and even by vascular endothelial cells. These structurally related cytokines with chemoattractive properties are responsible for the recruitment, adhesion, chemotaxis of leukocytes towards the site of inflammation (Eugenin et al. 2003). They also play an important role in immune homeostasis by driving the maturation, homing, and activation of leukocytes. With few exceptions, in vitro chemotaxis assays showed that CXC chemokines have an affinity to attract neutrophils, whereas CC chemokines act preferentially on monocyte/macrophages. However, the distinction between the two main subfamilies of chemokines is less well defined when the different subtypes of leukocytes are considered. See table 4 for a summary of the chemokines described in this chapter (Table 1.4).

Chemokines exert their biological actions by binding to G protein-coupled cell surface receptors (Haribabu et al. 1999; Murphy 1994). Although chemokine-receptor interactions exhibit a great degree of selectivity, there is a considerable amount of redundancy in these interactions with some chemokines binding to more than one receptor and vice versa.

Table 1.4. Systematic chemokine and chemokine receptor nomenclature. Modified from Le et al. and Murphy et al. (Le et al. 2004; Murphy et al. 2000).

Chemokines	**ELR**	**H/I**	**Synonyms**	**Major target cells**	**Chromosome**
CC chemokines					
CCL2	N/A	I	MCP-1, MCAF	Monocytes, T cells, basophils, NK cells, progenitors	17q11.2-q12
CXC chemokines					
CXCL8	ELR+	I	IL-8, NAP-1	Neutrophils, T cells, basophils, endothelial cells	4q21
Receptors	**Ligands**		**Synonyms**	**Receptor-expressing cells**	
CC Receptors					
CCR2	CCL2, 7, 8, 12, 13		CKR2, CC CKR2, CMKBR2	Monocytes, immature DCs, T cells, PMNs, eosinophils, platelets	3p21
CXC receptors					
CXCR1	CXCL2, 3, 5, 6 , 7, 8		IL-8RA, IL-8 R-I, IL-8R	PMNs, monocytes, astrocytes, endothelia, mast cells	2q34-q35

H: homeostatic chemokine; I: Inflammatory chemokine; ELR+: Presence of glutamic acid-lecutine-arginine tripeptide motif; ELR-: Absence of glutamic acid-lecutine-arginine tripeptide motif.

1.4.1. CXC chemokines and chemokine receptors

1.4.1.1. Interleukin-8

IL-8 was identified by several groups as a neutrophil activating peptide produced by a variety of cells that include, monocytes, T lymphocytes, neutrophils, fibroblasts, endothelial, and epithelial cells (Conlon et al. 1995). It is also known as CXCL8, or NAP-1. Among its many properties, which include chemoattraction of T lymphocytes, and histamine release from basophils, it is best known for being one of the main chemoattractants for neutrophils (Thorburn et al. 2009).

1.4.1.1.1. Association studies of IL-8 with disease

Most of the evidence for the role of IL-8 in the trafficking of neutrophils comes from experiments that document chemokine expression at different inflammatory foci. In order to examine functionality, Hull et al. identified a common polymorphism in the promoter region -251 T/A (rs4073) and examined IL-8 levels from LPS-stimulated whole blood. The IL-8 -251 A allele tended to be associated with increased IL-8 production, although it did not reach statistical significance (Hull et al. 2000). The authors in later study identified 9 SNPs in a 7.6Kb segment spanning the IL-8 gene and its promoter region, and used six of these SNPs to define the haplotypic structure of the IL-8 locus, with two common occurring; haplotype 1 (-251T/+396T/+781C/+1238insA/+1633C/+2767A), and haplotype 2 (-2251A/+396G/+781T/+1238delA/+1633T/+2767T) (Hull et al. 2001). Using primary respiratory epithelial cells stimulated with TNF they found a moderate increase of haplotype 2. After performing reporter analysis on the -251 T/A promoter SNP, they did not find a difference in promoter activity between the two alleles. Then they proceeded to systematically screen for allele-specific protein-DNA binding in the functional haplotype, which revealed significant differential binding with the +781 C/T allele (rs2227306), suggesting that the increased transcription from haplotype 2 may be mediated by interactions between a transcription factor binding complex and the promoter (Hacking et al. 2004).

Elevated levels of IL-8 have been found in bronchoalveolar lavage (BAL) fluid from patients with interstitial lung disease (Meloni et al. 2004), and not only correlate with the respective BAL cellular differentiation but also with worse lung-function parameters (Schmidt et al. 2009).

Similarly, IL-8 is upregulated in the kidney in IgA nephropathy, SLE associated nephritis, and ANCA-associated vasculitis, and its levels correlate with disease activity. Neutralisation of IL-8 attenuates glomerular neutrophil infiltration and glomerular tissue injury (Rovin et al. 2002).

Elevated levels of IL-8 in serum and CSF have been found in patients with ocular and CNS inflammation in the context of Behçet's disease (Zouboulis et al. 2000), in particular during the active phase of the disease (Saruhan-Direskeneli et al. 2003), suggesting a role in IL-8 as a possible marker of activity and predictor of vascular involvement in Behçet's disease (Durmazlar et al. 2009).
More importantly there is a significant correlation between AqH levels of IL-8 in patients with uveitis and increased disease activity, especially in patients with Behçet's disease, which may be related to other neutrophil chemoattractants that are involved in neutrophil recruitment in this particular condition. In a cohort of patients with different uveitis entities which included HLA-B27 related AU, systemic levels of IL-8 were found to be significantly elevated during exacerbations of the active ocular disease and were decreased during remission (Kramer et al. 2007).

1.4.1.1.2. Association studies of IL-8 polymorphisms with disease

The IL-8 gene is mapped to the 4q13-q21 and contains 4 exons and 3 introns (Mukaida et al. 1989).
Among all IL-8 SNPs described, the presence of -251A/T in the transcription site is known to exert a strong influence in protein synthesis, and its presence has been associated with a wide spectrum of disorders, some of an autoimmune nature. There is a wide heterogeneity among the distribution of this SNP throughout the world (Fujihara et al. 2007). Since the existence of -251A/T IL-8 polymorphism was reported, there have been reports showing a higher IL-8 production by allele A (Hull et al. 2000), while others showed the high production to be associated with the presence of allele T (Lee et al. 2005). More recent data revealed that the differences in IL-8 expression were linked to two main haplotypes which include up to six SNPs and constitute the so-called haplotype 2, which is significantly associated with higher IL-8 levels compared to its counteract haplotype 1 (Hacking et al. 2004). This would indicate that the presence of -251A allele in high haplotype producers of IL-8 has no effect on allele-specific level of transcription, and that the functional allele is probably in LD with haplotype 2 and that -251A/T is not the functional SNP. In keeping with these findings, the frequency of the haplotype TAT inferred from IL-8 -251A/T, -353A/T, and +678T/C is significantly higher in patients with Behçet's disease compared to healthy individuals (Lee et al. 2007).

Other SNPs associated with disease include the -845C/T in the IL-8 promoter region and severe renal injury in African-American patients with SLE nephritis (Rovin et al. 2002), or three other different SNPs located in intron or 2'UTR positions (+781T/C, +1633T/C, +2767T/A) found to be associated with asthma (Heinzmann et al. 2004).

1.4.1.2. CXCR1

It belongs to the G-protein coupled receptor superfamily (Dohlman et al. 1991). Also known as IL-8RA, IL-8R, IL-8R-I. It is expressed mostly by neutrophils and immunofluoresence staining techniques and flow cytometry analysis demonstrated the 70% to 90% of neutrophils bear the CXCR1. It has with more limited expression in T cells, monocytes, dendritic cells, or synovial fibroblasts. CXCR1 binds to two different types of chemokines, CXCL8/IL-8 and CXCL2/GCP2, but is only CXCL8/IL-8 that has been able to neutralize neutrophil adhesion as well as cause neutrophil infiltration (Pignatti et al. 2005).

It has been linked with inflammatory lung disease and Behçet's disease since the expression levels of CXCR1 and CCR5 were down-regulated on CD8(bright)$CD56^{+}$ T cells after systemic immunosuppressive treatment during active Behçet's uveitis (Ahn et al. 2006a).

1.4.2. CC chemokines and chemokine receptors

1.4.2.1. Monocyte chemoattractant protein-1

MCP-1 or MCAF now known as CCL2 was the first discovered human chemokine. It is a potent chemotactic factor for monocytes located on chromosome 17 (17q11.2-q21.1) and is 13Kd in size. It belongs to a family of at least four members (MCP-1, -2, -3, -4). It is produced by a variety of cell types either constitutively or induced by other cytokines, but monocytes/macrophages are found to be the major source of CCL2. It regulates migration and infiltration of monocytes, T cells and NK cells (Deshmane et al. 2009).

Human CCL2 gene transcription is regulated by two distinct areas of the 5' flanking region. The proximal region extends at least 150 bases upstream and contains a number of transcription factors binding sites that coordinate CCL2 gene response to other cytokines such as TNF and INF-γ. However it is the distal regulatory region that contains the -2518 A/G (rs1024611) polymorphism that regulates CCL2 expression (Rovin et al. 1999).

1.4.2.1.1. Association studies of CCL2 with disease

Macrophages, endothelial cells, and smooth muscle cells in inflammatory bowel disease (IBD) express CCL2 mRNA and protein; furthermore the use of immunosuppressive agents decreases MCP-1 expression and production in stimulated monocytes (Grimm et al. 1996). Atherosclerosis is accepted by many as an inflammatory condition where CCL2 as well as its receptor CCR2 has been found to play an important role in the recruitment of monocytes following injury to the vascular wall. There is evidence of CCL2 expression in human atheromatous plaques, which is essential for monocytes recruitment, angiogenesis and proliferation and migration of smooth muscle (Yla-Herttuala et al. 1991). In a similar fashion, in asthma sufferers the kinetics of production of MCP-1 correlates with the recruitment in their airways of specific leukocyte subsets expressing the CCL2 receptor (CCR2)

(D'Ambrosio et al. 2001). Patients with RhA were stratified for the presence for the presence of IgM rheumatoid factor and antibodies against cyclic citrullinated peptide (predictor for development of RhA) and CCL2 levels were found to be significantly increased (Rantapaa-Dahlqvist et al. 2007). Synovial production of CCL2 by local macrophages has been suggested to play an important role in the recruitment of mononuclear phagocytes during inflammation associated with RhA (Szekanecz et al. 2001).
In experimental autoimmune encephalitis (EAE), an animal model for multiple sclerosis (MS), the immune cells infiltrate in CNS consists of CD4, CD8 T cells, and macrophages which express CCL2 and have a direct correlation with disease severity (Jee et al. 2002). Tanuma et al. demonstrated increased immunoreactivity for CCL2 in hypertrophic astrocytes from demyelinating plaques in patients with MS (Tanuma et al. 2006).
Another example implicating CCL2 in the pathogenesis of chronic inflammatory conditions and disease severity is the direct relationship between plasma levels of CCL2 in patients with SLE and severe renal involvement (Lit et al. 2006). In a similar study Brown et al. found that SLE patients had higher CCL2 concentrations than healthy individuals, and that patients with nephritis had higher concentrations than those without nephritis (Brown et al. 2007).

1.4.2.1.1.1. CCL2 and uveitis

Further evidence of the role that CCL2 plays in autoimmune inflammatory diseases has been shown in Lewis rats injected with myelin basic protein (MBP) that developed inflammation in the spinal cord and AU (Adamus et al. 1997). Induction of the gene expression of CCL2 appears to contribute to the recruitment of inflammatory cells in the eye and CNS and also to correlate with disease severity (Adamus et al. 2001). In another murine model, CCL2 deficient mice that were injected Salmonella typhimurium LPS subcutaneously showed a significant reduction of intraocular inflammation when compared to the wild-type control mice. When CCL2 deficient mice were treated with recombinant rat MCP-1 (rRMCP-1) the inflammatory response was reconstituted intraocularly. Another observation from this study was the over expression of IFN gamma in serum, as well as IL-6, and an increased intraocular IFN gamma transcription in the CCL2 deficient mice (Tuaillon et al. 2002). In a similar study Fang et al. detected increased levels of CCL2 in AqH of Lewis rats immunized with melanin-associated antigen prior to the clinical onset of intraocular inflammation, as well as an upregulation of mRNA of CCL2 during the evolution of the EAAU, suggesting the involvement of this chemokine in the initial recruitment of inflammatory cells (Fang et al. 2004).

1.4.2.1.2. Association studies of CCL2 polymorphisms with disease

A variety of diseases which include SLE, juvenile rheumatoid arthritis, and HLA-B27 associated AU have been associated with this polymorphism. Patients of Caucasian origin diagnosed with SLE who carried the -2518 G allele had higher plasma concentrations of CCL2 than those who were homozygous for the allele A (Brown et al. 2007), and predisposed the development of renal involvement and the presence of cutaneous vasculitis (Aguilar et al. 2001; Tucci et al. 2004). Despite different groups having failed to prove an association between this functional polymorphism and susceptibility to RhA (Gonzalez-Escribano et al. 2003; Lee et al. 2003), when patients and controls were stratified according to HLA-DRB1, those patients who lacked the HLA shared epitope were significantly more likely to be homozygous for the allele G (Gonzalez-Escribano et al. 2003). Contrary to these findings, another study postulated the role of the -2518 AG polymorphism in HLA-B27 positive patients with associated AU (Wegscheider et al. 2005). These results are in concordance with these of other groups who looked at patients with Behçet's disease and associated uveitis (Chen et al. 2004; Cho et al. 2004).

The -362G/C polymorphism has also been examined in the model of atherosclerotic disease in addition to the -2578A/G SNP. This polymorphism lies next to a number of transcription factor-binding regions that are linked with an increase in transcription of CCL2 and biological activity of its receptor CCR2. The result of the activity of CCL2/CCR2 system is an increase risk for the development of atherosclerotic plaques (Nyquist et al. 2009).

1.4.2.2. CCR2

CCR2 or CKR2, CC CKR2, CMKBR2 exists in two forms CR2A and CCR2B. These two forms differ only in the amin acid sequence of their intracellular C-terminus (Charo et al. 1994). Therefore, their ligand binding specificities appear to be identical (Combadiere et al. 1995; Franci et al. 1995). It is mainly expressed on monocytes, B cells, activated T cells, immnature dendritic cells (Frade et al. 1997). CCR2 is involved in macrophage migration (Mackay 2001), and its ligands have been implicated in the pathophysiology of a wide number of disorders such as RhA, MS, organ transplant rejection, atherosclerosis. However probably it is better known for its role in viral pathogenesis, supported by in vitro observations that CCR2B can act as a fusion co-factor for HIV-1 (Doranz et al. 1996).

Jiang et al. demonstrated that induction of EAU in an acute model in the rat was accompanied by an increase in CCR2 levels in animals displaying clinical signs of the disease (Jiang et al. 1998). Another group demonstrated the upregulation of gene expression of CCL2 and CCR2 in the acute stages of EAU (Keino et al. 2003).

1.5. UVEITIS

The uvea, the middle vascular coat of the eye (from the Latin *uva* or grape) is composed by the iris, ciliary body and choroid, with each one of these components having different histology and function.

At present, the most widely accepted classification of uveitis is based on the primary anatomical location of the inflammation within the eye (Bloch-Michel et al. 1987; Deschenes et al. 2008) and not on the presence of structural complications, as suggested in the last International Workshop on Standardization of Uveitis Nomenclature (SUN) (Jabs et al. 2005) (Table 1.5).

Inflammation has also been categorised according to its time course and duration into acute, recurrent, subacute and chronic. These terms have been used inconsistently and therefore their use is reserved to describe the onset of the uveitis and the duration of the attack. Hence, AAU usually lasts days to weeks and resolves completely between episodes. Chronic anterior uveitis (CAU) by definition lasts longer than three months. It may follow an episode of AAU or begin insidiously as a low grade and occasionally asymptomatic (Table 1.6).

In order to assist in the evaluation and diagnosis of uveitis, the International Uveitis Study Group (IUSG) also recommended a clinical classification based on etiological criteria, as detailed in table 7 (Deschenes et al. 2008) (Table 1.7).

Table 1.5. The SUN working group anatomic classification of uveitis (Jabs et al. 2005).

Type	**Primary site of inflammation**	**Includes**
Anterior uveitis	Anterior chamber	Iritis, iridocyclitis, anterior cyclitic
Intermediate uveitis	Vitreous	Pars planitis, posterior cyclitic, hyalitis
Posterior uveitis	Retina or choroid	Focal, multifocal, or diffuse choroiditis, retinochoroiditis, retinitis, neuroretinitis
Panuveitis	Anterior chamber, vitreous, and retina or choroid	

Table 1.6. The SUN working group descriptors of uveitis (Jabs et al. 2005).

Category	**Descriptor**	**Comment**
Onset	Sudden	
	Insidious	
Duration	Limited	3 months or less of duration
	Persistent	More than 3 months of duration
Course	Acute	Episode characterized by sudden onset and limited duration
	Recurrent	Repeated episodes separated by periods of inactivity without treatment for at least 3months in duration
	Chronic	Persistent uveitis with relapse in less than 3 months after discontinuing therapy

Table 1.7. IUSG Clinical Classification of uveitis (Deschenes et al. 2008).

Infectious	Bacterial
	Viral
	Fungal
	Parasitic
	Others
Noninfectious	Known systemic associations
	Un-known systemic associations
Masquerade	Neoplastic
	Non-neoplastic

1.5.1. Anterior uveitis

AU refers to disease limited to the anterior segment of the eye. AU is the most prevalent form of intraocular inflammation. The symptoms of an acute episode of AU may include pain, typically in the periorbital region, redness and photophobia. Occasionally there may be associated epiphora, especially in bright light.

The signs of AAU include limbal conjunctival injection, also known as ciliary flush. The characteristic finding in the acute phase are the presence of cells and flare in the anterior chamber, which are the result of extravasated inflammatory cells and proteins respectively. Hypopyon formation occurs when the cells in the anterior chamber collect inferiorly, and it is a sign of severe inflammation. It is typically associated with HLA-B27 related uveitis and Behçet's disease (Ramsay et al. 2001). Keratic precipitates (KPs) are clusters of inflammatory cells deposited on the corneal endothelium. They most frequently coalesce in the inferior third of the cornea as a result of the convection current of the AqH in the anterior chamber, or they may be widely dispersed over the entire corneal endothelium as it is seen in Fuchs' Heterochromic Cyclitis (FHC). The mechanism by which these inflammatory cells attach to the corneal endothelial cells seems to be influenced by the effect of inflammatory cytokines on the expression of cell adhesion molecules. Iris nodules may appear along the pupillary margin (Koeppe) or in the iris stroma (Busacca). Posterior synechiae (PS) formation represents the adhesion of the posterior surface of the iris and the anterior surface of the lens. It tends to develop in untreated severe or persistent AU. When these adhesions occur along the entire 360˚ pupillary margin, the AqH outflow becomes compromised and the iris bows forward developing the so-called, iris bombé. The intraocular pressure may rise as a consequence of this pupillary block or as a result of the formation of peripheral anterior synechiae, between the peripheral iris and the angle structures. However, severe inflammation involving the ciliary body can affect the production of AqH lowering the intraocular pressure. It can also be secondary to ciliary body detachment caused by cyclitic membrane formation.

1.5.1.1. Structure and function of Human Leukocyte Antigen (HLA) molecules

The HLA system is genetically encoded in the chromosome 6 by the Major Histocompatibility Complex (MHC) in humans. The MHC is a system of glycoproteins molecules expressed on the surface membranes of most human cells.

The HLA genes that are involved in the immune response fall into two classes, I and II, which are structurally and functionally different.

The human class I region contains three main loci i.e.: HLA-A, HLA-B, and HLA-C. Each locus encodes the heavy chain of the MHC class I genes antigen. Other, less polymorphic class I genes include the HLA-E, -F, -G, and –H genes (Bjorkman et al. 1990). The class I HLA molecule consists of a non-covalently linked two-chain structure; a polymorphic heavy

chain or α chain encoded by a gene in the HLA class I region and a non-polymorphic β_2-microglobulin that is coded in the chromosome 15. The HLA molecule has a peptide-binding groove that acts as a binding site for short (8 to 12 amino acids) peptides. The complex of HLA molecule-antigen is then recognised by the T cell receptor of class I-restricted $CD8^+$ T cell (Klein et al. 2000). Class I HLA molecules are expressed on most somatic cells with different level of expression. Normal endothelial corneal cells do not express HLA class I molecules as part of their mechanism for immune privilege. Human uveal cells normally expressed HLA antigens at very low levels in normal eyes, but expression is upregulated during inflammation in AU (Abi-Hanna et al. 1989). On the other hand, class II genes code for the α and β polypeptide chains of the HLA-D molecules including HLA-DR, -DQ, DP families, and are expressed in a limited number of cells, which include B cells, monocytes, macrophages, and dendritic cells. They are also expressed by different pro-inflammatory stimuli as it has been observed in patients with uveitis on RPE cells (Chan et al. 1986; Liversidge et al. 1988) which then present retinal antigens to T lymphocytes via the HLA class II system .Finally, class III genes encode for the complement proteins, as well as TNF-α and –β.

The association between the MHC complex, HLA-B27 and its spectrum of HLA-B27-associated inflammatory diseases and uveitis was first reported almost forty years ago by Brewerton et al. (Brewerton et al. 1973) and it remains one of the strongest HLA-disease associations (Table 1.8).

The HLA-B locus is the most polymorphic of the HLA class I genes containing over 500 alleles (Robinson et al. 2006). Similarly, HLA-B27 is not a single allele but a family of at 38 different subtypes (Reveille et al. 2009), named HLA-B*2701 to HLA-B*2728 that have a wide varied racial distribution worldwide, differing from each other by one or a few amino acids, and binding different peptides with some overlapping among them. There is evidence of the diversity of strength of association between HLA-B27 subtypes and different conditions (Khan 2000), including AAU.

Table 1.8. HLA-B27 SpA and their association with AAU. Courtesy of Dr Chang, University of new South Wales, Sydney.

Disease	**HLA-B27 prevalence (%)**	**Proportion of patients with the systemic disease developing AAU (%)**	**Proportion of patients with AAU developing the systemic disease (%)**
AS	90	20-30	15-50 55-90 in HLA-B27 AAU
ReA	40-80	12-37	2-25 8-21 in HLA-B27 AAU
PsA	40-50	40-50	0-2 3-4% in HLA-B27 AAU
IBD	35-75	35-75	2-3 1-7 in HLA-B27 AAU
Undifferentiated spondyloarthropathy	70	70	4-12 5-21 in HLA-B27 AAU

AS: Ankylosing spondylitis; ReA: reactive arthritis; PsA: Psoriatic arthropathy; IBD: Inflammatory bowel disease.

One of the most widespread subtypes is the HLA-B*2705, which is present in nearly 90% of HLA-B27 positive healthy individuals form Northern Europe (Khan 2000), it is also believed to be the common ancestral type from which other subtypes have evolved.
Other subtypes present in Caucasian individuals of Northern European ancestry include the HLA-B*2702, which is present in 10% of HLA-B27 positive patients with AS, and AAU (Chang et al. 2005), and in a Dutch population the frequencies of HLA-B*2705 and HLA-B*2702 subtypes in patients with AAU were found not to be different from these found in HLA-B27 normal controls (Derhaag et al. 1988). Interestingly, in Asian populations, such as the Japanese and Chinese, there is a predominance of the HLA-B*2704 subtype, which in addition to the weaker association of this subtype of HLA-B27 and AAU, may explain the lower prevalence of HLA-B27 related AAU in these two populations.
However, despite an increase in the number of publications reporting the association between HLA-B27 related diseases and different B*27 subtypes, there remains no evidence of the predictive or diagnostic value of the various subtypes.

1.5.1.2. HLA-B27 associated anterior uveitis

HLA-B27 positive AAU is recognised as a distinct clinical entity with characteristic clinical features that makes it distinguishable from its HLA-B27 negative counterpart (Huhtinen et al. 2000). The typical phenotype of HLA-B27 positive AAU has extensively been reviewed in the literature (Chang et al. 2005), and it is characterised by an acute onset of a red, painful eye, with significant inflammatory cellular and proteinaceous extravasation into the AqH, including the development of hypopyon in the anterior chamber, and a tendency for recurrences, which may involve the fellow eye (Table 1.9).

Table 1.9. Clinical features of HLA-B27 positive patients compared to HLA-B27 negative AU. From Chang et al. (Chang et al. 2005).

Clinical features	**HLA-B27 positive AU**	**HLA-B27 negative AU**
Age at onset (years)	32-35	39-48
Gender (male preponderance)	1.5-2.5:1	1:1
Eye involvement	Unilateral (48-59%) Unilateral alternating (29-36%)	Bilateral (21-64%)
Pattern of uveitis	Acute (80-87%)	Chronic (43-61%)
Recurrence	Frequent	Uncommon
Fibrin in AC	25-56%	0-10%
Hypopion	12-15%	0-2%
Associated systemic disease	48-84%	1-13%
Familial aggregation	Yes	No

1.5.1.2.1. Anterior uveitis associated with seronegative arthropathies

The spondyloarthropathies (SpA) are group of chronic inflammatory disorders primarily affecting the axial skeleton and joints, and are genetically linked with the strongest known contributing factor being the MHC class I molecule HLA-B27. The National Arthritis Data Workgroup has estimated an overall prevalence of 1.3% with a worldwide distribution (Akkoc 2008). The SpAs include: AS, IBD-associated spondyloarthropathy, psoriatic arthropathy (PsA), and post-infectious or reactive arthritis. Due to the overlap of clinical manifestations within the group and often incomplete or atypical forms at presentation, two separate sets of diagnostic criteria, the European Spondyloarthropathy Study Group and the Amor criteria, which include both clinical and radiological features were developed in the early 90s'and still remain in use today (Amor et al. 1990; Dougados et al. 1991). Their clinical characteristics are summarised in table 1.10 (Table 1.10).

Among the extra-articular manifestations AU is one of the most common manifestations (Collantes et al. 2007). Although the clinical features of AU in some of the subtypes i.e., AS or PsA, is highly consistent with typical symptoms of AAU, in other subtypes such as IBD or reactive arthritis, the clinical characteristics are more variable (Martin et al. 2002).

Table 1.10. Summary of Clinical Features of Spondyloarthropathies. From Kataria et al. (Kataria et al. 2004).

Features	**AS**	**Reactive arthritis**	**PsA**	**IBD-associated spondyloarthropathy**
Prevalence	0.1-0.2%	0.1%	0.2-0.4%	Rare
Age at onset	Late teens to early adulthood	Late teens to early adulthood	35-45 years	Any age
Male to female ratio	3:1	5:1	1:1	1:1
HLA-B27	90-95%	80%	40%	30%
Sacroiliitis *Frequency* *Distribution*	 100% Symmetric	 40-60% Asymmetric	 40% Asymmetric	 20% Symmetric
Syndesmophytes	Delicate, marginal	Bulky, nonmarginal	Bulky, nonmarginal	Delicate, marginal
Peripheral arthritis *Frequency* *Distribution*	 Occasional Asymmetric, lower limbs	 Common Asymmetric, lower limbs	 Common Asymmetric, any joint	 Common Asymmetric, lower limbs
Enthesitis	Common	Very common	Very common	Occasional
Dactylitis	Uncommon	Common	Common	Uncommon
Skin lesions	None	Circinate balanitis, keratoderma blenorrhagicum	Psoriasis	Erythema nodosum, pyoderma gangrenosum
Nail changes	None	Onycholysis	Pitting, onycholysis	Clubbing
Oral	Ulcers	Ulcers	Ulcers	Ulcers
Cardiac	Aortic regurgitation, conduction defects	Aortic regurgitation, conduction defects	Aortic regurgitation, conduction defects	Aortic regurgitation
Pulmonary	Upper lobe fibrosis	None	None	None
Gastrointestinal	None	Diarrhoea	None	Crohn's disease, ulcerative colitis
Renal	Amyloidosis, IgA nephropathy	Amyloidosis	Amyloidosis	Nephrolithiasis
Genitourinary	Prostatitis	Urethritis, cervicitis	None	None

1.5.1.2.2. Ankylosing spondylitis

AS is a chronic systemic disease of unknown cause, characterized by the presence of sclerosis of the joint space of the axial skeleton. The prevalence of AS among Europeans is estimated to be 0.1-0.23% (Olivieri et al. 1998). There is a strong genetic link, with 90 to 95% of patients being HLA-B27 positive, and the risk of developing the disease of about 5% in healthy HLA-B27 positive individuals, and significantly higher in HLA-B27 positive relatives of patients (van der Linden et al. 1984). Although the importance of HLA-B27 is clear, there is now evidence of other genes associated with susceptibility to this disease, such as the TNF-α gene. Genome wide studies suggest the influence of other genes distributed on a number of different chromosomes and for example, the IL-1 gene cluster in chromosome 2 appears to be involved in disease susceptibility or disease severity (Maksymowych et al. 2003).
Furthermore, not only the HLA-B27 haplotype has a direct correlation with the presence of AU (Martinez-Borra et al. 2000), a different locus in chromosome 9p predisposes to the development of AU in patients with AS (Martin et al. 2005).
AS in its classical presentation remains the prototype of the B27 related group of disorders, with an insidious onset of lower back pain that persists longer than 3 months, associated with morning stiffness that improves with activity. Typically the most common ocular manifestation of AS is a non-granulomatous acute-recurrent AU. It is usually unilateral, but can also be alternating or bilateral. Characteristically, the cellular response is severe and it is one of the few non-infectious forms of uveitis that may present with a hypopyon (Ramsay & Lightman 2001; Zaidi et al. 2010). Severe fibrinous response can lead to the formation of PS. Rarely the posterior segment can show signs of inflammation such as, vitritis, CMO, papillitis and retinal vasculitis (Rodriguez et al. 1994). Nonetheless the presence of AAU and its severity does not correlate with that of the spondylitis.

1.5.1.2.3. Reactive arthropathy

The second type of arthritis in this group of SpA is part of a clinical triad consisting of arthritis, non-gonococcal urethritis and conjunctivitis, known as reactive disease. HLA-B27 is present in approximately 75 % to 90 % of patients (Amor 1998).The complete form also described as Reiter's syndrome. The diagnosis is usually clinical and it is summarised in table 11.
Over half of the patients with reactive disease will have ocular involvement with AU being the second most common form of ocular involvement after conjunctivitis. It may be seen in up to 12% of patients, especially in these patients who are HLA-B27 positive (Lee et al. 1986). The initial attack is often unilateral, non-granulomatous, of insidious inset with a mild to moderate cellular reaction with or without PS and typically lasting less than 3 months. The presence of posterior segment manifestations is uncommon; however Kiss et al. reported a

64% of cases with evidence of posterior segment inflammation at some point during their follow-up (Kiss et al. 2003).

1.5.1.2.4. Psoriatic arthritis

Psoriatic arthritis (PsA) has often been defined as a unique inflammatory arthritis associated with psoriasis in up to 30% of patients. It is defined by the triad of psoriasis, chronic-recurrent, erosive arthritis, and rheumatoid factor negative. The skin lesions often precede the onset of arthritis, but in up to 20% of cases the arthritis is the initial complaint. There are five types of recognised PsA: an oligoarticular (four or fewer joints involved), a polyarticular (five or more joints involved), a predominant distal interphalangeal joint involvement, arthritis mutilans, and psoriatic spondylitis. The oligoarticular pattern accounts for more than 70% of cases (Gladman et al. 2005). See table 1.10 for summary (Table 1.10)

Early studies on PsA associations with MHC alleles found a link between HLA-B27 positive patients and spinal involvement, whereas HLA-B38 and B39 were associated with peripheral disease (Gladman et al. 2003). Interestingly these latter two alleles are highly correlated with HLA-Cw*1203. Subsequent genome wide studies have identified the association between the psoriasis susceptibility locus 1 (PSORS 1) located in the MHC class I region and HLA-Cw*0602 and HLA-Cw*1203 (Veal et al. 2002). Furthermore, the presence of the shared epitope HLA-DRB1 from the MHC class II region is reported to be associated with radiological erosions in PsA (Korendowych et al. 2005). Other susceptibility genes such as TNF-α polymorphisms at the promoter positions -238 and -308 have been demonstrated to be in LD with HLA-Cw*0602, and TNF-857 allele T appears to be involved in the development of PsA independently of the PSORS1 allele (Reich et al. 2007).

With the exception of the MHC, linkages and association of other susceptibility genes have not yet been replicated in all cohorts. However, an association with alleles of the IL-23 receptor, which are also risk factor for other SpA have been found, suggesting a shared inflammatory pathway mediated by IL-23R in these group of disease (Nograles et al. 2009). Th17 cells are characterised by the production of IL-17 and IL-22 cytokines which have been found to be expressed in psoriatic skin lesions, however it is still unclear whether they play a role in the pathogenesis of joint disease. Nevertheless treatment with human 12/23 p40 monoclonal antibody has shown to reduce the signs and symptoms of PsA, supporting the role Th17 cytokines in influencing the clinical course of PsA (Nograles et al. 2009).

AU in the presence of PsA is more likely to have a more insidious onset, longer episodes, posterior segment involvement, and bilateral when compared with uveitis in patients with other spondyloarthropathies. It is also more likely to manifest in HLA-B27 positive patients with axial disease (Durrani et al. 2005; Paiva et al. 2000).

1.5.1.2.5. Inflammatory bowel disease

IBD comprises two different entities, Crohn's disease and ulcerative colitis. Arthritis manifests in two different ways, an axial and a peripheral. Whereas the latter has a predilection for the large joints of the lower extremities, and its activity parallels that of the intestinal symptoms often disappearing following proctocolectomy (Salvarani et al. 2001), the less common axial presentation mimics that seen in AS and its clinical course is unrelated to the gastrointestinal involvement.

Although the incidence of HLA-B27 and HLA-DRB1*0103 in patients with IBD is higher than in the normal population and is associated with the development of extra-intestinal disease manifestations, HLA associations in this subgroup have given inconclusive results. NOD 2 (nucleotide-binding oligomerisation domain protein 2)/CARD15 SNPs located in chromosome 16 have been associated with different clinical manifestations of Crohn's disease (Economou et al. 2004). In addition, there is evidence of TLR4 being involved in the pathogenensis of IBD, with the 299Gly allele frequency of the TLR4 gene and the T allele and TT genotype frequencies of the CD14 promoter were significantly higher in CD patients only compared to healthy individuals (Gazouli et al. 2005) and the Asp299Gly+/NOD2-polymorphism being a strong predictor of the stricturing disease phenotype (Brand et al. 2005).

Ocular involvement occurs in less than 10 % of cases of IBD (Mintz et al. 2004). A recent prospective randomized study conducted to evaluate the prevalence of ocular involvement in Turkish patients with IBD showed a higher prevalence of ocular inflammation in patients with Crohn's disease compared to these with UC, with conjunctivitis being the most common ocular complication seen in 9 % of patients, followed by uveitis in about 5 % of cases (Yilmaz et al. 2007). Compared to other HLA-B27 related spondyloarthropathies, the intraocular inflammation associated with IBD tends to be more insidious in onset and chronic in duration, and typically bilateral (Lyons et al. 1997). Nevertheless, the most common type of IBD-associated uveitis is a non-granulomatous, low-grade recurrent-AAU, seen in 60% of patients, with a third of patients manifesting with panuveitis (Salmon et al. 1991).

1.5.2. Fuchs' Heterochromic Cyclitis

FHC is a form of chronic low-grade AU of unknown aetiology. The prevalence of FHC varies from 1 % to 5 % of all cases of AU, although the prevalence might be higher due to the fact that the symptoms are subtle in the majority of cases. It is characterised by the presence of fine, white, non-pigmented, stellate KPs spread widely over the entire corneal endothelium, which are virtually pathognomonic. Atrophy of the iris may eventually result in the appearance of heterochromia and in some cases an irregular pupil or even a dilated pupil in

the affected eye. Typically, heterochromia is caused by the atrophy of the anterior layer of the iris stroma, which although it might make a brown iris look lighter, a blue iris will appear darker due to the revealing underlying iris pigmented posterior epithelium, resulting in a paradoxical “reverse heterochromia”. PS never develop in patients with FHC, unless the eye has had intraocular surgery (Rothova et al. 1994). Iris and trabecular meshwork show abnormal vessels (Bonfioli et al. 2005) that may sometimes lead to a hyphema or spontaneous haemorrhage (Amsler’s sign) in the anterior chamber following paracentesis in eyes with FHC. Cataract formation, secondary glaucoma and vitreous cells and opacities are the most common complications of this disorder. The presence of cataract formation in FHC is often present at the time of the diagnosis and often manifests as posterior subcapsular lens opacity (Jones 1991a), with a variable speed in its progression. Therefore, FHC should be considered in any young patient with unilateral cataract with no history of trauma or use of steroid. Cataract surgery in eyes with FHC has evolved considerably and although the incidence of complications reported in the literature varies considerably (Jakeman et al. 1990; Jones 1996; Razzak et al. 1990; Sherwood et al. 1992; Tejwani et al. 2006), cataract extraction and intraocular lens implant in eyes with FHC is a safe procedure with good visual outcome. Glaucoma is said to be the most damaging complication of FHC and occurs in about 25 % of patients (Jones 1991a). Although the mechanism of raised intraocular pressure in FHC remains poorly understood, the glaucoma is typical of chronic open-angle, but it can also be secondary to the presence of peripheral anterior synechiae, rubeosis, lens-induced angle closure and recurrent spontaneous hyphaema (Jones 1991b). The management of glaucoma in these patients is one of the most difficult challenges, and although the majority of patients show a good response to topical medication alone, a significant proportion of eyes will require filtration surgery in order to control the progression of visual field loss (Al-Mansour et al.)

Vitreous opacities are present in 50%of patients (Al-Mansour et al.) but in contrast to intermediate uveitis there is no macular oedema. Even if their presence is not found to be generally symptomatic, in some patients these can be quite debilitating even if their Snellen visual acuities appear to be relatively good. Two retrospective studies of patients with FHC who underwent pars plana vitrectomies for removal of vitreous floaters showed not only an excellent visual outcome, but also appeared to be safe with no exacerbation of any pre-existing intraocular inflammation (Scott et al. 2001).

The aetiology is unknown and many pathogenic mechanisms have been proposed. Although the presence of peripheral chorioretinal scars similar to the ones seen in Toxoplasma retinochoroiditis has raised the hypothesis of an infective cause (Toledo de Abreu et al. 1982), there has been no proof of such relationship after analyzing humoral and cell-mediated

immunity against toxoplasma gondii in blood and AqH in patients with uveitis and a control group (La Hey et al. 1992). Other theories include herpes simplex and rubella infection as possible agents in the pathogenesis of the disease, and a recent study correlating the percentage of new cases of idiopathic intermediate uveitis including FHC before and after the rubella vaccination program in the United States, showed a decrease in the number of new cases following the introduction of the US rubella vaccination scheme, supporting the link between rubella infection and FHC (Birnbaum et al. 2007). The cellular phenotype and cytokine profile in the AqH of patients with FHC and idiopathic AAU has been compared (Muhaya et al. 1998). The higher $CD8^+$ T cell and IL-10 levels seen in FHC compared to the idiopathic AAU, where there was a predominance of $CD4^+$ T cells and IL-2 levels might explain the low-grade inflammation seen in eyes of patients with FHC compared to that seen in idiopathic AAU.

The course of the inflammatory process is unchanged by the use of corticosteroids and the management of this condition should be aimed to the treatment of its complications i.e., cataract, glaucoma, and vitreous opacities.

1.6. BEHÇET'S DISEASE

Behçet's disease is a chronic, relapsing multisystem, inflammatory disorder of unknown aetiology with systemic and ocular manifestations.

It has a worldwide distribution but it is more frequently seen in the countries of the Eastern Mediterranean and Asia that correspond to the old Silk Route (Verity et al. 1999a), and interestingly patients who are HLA-B5 distribute among the same areas. The incidence varies from approximately one case per 1,000 population in Turkey which has the highest incidence, to one in 500,000 in the UK (Suzuki Kurokawa et al. 2004). Although many reports have shown a higher prevalence in young male patients, the number of cases of female patients has increased, reaching a male female ratio of almost one to one (Zouboulis et al. 1997).

The disease is rather heterogeneous with variable involvement of many organ systems. The diagnosis is based on the presence of different clinical findings, and it is characterised by the triad of recurrent oral and genital aphthous ulcers, ocular inflammation, and skin lesions such as erythema nodosum or acneiform eruptions. The most widely used diagnostic criteria in the UK is the one published by the International Study Group for Behçet's Disease (Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease 1990), originally developed as a tool for aid classifying patients with the disease rather than that a diagnostic criteria (Table 1.11). In order to improve the accuracy of the diagnosis, the Behçet's Disease Research Committee of Japan has revised the criteria using clinical and laboratory data and includes a list of major diseases for the differential diagnosis. The main characteristics of these revised criteria include recurrent aphthous ulcers on the oral mucosa,

genital ulcers, skin lesions and ocular lesions. Additional symptoms include arthritis, epididymitis, gastrointestinal, vascular and central nervous system lesions. The revised criteria includes clinical and laboratory data such as, pathergy test and prick test for dead Streptococcus, HLA-B51 and pathological findings of erythema nodosum divides the disease into four types: complete, incomplete, suspected or special lesions (Suzuki Kurokawa & Suzuki 2004)

Table 1.11. International criteria for diagnosis of Behçet’s disease (Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease 1990).

Recurrent oral ulceration	Minor aphthous, major apthous, or herpetiform ulceration observed by physician or patient, which recurred at least three times in one 12 month period
Plus two of:	
Recurrent genital ulceration	Aphthous ulceration or scarring, observed by physician or patient
Eye lesions	Anterior uveitis, posterior uveitis, or cells in vitreous on slit lamp examination; or retinal vasculitis observed by ophthalmologists
Skin lesions	Erythema nodosum observed by physician or patien, pseudofolliculitis, or papulopustular lesions; or acneiform nodules observed by physician in postadolescent
Positive pathergy test	Read by physician at 24-48 hours (findings applicable only in absence of other clinical explanations)

So far, the pathogenesis of Behçet's disease remains unclear. It has been accepted that an exaggerated response to a microbial, viral or auto-antigen stimuli with increased cytokines and chemokines production and function is responsible of the clinical manifestations of the disease, it is not clear what is responsible for he initial onset and persistence of the disease.

Behçet's disease has a wide spectrum of clinical manifestations, but oral ulceration remains the most common and defining feature being present in over 95 % of cases (Yazici et al. 1999). Genital ulcers are less common than oral ulcers and are present in up to 80 % of cases. Skin lesion area present in about 80 % of patients and can be divided into erythema nodosum and papulo-pustular, acneiform lesions. Another form of skin lesion is the pathergy reaction, which represents a hyper-reactivity of the skin to minor trauma. Various other organs can be involved among different populations. Joint involvement is seen in about half of patients, typically as a non-erosive, non-deforming arthritis with an oligoarticular pattern. It has a transient nature involving peripheral joints, such as knees, ankles, and wrists (Kim et al. 1997). A widespread vasculitis of vessels of all sizes, both arteries and veins may involve up to one quarter of patients. Neurological involvement is not uncommon, occurring in approximately 5 % of cases (Kontogiannis et al. 2000). It can be related to primary central nervous system involvement, most frequently of the brain stem but the hemispheres, meninges and spinal cord can be affected. It can also be secondary to the vasculitis causing arterial occlusions or dural sinus thrombosis. Although, cardiac involvement was thought to be unusual, Morelli et al. reported mitral valve prolapse and dilatation of the proximal aorta in 50 % and 30 % of patients respectively (Morelli et al. 1997).
Ocular involvement occurs in approximately 75% of patients with Behçet's disease, being the presenting feature in up to 20% of patients (Kontogiannis & Powell 2000), and is the major cause of morbidity of the disease. Typically has a relapsing, remitting course and first manifests with an attack of severe AAU with white non-pigmented KPs' and hypopyon formation. The classical finding of AAU with hypopyon formation is present in 19% to 31% of patients with the disease (Deuter et al. 2008). PS with or without iris bombé, iris atrophy and secondary glaucoma may develop as a result of repeated attacks. The natural history of Behçet's disease is characterised by explosive recurrent attacks of intraocular inflammation that may be confined to the anterior segment or may also affect the posterior segment. The retinal disease is characterised by an occlusive vasculitis of arteries and veins with associated vitritis, retinal haemorrhages with or without neovascularisation. Macular oedema is a major cause of visual impairment in uveitis associated to Behçet's disease even in these cases with isolated AU. The end-stage of the disease is characterised by retinal and optic nerve atrophy with attenuation of the retinal vessels.

1.7. SARCOIDOSIS

Sarcoidosis is a multisystem granulomatous disease of unknown cause characterised pathobiologically by non-caseating granuloma formation in different organs. Sarcoidosis occurs worldwide; affecting all races but with a predilection for certain ethnic groups, such as African-Americans (Rybicki et al. 1997). It affects both genders but with a higher incidence of the disease in women and has a tendency to affect young adults under the age of 40. The clinical features and the natural history are variable, making the prevalence and incidence of the disease difficult to determine.

The clinical manifestations of sarcoidosis can be widespread or may involve only one organ system. The majority of patients present with non-specific constitutional symptoms such as fever, weight loss, fatigue, and malaise. The most common involved organs are the lung, lymph nodes, skin and eye. Pulmonary involvement is almost universal at some point in the course of the disease. Most typically, sarcoidosis is an interstitial disease, bilateral involving alveoli, blood vessels and bronchioles leading to characteristic radiological features. Intra-thoracic and peripheral lymphadenopathy is common, with hiliar-node enlargement seen in up to 90 % of patients. Frequently the disease is asymptomatic and discovered by chest radiography. The radiographic features are usually used to classify the different stages of pulmonary sarcoidosis: stage 1 represents bilateral hiliar lymphadenopathy without parenchymal involvement, stage 2 bilateral adenopathy with parenchymal involvement, stage 3 is pulmonary infiltrates without associated lymphadenopathy including cystic changes (Miller et al. 1995). Approximately 25 % of patients will manifest skin lesions during the course of the disease. These range from erythema nodosum to a wide variety of macular, papules, or subcutaneous nodules. The clinical presentation of erythema nodosum is that of an erythematous, warm, raised, and tender swelling typically over the anterior aspects of the lower legs. Multiple lesions can be present at different stages. The most easily recognizable form of cutaneous sarcoidosis is lupus pernio, which consists of purple papules involving the nose, cheeks, lip, ears, eyelids, and sometimes the tips of fingers. It is often associated with the presence of lung disease in up to 74 % of patients, and has a strong association with the presence of ocular involvement (James 1992). Less frequently seen cutaneous manifestation of sarcoidosis may also include nail dystrophy, alopecia, areas of hypo or hyperpigmentation, and leukocytoclastic vasculitis. Non-specific arthralgia occurs in up to one quarter of patients. It manifests as an acute migratory polyarthropathy affecting most commonly the ankles and knees. It tends to resolve completely, although it may recur. Chronic arthritis has a similar distribution to the acute form but it may also affect the metacarpophalangeal and interphalangeal joints (Thelier et al. 2008).

Ocular disease may be the initial manifestation and may progress to severe visual impairment (Lobo et al. 2003). There are two peaks of incidence of ocular sarcoidosis, the first at the ages of 20 to 30 and the second at ages 40 to 50 years (Rothova 2000). Depending on the population studied the incidence of ocular involvement ranges from 25 % to 60 % (Rothova 2000). AU is the most common ocular manifestation, affecting around two thirds of patients with systemic sarcoidosis (Jabs et al. 1986). It characteristically presents as a bilateral granulomatous uveitis, but it can also manifests as CAU together with skin lesions, such as erythema nodosum and bilateral hiliar lymphadenopathy.

The 1st International Workshop on Ocular Sarcoidosis (Herbort et al. 2009) has laid down a diagnostic criteria based on a combination of ophthalmic signs and laboratory investigations (Tables 1.12-1.14).

Table 1.12. Diagnostic criteria for ocular sarcoidosis.

Definite	Biopsy-supported diagnosis with a compatible uveitis
Presumed	Biopsy not done; presence of BHL with compatible uveitis
Probable	Biopsy not done; BHL negative; presence of three suggested intraocular signs and two positive investigational tests
Possible	Biopsy negative; four suggested intraocular signs and two positive investigational tests
All other possible causes of uveitis, in particular tuberculosis have to be previously excluded	

BHL: Bilateral Hiliar Lymphadenopathy

Table 1.13. Clinical signs suggestive of ocular sarcoidosis.

Mutton fat KPs and/or iris nodules at pupillary margin or stroma
Tabecular meshwork nodules and/or tent-shaped peripheral anterior synaechia
Snowballs/string of pearls vitreous opacities
Multifocal peripheral chorioretinal lesions (active and atrophic)
Nodular and/or segmental periphlebitis(with or without candle-wax exudates) and/or macroaneurysm
Optic disc nodules/granuloma and/or solitary choroidal nodule
Bilateral inflammation (evident on clinical examination or on investigational imaging)

Table 1.14. Laboratory investigations in suspected ocular sarcoidosis.

Negative tuberculin test in a patient who either had BCG vaccination or previously had a positive tuberculin test
Elevated sACE and/or elevated serum lysozyme*
Chest X-ray showing BHL
Abnormal liver enzyme tests (any two of: alkaline phosphatase, aspartate transaminase, alanine transaminase)
Chest CT-scan in patients with normal chest X-ray

* Lysozyme required in patients taking ACE inhibitors

BHL: Bilateral Hiliar Lymphadenopathy

The KPs tend to have the mutton-fat appearance, and it is characterised by the presence of iris nodules. A Japanese study revealed the presence of nodules or granulomas on the trabecular meshwork in nearly half of the patients (Mizuno et al. 1986). Patients with CAU may develop macular oedema more frequently than patients with acute or relapsing disease; corneal band keratopathy may also develop. Occasionally intermediate uveitis with vitritis with or without peripheral vasculitis and snowball infiltrates is seen. This type of uveitis may precede more severe posterior segment changes (Rothova 2000). Posterior segment involvement include the presence of periphlebitis with typical segmental cuffing or more extensive perivenous exudates, in addition to these features the presence of multiple small round chorioretinal lesions mostly in the peripheral retina not associated with retinal vessels are seen. Optic nerve leakage on fluorescein angiography might be seen and it should be differentiated from true optic nerve involvement, since it appears to be a higher involvement of the central nervous system in sarcoidosis in the presence of posterior uveitis (Menezo et al. 2009).

Systemic investigations that help in the diagnosis of sarcoidosis are chest radiograph. Findings on chest x-ray suggestive of sarcoidosis have been classified in a staged system from 0 to III (Miller et al. 1995). Laboratory values are affected to some extent by the epidemiology of uveitis of the geographical area were they are performed. Among them, levels of serum angiotensin-convertase enzyme (sACE) produced by local macrophages appears to correlate with disease activity (Weinreb et al. 1984), however the test is not useful in patients who are taking ACE inhibitors. In these cases determining lysozyme levels in serum would be recommended. In fact a Japanese study has shown lysozyme levels to have a better predictive value than sACE (Kawaguchi et al. 2007).
Chest CT-scans have been shown to be helpful in those cases where chest radiography fails to provide diagnostic support and can assist targeting of the biopsy site (Kaiser et al. 2002). Newer functional imaging techniques such as PET-scan allow not only detecting localised foci of inflammation; assess activity and determining treatment efficacy (Shulman et al. 2009).
The gold standard for confirmation of the diagnosis of sarcoidosis is the histological demonstration of non-caseating granuloma formation in the involved tissue, together the presence of other clinical manifestations. The presence of non-caseating granuloma on tissue biopsy and clinical features compatible with sarcoidosis are considered a proof of a definite diagnosis. Histological confirmation can be challenging, and therefore more accessible sites such as skin lesions or the conjunctival mucosa have been proposed (Bastiaensen et al. 1985; Leavitt et al. 1998; Spaide et al. 1990). Fibreoptic bronchoscopy with transbronchial biopsy has reported to be positive in up to 90 % of patients with positive findings on chest x-ray

(Koerner et al. 1975). In these cases with negative findings in transbronchial biopsy, BALmay be of value (Society 1990; Vallee et al. 1990).

1.8. RESEARCH HYPOTHESIS

1.8.1. AIM OF STUDY

The study aims to evaluate the role of specific gene polymorphisms in determining the outcome of patients with AU. The specific hypothesis to be tested is:

1. Polymorphisms of certain key cytokine(s) gene(s) are associated with the development of complications leading to a poorer visual outcome including macular oedema, glaucoma and cataract in patients with AU, and
2. That posterior synaechia formation is associated with an increased complication rate.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. PATIENT CLASSIFICATION

Patients with uveitis presenting to Professor Lightman's uveitis clinics at Moorfields Eye Hospital clinic were characterised on clinical grounds, and were divided into those with acute-recurrent AU, CAU, and those with FHC.

The groups of patients identified were idiopathic acute-recurrent AU (n=98), acute-recurrent AU associated with systemic disease (n=30), idiopathic CAU (n=21), CAU associated with systemic disease (n=7), FHC (n=11). AU with associated disease was defined as AU related to systemic conditions such as AS (n= 19), Reiter's syndrome (n=4), reactive arthritis (n=3), psoriatic arthropathy (n=3), IBD (n= 1), sarcoidosis (n=4), Behçet's disease (n=1), systemic vasculitis (n=1), multiple sclerosis (n=1).

All patients had a complete ocular examination including best corrected Snellen visual acuity, biomicroscopy, Goldmann applanation tonometry and dilated fundus examination. Fluorescein angiography, visual fields and ultrasonography were performed when indicated. Initial laboratory investigations included full blood cell count, renal and liver function, treponema serology, serum angiotensin-convertase enzyme and HLA-B27 typing. Chest x-rays were requested for these patients suspected of having associated sarcoidosis. Patients with clinical and laboratory evidence of associated systemic disease were referred to the appropriate medical team for further evaluation.

Patients were divided further into either a uveitis-related complication group or a no complication group. These complications would have been detected on the day of clinical phenotyping or venepuncture. The ocular complications analysed were the presence of posterior iris synaechia, posterior subcapsular lens opacity, ocular hypertension and/or glaucoma, steroid response, and clinical or angiographic macular oedema. Posterior iris synaechia was defined as the presence of iris adhesions to the anterior lens capsule post-dilatation. Only the presence of posterior subcapsular lens opacities was considered as cataract secondary to uveitis. Ocular hypertension was defined as intraocular pressure greater than 21mmHg without associated optic disc or visual field damage prior to treatment with corticosteroids. Glaucoma secondary to uveitis consisted of raised intraocular pressure over 21 mmHg with associated optic disc damage and/or correspondent visual field defect. Clinically significant macular oedema was considered when thickening of the central macula and or loss of the normal foveal depression was present on biomicroscopy confirmed or not by fluorescein angiography. Patients with uveitis of infectious aetiology (i.e., herpetic simplex or zoster uveitis), postoperative uveitis, or those with a previous history of glaucoma, macular and or other retinal disease were excluded.

This study was approved by the Ethics Committee of Moorfields Eye Hospital and was conducted according to the Tenents of the Declaration of Helsinky.

2.2. CONTROL POPULATION

A total number of 586 subjects from an ethnically matched population were recruited. All the controls were of Caucasian origin from the Southeast of England to avoid any ethnic genetic differences (Grutters et al. 2002; Pantelidis et al. 2001). Peripheral blood was collected from patients undergoing routine cataract surgery for age related cataract and from blood and organ donors. They were screened to have no past or present ocular or medical history.

Data from the control population described in chapter 5 was collected from 66 patients who underwent routine cataract surgery and who had no previous history of ocular disease or related systemic co-morbidity.

Control data described in chapters 4 and 6 included the 66 patients described in chapter 5 and in addition to these the remaining group of control subjects came from blood and organ donors from South England, as well as cadaver donors from Oxford, England.

2.3. PERIPHERAL BLOOD SAMPLES

Fifteen ml of peripheral blood were obtained by venepuncture from patients undergoing cataract surgery who had no previous history of ocular disease or autoimmune related systemic disorders. Willing patients were asked to sign a control sample specific consent form [Appendix 1] and an ethics approved information leaflet was given to each patient [Appendix 2]. A record of the patient's ethnic group was kept. All blood samples were anticoagulated with Na_3citrate or Na_2EDTA in four 4.5ml BD Vacutainer TM or polypropylene tubes and kept on ice before being frozen and stored at minus 20°C for later manipulation. Samples were not stored in heparin coated tubes as an anticoagulant since it inhibits both reverse transcriptase and Taq polymerase reactions (Beutler et al. 1990; Wang et al. 1992; Willems et al. 1993).

2.4. GENETIC ANALYSIS

2.4.1. DNA extraction methods

Genomic DNA from all frozen bloods were extracted either using a salting out DNA extraction method or by using the commercial DNA extraction kit, QIAamp®DNA Blood Maxi Kit (QIAGEN Ltd., UK). No significant difference in the quality of the DNA was seen between the two methods used.

2.4.1.1. Salting-Out DNA Extraction Method

A modified salting-out method originally described by Miller et al. (Miller et al. 1988) was used for DNA extraction. Assuming 20 ml of blood (scaleable as required), anticoagulated blood was thawed in room temperature and decanted into a 50 ml centrifuge tube. The tube was then top up to 50 ml with fresh TE solution (5 ml 1M Tris, pH 7.35; 1 ml 0.5M EDTA, pH 8.0). The mixture was then centrifuged at 2500 rpm for 20 minutes at room temperature. Thirty ml of the supernatant was then decanted into Virkon or Hycolin and left for 2 hours before discarding. The residual pellet after centrifuge was resuspended to 40 ml of swelling buffer and left on ice for 20 minutes. The mixture was then centrifuged at 2500 rpm for 15 min at 4 °C (step 1). The supernatant was poured off and discarded after. The residual pellet is re-suspended in 10 ml of red cell lysis buffer and left at room temperature for 15 minutes (step 2). The mixture was further centrifuged at 2500 rpm for 15 minutes at room temperature. After which, the supernatant was discarded. The pellet should be white with a pink halo. If the pellet was still dark in colour at this stage, steps 1 and 2 were repeated until it was. When the pellet was homogenously white, it was either stored at minus 70 °C to be processed another day or processed immediately. The pellet was resuspended in 3 ml nuclei lysis buffer, 200 c of 10% w/v sodium dodecyl sulphate (SDS) and 600 ml Proteinase K solution (10 mg/ml) in a sterile 15 ml tube and left overnight at 37°C. The mixture was later added with 1 ml of 6M NaCl and shaken vigorously for 30 seconds. It was then centrifuged at 3000 rpm for 30 minutes. The supernatant was aspirated into a sterile 15 ml tube, added with 10 ml cold absolute ethanol, and mixed gently until the DNA was precipitated. The precipitated DNA was removed and placed into a sterile tube and washed with 75% ethanol. The mixture was then left to dry with the lid open. Finally, the DNA was re-suspended in an appropriate volume of sterile water, approximately 400 µl, and stored at minus 20 °C.

2.4.1.2. QIAmp®DNA Blood Maxi Kit

Five hundred µl of QIAGEN protease were pipetted into the bottom of a 50 ml centrifuge tube and 8 ml of blood were added and vortexed briefly. 9.6 ml of Buffer AL were added (6ml Buffer when using 5 ml of peripheral blood) and mixed by vortexing three times, for at least 5 seconds each time. This mixture was then incubated at 70 °C for 10 minutes, as DNA yield reaches a maximum after lysis for 10 minutes at 70 °C. Eight ml of ethanol (96-100 %) were added to the mixture and vortexed. When the initial sample was 5ml of peripheral blood, the volume of ethanol was reduced to 5 ml. One half of the solution was transferred into the QIAamp Maxi column placed in a 50 ml centrifuge tube and centrifuged at 3000 rpm (1850 g) for 3 minutes. The QIAamp Maxi column was removed, the filtrate discarded into Virkon and the column was placed back again in the tube. The remainder of the solution was loaded and centrifuged again at 3000 rpm for another 3 minutes. Five ml of Buffer AW1 were added to

the QIAamp Maxi column and centrifuged at 5000 rpm (4500 g) for 1 minute. Without discarding the flow-through from the centrifuge tube, 5 ml of Buffer AW2 were added to the QIAamp and centrifuged at 5000 rpm (4500 g) for 15 minutes. The QIAamp column was then placed in a clean 50 ml centrifuged tube. 960 µl of Buffer AE equilibrated at room temperature was pipetted directly onto the membrane of the QIAamp Maxi column and incubated at room temperature for 5 minutes and centrifuged at 5000 rpm for 5 minutes. In order to maximise the DNA concentration the eluate containing the DNA was reloaded onto the membrane of the QIAamp Maxi column, incubated at room temperature for 5 minutes and centrifuged at 5000 rpm for another 5 minutes. This eluate containing the DNA was then stored in Eppendorf tubes at minus 20 °C.

2.4.2. Determination of the concentration of the DNA extracted

The concentration of the DNA extracted was determined by means of spectrophotometry.

For quantifying the amount of DNA readings should be taken at wavelengths of 260 nm and 280 nm. The reading at 260 nm allows calculation of the concentration of the nucleic acid in the sample, and the ratio between the readings at 260 nm and 280 nm provides an estimate of the purity of the nucleic acid.

It was anticipated that spectrophotometric reading of Absorbance260/280 would be in the range 1.7–2.0. In order to determine the Absorbance260/280 ratio, 69µl of sterile water for injection and 1µl of the extracted sample DNA were transferred using a Gilson pipette (P100) into a 0.5ml Eppendorf TM tube. The concentration of DNA was calculated multiplying the Absorbance260/280 ratio by 50 and by 70 as dilution factor. The concentration of DNA was required so that the DNA obtained could be diluted to a concentration of 50 ng/µl, as required by the genotyping protocol at the Division of Genomic Medicine, University of Sheffield. There was no need for dilution whenever a sample was found to have a concentration of less than 50ng/µl.

2.4.3. Genotyping of single nucleotide polymorphisms. PCR analysis

Polymerase chain reaction (PCR) is a technique designed to allow for selective amplification of a target DNA sequence within a heterogeneous collection of DNA sequences by in vitro enzymatic replication. Since its original description in 1985, PCR has evolved into a group of different technologies almost universally used in diagnostic or clinical research.
PCR involves the enzymatic synthesis of millions of copies of a specific DNA segment. The exponential amplification of the template DNA is carried out by heat-stable DNA

polymerases with a 3'→5' exonuclease activity, which will result in the synthesis of defined portions of the original DNA sequence.
It is based on three steps: denaturation of the template into single strands by heating the template of DNA to high temperature, annealing of primers to each original strand for new strand synthesis, and extension of the new DNA strands from the primers.
Double-stranded DNA containing the sequence to be copied and amplified is mixed with a large molar excess of two single-stranded DNA oligonucleotides or primers. The first primer is identical to the 5' end of the sense strand of the DNA to be copied, and the second primer is identical to the anti-sense strand at the 3' end of the sequence.
The PCR reaction is initiated by melting the double-stranded DNA at high temperature (typically 90 to 95°C) and then cooling the mixture to allow DNA annealing, so the first primer will hybridize to the 3' end of the anti-sense strand and the second primer will hybridize to the 3' of the sense strand. This annealed mixture is then incubated with DNA polymerase and all four deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP) to allow new DNA to be synthesized. The original sense strand is used as a template to make the new anti-sense strand and then allowed to re-anneal with the primers.

A second cycle commences when the reaction mixture is re-melted and the allowed to re-anneal with the primers. In this synthetic step of the second cycle, each strand synthesized in the first cycle serves as an additional template after having hybridized with the appropriate primers. This second cycle is completed when DNA polymerase extends the primers to synthesize the complement of the templates. These reactions are performed at the optimum temperature for the heat-stable polymerase (68 to 72°C) automated by temperature controlled cyclers that regulate denaturation of the template into single strands, annealing and DNA synthesis. The most commonly used polymerase is the enzyme isolated from *Thermus aquaticus* (*Taq* DNA polymerase).
The three-stage cycle of denaturation, annealing and primer extension is repeated 25 to 40 times in a typical PCR procedure. The product of such reaction is a large quantity of double-stranded DNA whose length is determined by the distance between the primer sites on the original template molecule.

2.4.3.1. Components

2.4.3.1.1. DNA polymerase

The most commonly used DNA polymerase for a wide range of DNA polymerases is a cloned heat-stable DNA polymerase from *Thermus aquaticus*.

2.4.3.1.2. Oligonucleotide primers

PCR primers are normally between 18 and 30 nucleotides in length and should have a guanine and cytosine context of about 50%. The melting temperature at which half the oligonucleotide is annealed to its exact complement (T_m) should be similar for the two primers in a reaction and about 5°C above the annealing temperature. Despite software being currently available to calculate the ideal T_m, empiric testing of annealing temperatures is often necessary for a well-optimized PCR assay.

2.4.3.1.3. Thermal cycling equipment

PCR process involves thermal cycling between the different cycles of denaturation, annealing and extension. During denaturation temperatures of 94°C are often required, although after several cycles, it can be lowered to 90 to 92°C to reduce loss of polymerase activity during the course of PCR. The choice of annealing temperature is in the range of 50 to 60°C, but will ultimately depend on primer T_m. Finally, samples will be heated at 68 to 72°C during extension.

Thermal cyclers that can monitor progress of the amplification reaction while it is taking place are called real-time thermal cyclers. They monitor the emission of fluorescence from labels whose emission intensity is proportional to the amount of amplified DNA. When *Taq* DNA polymerase cleaves a probe labelled with a fluorophore it will dissociate the probe from the quencher, and hence amplification will be detected by the correspondent increase in fluorescence.

2.4.3.1.4. Post-PCR analysis

Traditionally, once PCR reaction is complete the amplified DNA fragments are analysed and visualised by gel electrophoresis. However, Real-Time PCR allows the analysis of the products while the reaction is actually in progress and facilitates the quantitation of the DNA. This is achieved by using various fluorescent dyes which react with the amplified product and can be measured simplifying the process of quantification. Identification of the amplification products by probe detection in real-time is highly accurate compared with size analysis on gels, and since it is not necessary to perform electrophoresis or other manipulations after the DNA amplification reaction Real-Time PCR assays can be completed very rapidly (Heid et al. 1996).

Another variant of PCR, allele-specific PCR is designed to amplify a DNA sequence while excluding the possibility of amplifying other alleles. It is based on the requirement for precise base matching between the 3’ end of a PCR primer and the target DNA.

2.4.3.1.5. TaqMan

The TaqMan system is a method of following real time-PCR, used for the analysis of polymorphisms. We used the 5' nuclease (TaqMan®) allelic discrimination assay for genotyping cytokine polymorphisms in which the Taq polymerase cleaves the so-called TaqMan probe during the extension phase of the PCR (Hui et al. 2008).

With this method, the region flanking the polymorphism is amplified in the presence of two probes since we are dealing with a bi-allelic system. There is one specific probe to an allelic variant, and they were labelled with a different reporter dye, 6-carboxy-fluorescein (FAM) and tetrachloro-6-carboxy-fluoresecein (TET). These probes consists of an oligonucleotide that is complementary to the sequence of interest located over the SNP, labelled with the reporter dye at the 5' end and with the quencher dye 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. As long as the probe is intact the proximity between the reporter and quencher dyes reduces the emission of fluorescence. During the degradation of the probe by the 5'→ 3'exonuclease activity, the reporter and quencher dyes are separated resulting in an increase of the reporter fluorescence emission. In this way, fluorescence is a measure of each probe binding to a specific allele.

As well as the samples being analysed, a series of controls were included. No template control containing no DNA. Allele 1 control where a DNA sample homozygous for allele 1, and allele 2 control in which the DNA sample is homozygous for allele 2 are amplified. The fluorescence levels in the allele 1 and 2 control reflect the cleavage of the allele specific fluorophore.

This process is measured by the ABI PRISM™ 7200 Sequence Detector (Applied Biosystems, Warrington ,UK).

2.4.3.1.5.1. Primer design and optimization

Probes and primers for detection of the SNP were designed using the Primer Express® software (Applied Biosystems) (Table 2.1). We followed the guidelines for oligonucleotide design in Primer Express® using the same principles as in the design of standard PCR primers. The optimal probe concentrations are determined by comparing a range of TET concentrations against a constant FAM concentration prior to PCR, and identifying those who produce approximately equal levels of fluorescence signal.

Table 2.1. TaqMan probes and primers sequences.

	Sequence (All 5' - 3')	N bases
TNF-α (-308)		
Allele 1 (TET Label)	ACC CCG TCC CCA TGC CC	17
Allele 2 (FAM Label)	AAC CCC GTC CTC ATG CCC C	19
Forward Primer	GGC CAC TGA CTG ATT TGT GTG T	22
Reverse Primer	CAA AAG AAA TGG AGG CAA TAG GTT	24
TNF-α (-238)		
Allele 1 (TET Label)	CCT CCC TGC TCC GAT TCC G	19
Allele 2 (FAM Label)	TCC TCC CTG CTC TGA TTC CGA	21
Forward Primer	GCA TCA AGG ATA CCC CTC ACA	21
Reverse Primer	ATC AGT CAG TGG CCC AGA AGA	21
IL1ra(+2018)		
Allele 1 (FAM Label)	AAC AAC CAA CTA GTT GCT GGA TAC TTG CAA	30
Allele 2 (TET Label)	ACA ACC AAC TAG TTG CCG GAT ACT TGC	27
Forward Primer	GGG ATG TTA ACC AGA AGA CCT TCT ATC T	28
Reverse Primer	CAA CCA CTC ACC TTC TAA ATT GAC ATT	27
IL-10(-1082)		
Allele 1 (FAM Label)	CTA CTT CCC CCT CCC AAA GAA GCC T	25
Allele 2 (TET Label)	CCT ACT TCC CCT TCC CAA AGA AGC C	25
Forward Primer	GAT AGG AGG TCC CTT ACT TTC CTC TTA	27
Reverse Primer	CAC ACA CAA ATC CAA GAC AAC ACT AC	26
IL-6(-174)		
Allele 1 (FAM Label)	ACG TCC TTT AGC ATC GCA AGA CAC AAC	27
Allele 2 (TET Label)	ACG TCC TTT AGC ATG GCA AGA CAC AAC	27
Forward Primer	GCT GAT TGG AAA CCT TAT TAA GAT TGT	27
Reverse Primer	AAT GAC GAC CTA AGC TGC ACT TT	23

2.4.3.1.5.2. PCR conditions

All reactions were performed using a 96-well PCR plate. Each reaction well represented one of the specific allelic polymorphism for the DNA sample of each patient and a template control.

Each well contained 7.5 µl of TaqMan Mastermix. The volume for each primer and probes for the different cytokine polymorphisms were optimized as follow:

TaqMan 7200 Protocol for IL-1ra (+2018):

0.375 µl of forward primer, 0.375 µl of reverse primer, 0.15 µl of FAM probe, 0.525 µl of TET probe and 4.875 µl of distilled H_2O.

TaqMan 7200 Protocol for IL-6 (-174):

0.025 µl of forward primer, 0.025 µl of reverse primer, 0.09 µl of FAM probe, 0.18 µl of TET probe 5.88 µl of distilled H_2O.

TaqMan 700 Protocol for IL-10 (-1082):

0.075 µl of forward primer, 0.45 µl of reverse primer, 0.15 µl of FAM probe, 0.375 µl of TET probe 5.25 µl of distilled H_2O.

TaqMan 7200 Protocol for TNF-α (-238):

1.35 µl of forward primer, 1.35 µl of reverse primer, 0.15 µl of FAM probe, 0.225 µl of TET probe 3.225 µl of distilled H_2O.

TaqMan 7200 Protocol for TNF-α (-308):

0.45 µl of forward primer, 0.45 µl of reverse primer, 0.15 µl of FAM probe, 0.375 µl of TET probe 4.875 µl of distilled H_2O.

All reactions were carried out using a DNA Engine Tetrad Machine PTC-225 Peltier Thermal Cycle and run according to the following protocols (Tables 2.2-2.6):

Table 2.2 TaqMan 7200 Protocol for IL-1ra (+2018).

Step 1	2 minutes	50°C
Step 2	10 minutes	95°C
Step 3	15 seconds	95°C
Step 4	1 minute	64°C
Step 5	Go to step 3 and repeat 40 times	
Step 6	Hold	15°C
Step 7	End	

Table 2.3. TaqMan 7200 Protocol for IL-10 (-1082).

Step 1	2 minutes	50°C
Step 2	10 minutes	95°C
Step 3	15 seconds	95°C
Step 4	1 minute	62°C
Step 5	Go to step 3 and repeat 40 times	
Step 6	Hold	15°C
Step 7	End	

Table 2.4. TaqMan 7200 Protocol for IL-6 (-174).

Step 1	2 minutes	50°C
Step 2	10 minutes	95°C
Step 3	15 seconds	95°C
Step 4	1 minute	62°C
Step 5	Go to step 3 and repeat 40 times	
Step 6	Hold	15°C
Step 7	End	

Table 2.5. TaqMan 7200 Protocol for TNF-α (-238).

Step 1	2 minutes	50°C
Step 2	10 minutes	95°C
Step 3	15 seconds	95°C
Step 4	1 minute	58°C
Step 5	Go to step 3 and repeat 40 times	
Step 6	Hold	15°C
Step 7	End	

Table 2.6. TaqMan 7200 Protocol for TNF-α (-308).

Step 1	2 minutes	50°C
Step 2	10 minutes	95°C
Step 3	15 seconds	95°C
Step 4	1 minute	58°C
Step 5	Go to step 3 and repeat 40 times	
Step 6	Hold	15°C
Step 7	End	

2.4.3.1.6. PCR-SSPs

PCR with sequence-specific primers (PCR-SSP) uses sequence-specific primers with 3' end mismatches and identifies the presence of specific allelic variants through PCR amplification.

2.4.3.1.6.1. Primer design and optimization

TNF, LT-α, TNFR primer design

Seventeen SNPs were investigated in this study, including *TNF* -1031, -863, -857, -308, -238 (all promoter, NCBI RefSNP: rs1799964, rs1800630, rs1799724, rs361525, and rs1800629), *LTA* +720 (exon 3, NCBI RefSNP: rs1041981), +365 (intron 1, RefSNP: rs746868), +249 (intron 1, RefSNP: rs909253); *TNFRSF1A* -201, -230, -845, -839, -1135 (all promoter, RefSNP: rs4149570, rs4149621, rs767455, rs4149584, and rs1800692); *TNFRSF1B* +1663 (exon 10, RefSNP: rs1061624), +1668 (exon 10, RefSNP: rs5030792), +1690 (exon 10, RefSNP: rs3397), +676 (exon 6, RefSNP: rs1061622). For the polymorphisms in the TNF-α and LT-α genes, we used the primer sequences and primer mixtures previously described by Fanning et al. and Grutters et al. (Fanning et al. 1997; Grutters et al. 2002) (Tables 2.7-2.9). The primer sequences used for polymorphisms of TNFR1 gene are shown in table 2.10 (Table 2.10). For identifying the polymorphisms in the TNFR2 gene, we used the primer sequences and primer mixtures described by Pantelidis and colleagues (Table 2.11) (Pantelidis et al. 1999).

Table 2.7. TNF-1031, -863, -857 primer sequences. From Grutters et al. (Grutters et al. 2002).

Primer	**TNF-α**	Sequence	Conc. (ng/µl)	Product size (bp)
	(-1031)			
1	T	CAAAGGAGAAGCTGAGAAGAT		
2	C	CAAAGGAGAAGCTGAGAAGAC		
3	Reverse primer	CCGGGAATTCACAGACCCC	20	433
	(-863)			
4	C	CGAGTATGGGGACCCCCC		
5	A	GAGTATGGGGACCCCCA		
6	Forward primer	CCGGGAATTCACAGACCCC	20	263
	(-857)			
7	C	CTACATGGCCCTGTCTTCG		
8	T	TCTACATGGCCCTGTCTTCA		
9	Forward primer	AAGGATAAGGGCTCAGAGAG	10	270
All 5'→3'				

Conc.: Concentration

Table 2.8. TNF-238, -308 primer sequences. From Fanning et al. (Fanning et al. 1997).

Primer Mix No	TNF gene SNPs		Primer No*	Conc(µM)	Primer No*	Conc(µM)	Product Size (bp)
	238	308					
1	A	-	1	0.58	5	0.57	763
2	G	-	1	0.58	6	0.57	763
3	A	-	2	0.47	5	0.57	763
4	G	-	2	0.47	6	0.57	763
5	G	G	3	0.49	7	0.56	109
6	A	G	4	0.62	7	0.56	109
7	G	A	3	0.49	8	0.56	109
8	A	A	4	0.62	8	0.56	109
9	-	G	1	0.58	7	0.56	835
10	-	A	1	0.58	8	0.56	835
11	-	G	2	0.47	7	0.56	835
12	-	A	2	0.47	8	0.56	835

Conc.: Concentration

* Primer 1: 5’ GCA TCC CCG TCT TTC TTC AC

Primer 2: 5’ GCA TCC CCG TCT TTC TCC AT

Primer 3: 5’ CTC CCC ATC CTC CCT GCT CC

Primer 4: 5’ CTC CCC ATC CTC CCT GCT CT

Primer 5: 5’ GAA GCA CCC CCT CGG AAT CA

Primer 6: 5’ GAA GCA CCC CCT CGG AAT CG

Primer 7: 5’ ATA GGT TTT GAG GGG CAT CG

Primer 8: 5’ ATA GGT TTT GAG GGG CAT CA

Table 2.9. LT-α primer sequences. From Fanning et al. (Fanning et al. 1997).

Primer Mix No	LT-α gene SNPs		Primer No*	Conc(µM)	Primer No*	Conc(µM)	Product Size (bp)
	365	249					
1	G	-	1	0.6	5	0.64	390
2	C	-	1	0.6	6	0.61	390
3	G	-	2	0.6	5	0.64	390
4	C	-	2	0.6	6	0.61	390
5	G	A	3	0.7	7	2.3	153
6	C	A	4	2.2	7	2.3	153
7	G	G	3	0.7	8	1.7	153
8	C	G	4	2.2	8	1.7	153
9	-	A	1	0.6	7	2.3	507
10	-	G	1	0.6	8	1.7	507
11	-	A	2	0.6	7	2.3	507
12	-	G	2	0.6	8	1.7	507

Conc.: Concentration

* Primer 1: 5’ GAG CAG CAG GTT TGA GGG

Primer 2: 5’ GAG CAG CAG GTT TGA GGT

Primer 3: 5’ GAC CTC CCG CCC TGG GAG AC

Primer 4: 5’ GAG CTC CCG CCC TGG GAG AG

Primer 5: 5’ GGG GTC GGG GGG TGC TG

Primer 6: 5’ GGG GTC GGG TGC TC

Primer 7: 5’ ATT CTC TGT TTC TGC CAT

Primer 8: 5’ ATT CTC TGT TTC TGC CAT GG

Table 2.10. TNFR1 primer sequences.

Primer Mix No	TNFR1 gene SNPs					Primer No*	Conc(Mm)	Primer No*	Conc(mM)	Product Size (bp)
	201	230	845	839	1135					
1	G	-	G	-	-	1	0.82	5	1.05	681
2	G	-	A	-	-	1	0.82	6	0.98	681
3	T	-	G	-	-	2	0.76	5	1.05	681
4	T	-	A	-	-	2	0.76	6	0.98	681
5	-	G	G	-	-	3	0.69	5	1.05	657
6	-	G	A	-	-	3	0.69	6	0.98	657
7	-	A	G	-	-	4	0.67	5	1.05	657
8	-	A	A	-	-	4	0.67	6	0.98	657
9	-	-	-	G	C	7	0.63	9	0.68	300
10	-	-	-	A	C	8	0.50	9	0.55	300
11	-	-	-	G	T	7	0.63	10	0.65	300
12	-	-	-	A	T	8	0.50	10	0.52	300

Conc.: Concentration

* Primer 1: 5’ TGGAAAACAGATCCAGACAGG (sense)

Primer 2: 5’ ATTGGAAAACAGATCCAGACAGT (sense)

Primer 3: 5’ GTTATGTGTCTGAGAAGTTCATTTG (sense)

Primer 4: 5’ AGTTATGTGTCTGAGAAGTTCATTTA (sense)

Primer 5: 5’ TCCCTGGTCTCACCAGC (antisense)

Primer 6: 5’ GTCCCTGGTCTCACCAGT (antisense)

Primer 7: 5’ TCTTCTTGCACAGTGGACCG (sense)

Primer 8: 5’ TCTTCTTGCACAGTGGACCA (sense)

Primer 9: 5’ CGGCACAGCTAAAGGAGG (antisense)

Primer 10: 5’ GCGGCACAGCTAAAGGAGA (antisense)

Table 2.11. TNFR2 primer sequences. From Pantelidis et al. (Pantelidis et al. 1999).

Primer No	TNFR2 gene SNPs				Concentration (µM)	Exon
	1663	1668	1690	676		
1	G	-	-	-	0.92	10
2	A	-	-	-	3.2	10
3	-	T	-	-	1.38	10
4	-	G	-	-	1.38	10
5	-	-	C	-	1.34	10
6	-	-	T	-	0.9	10
7	-	-	-	T	0.69	6
8	-	-	-	G	0.69	6

Primer 1: 5’ AGAGCAGAGGCACGG (sense)

Primer 2: 5’ AGAGCAGAGGCAGCGA (sense)

Primer 3: 5’ AGAGGCAGCG(A/G)GTTGT (sense)

Primer 4: 5’ AGAGGCAGCG(A/G)GTTGG (sense)

Primer 5: 5’ GCCTCTGCTGCCATGGC (sense)

Primer 6: 5’ GCCTCTGCTGCCATGGT (sense)

Primer 7: 5’ GACGTGCAGACTGCATCCA (antisense)

Primer 8: 5’ GACGTGCAGACTGCATCCC (antisense)

IL-8, CXCR1, MCP-1, CCR2 primer design

To locate potential SNPs, multiple sequences for IL-8, CXCR1, MCP-1 and CCR2 deposited in GenBank were analysed. Chemokine polymorphisms affecting the promoter region and exon were preferably selected, and where it was not possible, UTR (untranslated region) and intron polymorphisms were selected. To verify the presence of these polymorphisms, sequence specific primer PCR (SSP-PCR) was used (Table 2.12).

Figure 11. CC and CXC chemokine and receptor primer sequences.

No.	Gene	Chromosome	Polymorphism Site	Locus	NCBI SNP Cluster ID	GenBank Accession	Primer Type	S/AS	5'-3' Primer Sequence	Concentration
1	IL-8	4q13-q21	38366 C/T	Intron	rs2227543	AC112518	Specific C	as	CTA TGT ATG GTC TTT CTG GTC ATG	9 μL/ml
							Specific T	as	CTA TGT ATG GTC TTT CTG GTC ATA	9 μL/ml
							Consensus	s	TCA GGA ATG AGT TCA CTA GAA ACA	9 μL/ml
2	IL-8	4q13-q21	37511 C/T	Intron	rs2227306	AC112518	Specific C	as	AGT CAT AAC TGA CAA CAT TGA ACG	5 μL/ml
							Specific T	as	CAG TCA TAA CTG ACA ACA TTG AAC A	5 μL/ml
							Consensus	s	CGG AGC ACT CCA TAA GGC A	5 μL/ml
3	IL-8	4q13-q21	36849 C/T	Exon, Coding Sequence	rs1803205	AC112518	Specific C	as	GCT GCC AAG AGA GCC ACG	5 μL/ml
							Specific T	as	GCT GCC AAG AGA GCC ACA	5 μL/ml
							Consensus	s	CTA GAT CCC CCA CAT TAC TCA	5 μL/ml
4	IL-8	4q13-q21	36831 C/T	Exon, Coding Sequence	rs2227538	AC112518	Specific C	s	GAA CCA TCT CAC TGT GTG TAA AC	5 μL/ml
							Specific T	s	GAA CCA TCT CAC TGT GTG TAA AT	5 μL/ml
							Consensus	as	ACA GCT CTG CCA GCT ACT TC	5 μL/ml
5	IL-8RA	2q35	4205 G/A	3' UTR	rs1567868	AC097483	Specific G	s	TGA TGG AGT TGT CCT TGC AGG	5 μL/ml
							Specific A	s	TGA TGG AGT TGT CCT TGC AGA	5 μL/ml
							Consensus	as	CAC TTA TTA AGT TTG CCT TTG TGG	5 μL/ml
6	IL-8RA	2q35	6694 C/G	Exon, Coding Sequence	rs2234671	AC097483	Specific C	s	TGT TGC GGC GCT CAC AGC	4 μL/ml
							Specific G	s	TGT TGC GGC GCT CAC AGG	4 μL/ml
							Consensus	as	AAA TTT CAC TGG CAT GCC ACC T	3 μL/ml
7	IL-8RA	2q35	10188 T/C	Promoter	rs2671222	AC097483	Specific T	s	CCC ATC TCA GTA ACG GAG AAT	5 μL/ml
							Specific C	s	CCC ATC TCA GTA ACG GAG AAC	5 μL/ml
							Consensus	as	AGA GCC CAG GAG GAA AGC A	5 μL/ml
8	MCP-1	17 q11.2-q21.1	62534 A/T	5' UTR	rs2857655	AC005549	Specific A	s	CTG GCT TCC TTC TTA ATT TCC AA	5 μL/ml
							Specific T	s	CTG GCT TCC TTC TTA ATT TCC AT	5 μL/ml
							Consensus	as	GCA GGG CTC GAG TTG ATT TG	5 μL/ml
9	MCP-1	17 q11.2-q21.1	63997 C/T	Promoter	rs1024611	AC005549	Specific C	s	AAG AAA GTC TTC TGG AAA GTG AC	7 μL/ml
							Specific T	s	AAA AGA AAG TCT TCT GGA AAG TGA T	14μL/ml
							Consensus	as	GAT TCT GGA CAG CAT CAG AG	10 μL/ml
10	MCP-1	17 q11.2-q21.1	63555 A/T	Promoter	rs1024610	AC005549	Specific A	as	TTC ATG GTA AAG GAT GCA CTA ACT	9 μL/ml
							Specific T	as	TTC ATG GTA AAG GAT GCA CTA ACA	9 μL/ml
							Consensus	s	TCC CAG AGC AGA GAC TCT AT	9 μL/ml
11	CCR2	3p21	50490 T/A	3' UTR	rs1034382	U95626	Specific T	s	TCG AGT TAA GCA GGT GGA AGT	5 μL/ml
							Specific A	s	TCG AGT TAA GCA GGT GGA AGA	5 μL/ml
							Consensus	as	AAG GCC CCA TTG AAA CAA TGA C	5 μL/ml
12	CCR2	3p21	49776 C/G	3' UTR	rs762790	U95626	Specific C	as	CTG TGT CTT CTC ATT CAC CAG	4 μL/ml
							Specific G	as	CTG TGT CTT CTC ATT CAC CAC	4 μL/ml
							Consensus	s	TCG CTG TCA TCT CAG CTG GA	4 μL/ml
13	CCR2	3p21	49715 A/G	3' UTR	rs762789	U95626	Specific A	s	GAC ATC TGC CTC ATC CAA GCA	3 μL/ml
							Specific G	s	ACA TCT GCC TCA TCC AAG CG	3 μL/ml
							Consensus	as	TAT GCC AAG ACC CTT CCT TAC	4 μL/ml
14	CCR2	3p21	49652 T/C	3' UTR	rs762788	U95626	Specific T	s	TAC AGG CCA CAC AAC CCC AT	5 μL/ml
							Specific C	s	CAG GCC ACA CAA CCC CAC	5 μL/ml
							Consensus	as	CCT GCT TAA CTC GAA CAG CC	4 μL/ml
15	CCR2	3p21	49105 A/G	Exon	rs743660	U95626	Specific A	s	CCA GTG GGA ACT CCT AAA TCA AA	5 μL/ml
							Specific G	s	CAG TGG GAA CTC CTA AAT CAA G	5 μL/ml
							Consensus	as	ATA GGT AGA CCC TCC GGG AT	5 μL/ml
16	CCR2	3p21	46295 G/A	Exon, Coding Sequence	rs1799864	U95626	Specific G	as	TTT TTG CAG TTT ATT AAG ATG AGG AC	15 μL/ml
							Specific A	as	CTT TTT GCA GTT TAT TAA GAT GAG GAT	15 μL/ml
							Consensus	s	GAA GGC AGA AGG TGA ATA GTT C	15 μL/ml
17	CCR2	3p21	39353 A/G	Promoter	rs768539	U95626	Specific A	as	AAC CAG ATG GGA AGA GGG AAT	5 μL/ml
							Specific G	as	ACC AGA TGG GAA GAG GGA AC	5 μL/ml
							Consensus	s	GGA AAT TGC TAA GGG CAT CGT T	5 μL/ml
18	MHC Class I Antigen	6p21.3	HLA B27	-	-	-	Specific	as	CTC GGT CAG TCT GTG CCT T	10 μL/ml
							Specific	as	TCT CGG TAA GTC TGT GCC TT	10 μL/ml
							Consensus	s	GCT ACG TGG ACG ACA CGC T	10 μL/ml

* s = sense, as = antisense

2.4.3.1.6.2. PCR conditions

All PCR amplifications were carried in a PTC200 thermal cycler (MJ Research, GRI, UK). Two different cycle parameters were used.

PCR amplifications for the TNF-α promoter polymorphisms (-1031, -863, -857, -308, and -238), Lymphotoxin-α (+720, +365, + 249), TNFRSF1A (-201, -230, -845, -839, -1135), TNFRSF1B (+1663, +1668, +1690, +676) were as follow:

The DNA mixtures were heated at 96°C for 1 min in the first round of denaturation and then subjected to 5 cycles of 25 sec at 96°C, 45 sec at 70°C, and 25sec at 72°C; then followed by 21 cycles of 25 sec at 96°C, 50 sec at 65°C, 30 sec at 72°C; and 4 cycles of 30 sec at 96°C, 60 sec at 55°C, 90 sec at 72°C. Ten μL Orange-G loading buffer (0.5mM) was added into the final PCR mixtures and they were loaded onto a 1% agarose-0.5× Tris-borate-ethylenediamine tetra-acetic acid gel containing 0.14 μg/mL ethidium bromide. Electrophoresis was performed for about 20 minutes at 200 V/cm^2 in 0.5× TBE buffer (1× TBE = 89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0). The gels were visualized over ultraviolet light (320nm) and photographed.

PCR amplifications for the IL-8 polymorphisms (38366, 37511, 36849, 36831), CXCR1 (4205, 6694, 10188), MCP-1 (62534, 63997, 63555), CCR2 (50490, 49776, 49715, 49652, 49105, 46295, 39353) were as follow:

The cycling parameters for 23 μl reactions consisted of a first round of denaturation at 96°C for 1 min, followed by 5 cycles of 25 seconds at 96°C, 45 seconds at 70°C, 45 seconds at 72°C followed then by 21 cycles of 25 seconds at 96°C, 50 seconds at 65°C, 30 seconds at 72°C followed by 4 cycles of 30 seconds at 96°C, 60 seconds at 55°C, and 120 seconds at 72°C (Bunce et al. 1995).

After PCR amplification, the sealing mat was removed and 10 μl of Orange G loading buffer consisting of 150 ml of glycerol, 350 ml 0.5× TBE (1L of 0.5× TBE = 54g of Tris base, 27.5g of orthoboric acid and 20 ml of EDTA (0.5M, pH 8.0) or 3.72g of EDTA Na-dihydrate) and 0.125 g Orange G were added to each well to allow visualization during agarose gel electrophoresis.

The 1.5% agarose gel was prepared by dissolving 7.5g agarose powder (Baxter, UK) in 500 ml of 0.5x TBE aided by heating in a microwave. Fourteen μl of ethidium bromide were then added to the dissolved agarose mixture and the final mixture is then left to set and solidify at room temperature. The agarose gel is then loaded onto a gel tank (Flowgen model MH3025 with 26 well combs) containing 0.5 x TBE. After which the mixture in each reaction well

from the PCR plate was loaded into the agarose gel and electrophoresed for 15 minutes at 200V. The agarose gel was then photographed over ultraviolet light.

A positive reaction was defined as the presence of an allele-specific band of the expected size, in conjunction with a control band. The absence of an allele-specific band in the presence of a control band was considered to be a negative reaction.

CHAPTER THREE

3. THE DEVELOPMENT OF COMPLICATIONS IN PATIENTS WITH CHRONIC ANTERIOR UVEITIS

3.1. INTRODUCTION

AU is the most common form of intraocular inflammation seen in the clinical setting and the duration of the attack of inflammation defines whether the disease is acute or chronic (Rodriguez et al. 1996; Rothova et al. 1996). Specifically an attack that lasts longer than three months is termed CAU and distinguishes the disease process from those with acute inflammation in whom the inflammatory process resolves in less than 3 months although it may recur. The explanation for why some patients develop chronic anterior disease whereas others do not is unknown but it is not due to inadequate treatment. In some patients, it is associated with a systemic disease such as sarcoidosis but in many it is idiopathic with no detectable evidence of any systemic inflammatory process (Weiner et al. 1991). Uveitis patients with associated spondyloarthropathy are nearly always HLA-B27 positive and usually present with a sudden onset of AU, whereas patients with IBD associated uveitis are less frequently HLA-B27 positive and have a more insidious onset of uveitis and more chronic course (Feltkamp et al. 1998). Since the HLA-B27 antigen is less frequently found associated with other specific uveitis entities and systemic diseases such as sarcoidosis, than in uveitis associated with spondyloarthropathies, this suggests that the development of chronicity is not related to the presence of HLA-B27.

The primary aim of this study was to determine the visual outcome and long-term complications of patients with CAU treated aggressively as described above to achieve the best control of the inflammation.

3.2. METHODS

The clinical records of a total of 68 patients (91 eyes) consecutive patients with CAU were reviewed from Professor Lightman uveitis clinics at Moorfields Eye Hospital between January 1999 and June 2004. In all cases the intraocular inflammation was active for more than 3 months despite treatment. All patients had a complete ophthalmic examination including best corrected visual acuity, biomicroscopy, applanation tonometry and fundus examination. Fluorescein angiography, visual fields and ultrasonography were performed when indicated. Laboratory investigations included full blood cell count, renal and liver function, treponemal serology, and serum angiotensin-convertase enzyme. Chest or sacroiliac spine x-rays were requested for these patients suspected of having associated sarcoidosis or seronegative spondyloarthropathy respectively. HLA-B27 typing was performed according to the clinical features and the absence of coexistent systemic disease i.e., sarcoidosis. Patients

with clinical and laboratory evidence of associated systemic disease were referred to the appropriate medical team for further evaluation.

For the purpose of the study AU was defined as the presence of inflammatory cells in the anterior chamber and corneal endothelium (KPs) with or without flare and absence of posterior vitreous cells and other features of intraocular inflammation other than macular and optic disc edema. Other indicators such as ciliary flush, pain and photophobia, common features of AAU, were absent in most of the cases. When the intraocular inflammation persisted for more than three months it was categorized as CAU. Idiopathic uveitis was defined on the basis of the absence of a detectable associated systemic disease regardless of whether the patient was HLA-B27 positive or not. Visual acuity wit best spectacle correction was recorded with Snellen chart (logMAR visual acuity was calculated from the relation; logMAR visual acuity = Log of the reciprocal of Snellen visual acuity). Poor outcome was defined as visual acuity of 0.3 logMAR (6/12 Snellen equivalent) visual acuity or worse.

The ocular complications analyzed were the presence of posterior iris synechiae, posterior subcapsular lens opacity, ocular hypertension and/or glaucoma, and clinical or angiographic macular edema. Posterior iris synechiae (PS) was defined as the presence of iris adhesions to the anterior lens capsule post-dilatation. Only the presence of posterior subcapsular lens opacities was considered as cataract secondary to uveitis. Ocular hypertension was defined as intraocular pressure greater than 21mmHg without associated optic disc or visual field damage prior to treatment with corticosteroids. Glaucoma secondary to uveitis consisted of raised intraocular pressure over 21 mmHg with associated optic disc damage and/or correspondent visual field defect. Clinically significant macular edema was considered when thickening of the central macula and or loss of the normal foveal depression was present on biomicroscopy confirmed or not by fluorescein angiography. Patients with a previous history of glaucoma, macular and or other retinal disease were excluded from the study.

The medical treatment of all patients during the acute attack consisted of intensive topical corticosteroids followed by a slow reduction and in addition to this all patients received topical mydriatics for at least one month. When either clinical or angiographic macular edema was present, patients were offered periocular or systemic corticosteroids. Some patients also required systemic corticosteroids for their systemic disease. Patients with raised intraocular pressure only during the acute episodes of uveitis were treated by increasing the frequency of topical corticosteroids with or without anti-hypertensive drops. When specific anti-hypertensive therapy was required the drug of choice was a topical non-selective beta-adrenergic antagonist e.g., levobunolol 0.5% bid. The prostaglandin analogue latanoprost or

the α-adrenergic agonist apraclonidine for short-term management were the preferred second line agents (Sacca et al. 2001). Systemic carbonic-anhydrase inhibitors were given for the short-term management of raised intraocular pressure over 35mmHg.
Patients with evidence of clinical or angiographic macular edema at any time during the disease who were listed for cataract surgery were given systemic corticosteroids (40mg/daily) for two weeks before surgery followed by the reduction of 5mg per week starting at the beginning of the second postoperative week (Okhravi et al. 1999).

3.3. STATISTICAL ANALYSIS

For statistical purposes patients were divided into those with idiopathic CAU, those with associated systemic disease, and those with FHC. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 9.05, SPSS Inc., Chicago, ILL for Windows).
The distributions of demographic, ocular and systemic characteristics at base line were compared using chi-square or by Fisher's exact test. Product-limit or Kaplan –Meier method was used to estimate survival rates (Mathew et al. 1999). Log-rank test was used to compare survival curves among the different groups. *P* values less than 0.05 were considered statistically significant. No computation was carried out for missing data.

3.4. RESULTS

A total of 91eyes of 68 patients had AU that lasted more than 3 months. Twenty four patients (35.3%) were male and 44 (64.7%) were female. The median duration of follow up was 7.6 years (1.4 to 39.9 years). The median age was 55.5 years +/- 14.1 SD (range 24 to 74). The commonest ethnic group was Caucasian (60.3%), followed by patients from the Indian subcontinent (19%) and of Afro-Caribbean origin (10.3%). About one third of the patients (35.3%) were diagnosed with FHC. Patients with CAU (64.7%) were subdivided into those with idiopathic CAU (50%) and those where the intraocular inflammation was associated to a systemic disease (14.7%) that may account for the uveitis. Sarcoidosis associated AU and the seronegative spondyloarthropathy groups with 5 patients in each group (7.4%) were the commonest systemic disease associations identified.
The best corrected mean (+/- SD) logMAR visual acuity at presentation was 0.27 +/- 0.45. Fifty six eyes (61.5%) had a logMAR visual acuity at presentation of 0.17 or better. The frequency of complications is shown in table 3.1 (Table 3.1). Nineteen eyes (20.9%) presented with posterior subcapsular lens opacities. Overall, visual acuity was significantly worse in these patients with had evidence of cataract formation when they first presented *(p = 0.005 OR 5.92 95% CI 1.7-20.7).* There were no significant differences between patients with CAU and those with FHC or those with idiopathic CAU compared to those with non-

idiopathic CAU. Nineteen eyes (20.9%) had evidence of PS formation at presentation. As expected, compared to these eyes with CAU none of them had a diagnosis of FHC, however no significant difference was found between patients with idiopathic CAU and those with CAU associated to a systemic disease. Fourteen eyes (15.4%) had an intraocular pressure of 22mmHg or higher when they first presented, but only 2 eyes (2.2%) showed glaucomatous disc cupping. There was only one patient who presented with raised intraocular pressure, and he had a diagnosis of FHC. There was only one patient who presented with cystoid macular edema in one eye. There were no other significant differences between patients with CAU and those with FHC, or between those patients with idiopathic CAU compared to those with an associated systemic disease.

Table 3.1. Complications seen at presentation in patients with CAU.

	CAU (n=66)		FHC (n=25)
	Idiopathic CAU (n=48)	Non-idiopathic CAU (n=18)	
Raised IOP	10	3	1
PSCLO	9	3	7
PS	15	4	0
CMO	0	1	0
Cupped optic disc	2	0	0

IOP: Intraocular pressure; PSCLO: Posterior subcapsular lens opacification; PS: Posterior synechiae; CMO: Cystoid macular oedema.

Although there was no difference in the presence of PS between patients with CAU and those with FHC, cumulative survival curve at 5 years of follow-up showed a significant risk of developing PS in patients with CAU *(log-rank = 3.98, p = 0.045)*, and it was increased 3 years after i.e., 8 years after first presentation *(log-rank = 5.23, p = 0.022)* (Figures 3.1 and 3.2). Although there were no significant differences at 5 years in terms of raised intraocular pressure, a trend in patients with CAU to develop glaucoma was found *(log-rank = 3.08, p = 0.08)*. This was maintained 3 years later *(log-rank = 2.69, p = 0.100)*. When we looked at the risk of developing CMO, we found there were no significant differences between patients with CAU and those with FHC at 5 or 8 years follow-up *(log-rank = 1.96, p = 0.161 and log-rank = 2.15, p = 0.142 respectively)*. We then, compared the 2 subgroups of patients with CAU. There was no significant difference between patients with idiopathic uveitis compared to those with associated systemic disease in developing PS at 5 or 8 years of follow-up *(log-rank = 0.47, p = 0.49 and log-rank = 0.4, p = 0.52 respectively)*. There was a trend towards developing CMO after 5 and 8 years in patients with non-idiopathic CAU compared to these patients with idiopathic disease (log-rank = 2.17, p = 0.14 and log-rank = 2.43, p = 1.12 respectively) (Figures 3.3 and 3.4).

When we looked at visual outcome at 5 and 8 years after patients first presented in our department, we did not find any significant differences between patients with CAU and FHC or between patients with idiopathic CAU and non-idiopathic CAU.

Figure 3.12 Kaplan-Meier curve showing cumulative survival of eyes with a potential for developing posterior iris synechiae in CAU (dashed line) versus FHC (solid line) after 5 years of follow-up. x=CAU censored.

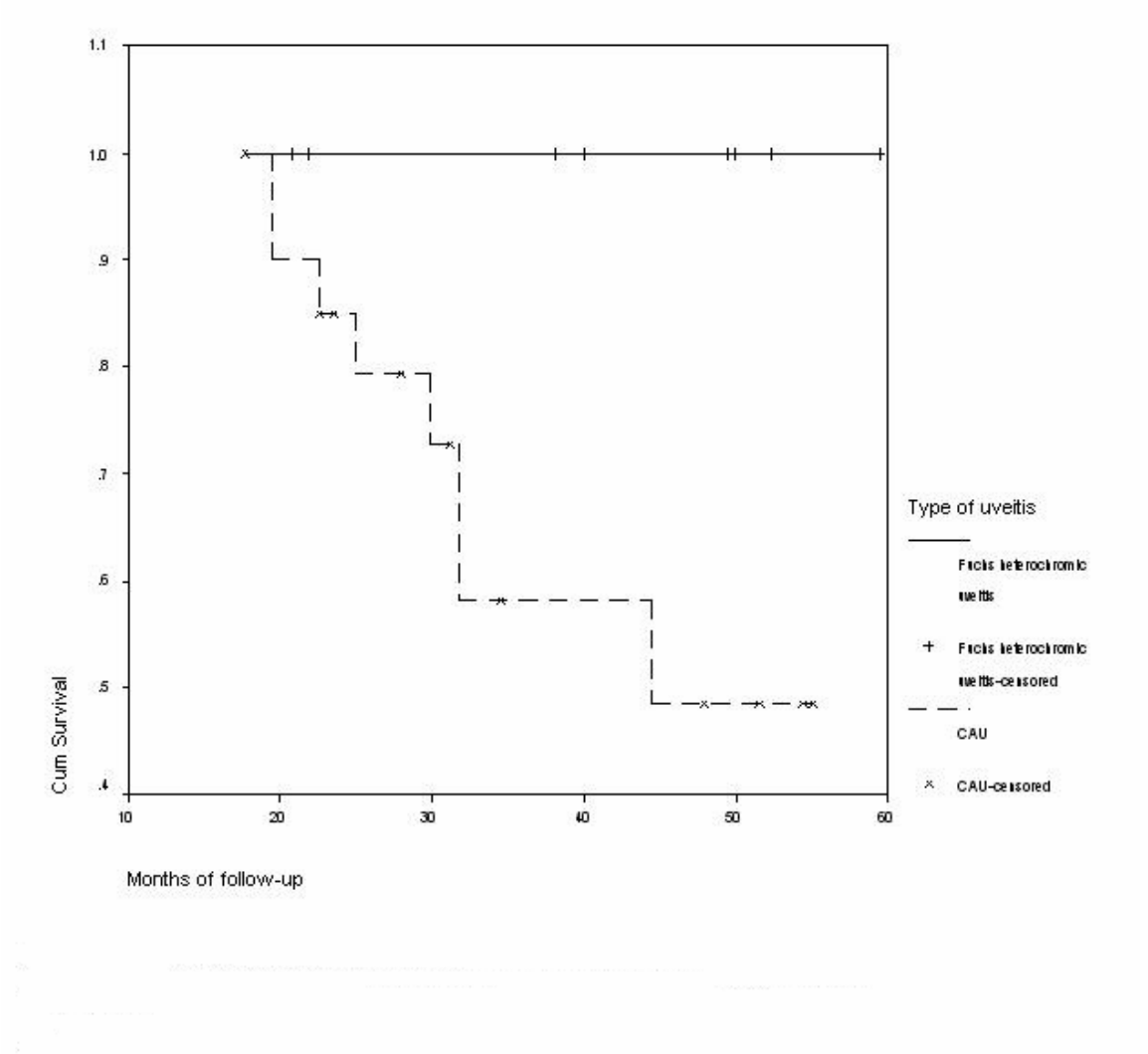


Figure 3.2. Kaplan-Meier curve showing cumulative survival of eyes with a potential for developing PS in CAU (dashed line) versus FHC (solid line) after 8 years of follow-up.

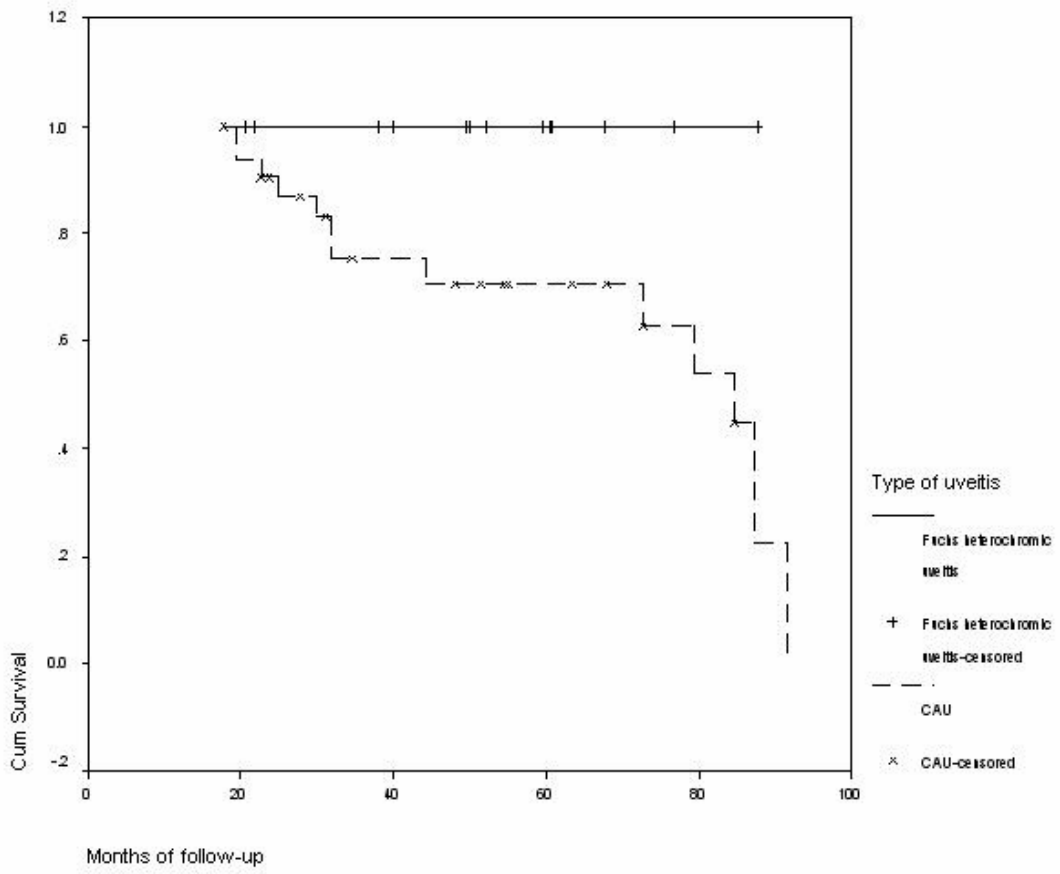


Figure 3.3. Kaplan-Meier curve showing cumulative survival of eyes with a potential for developing CMO in idiopathic CAU (I-CAU, solid line) versus non-idiopathic CAU (NI-CAU, dashed line) after 5 years of follow-up.

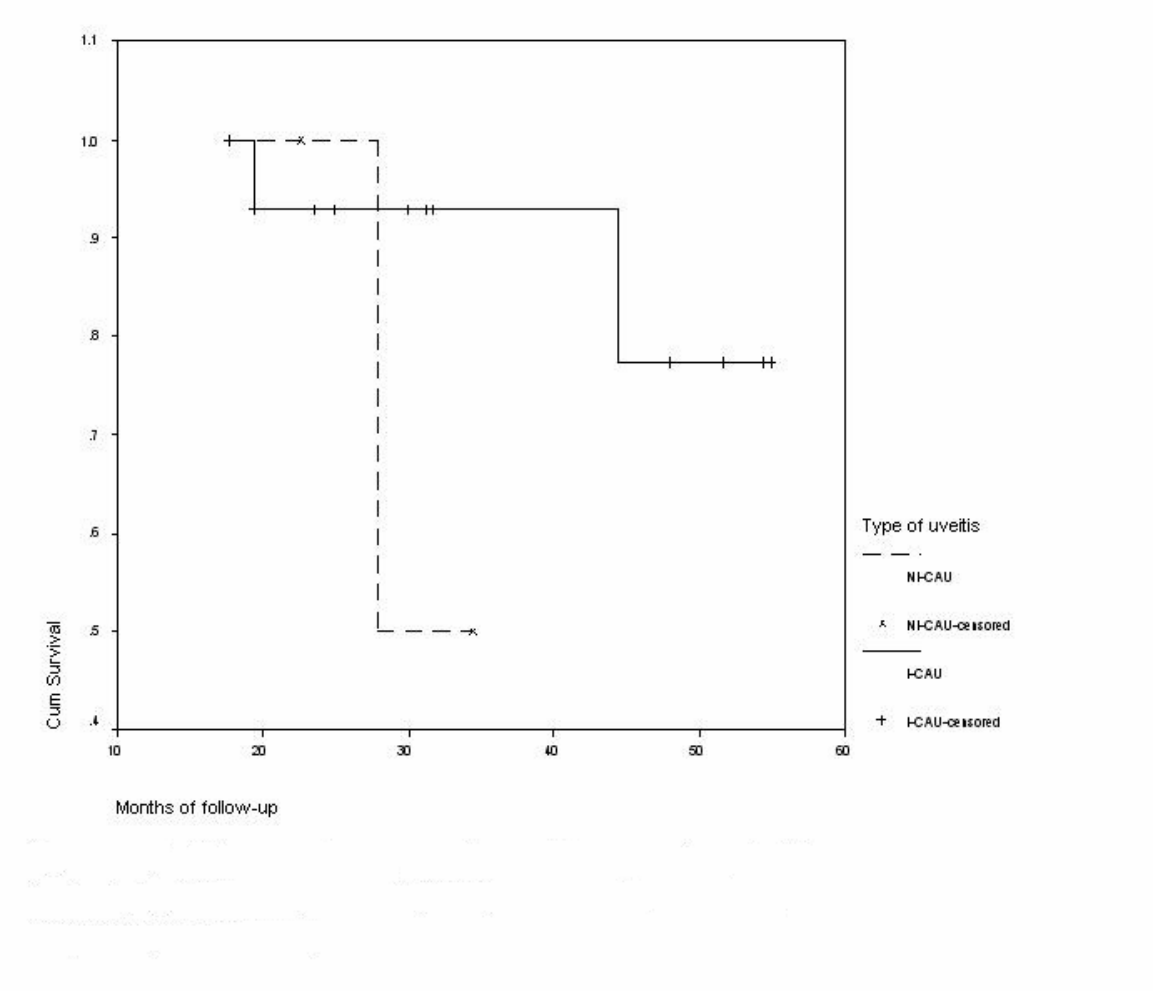
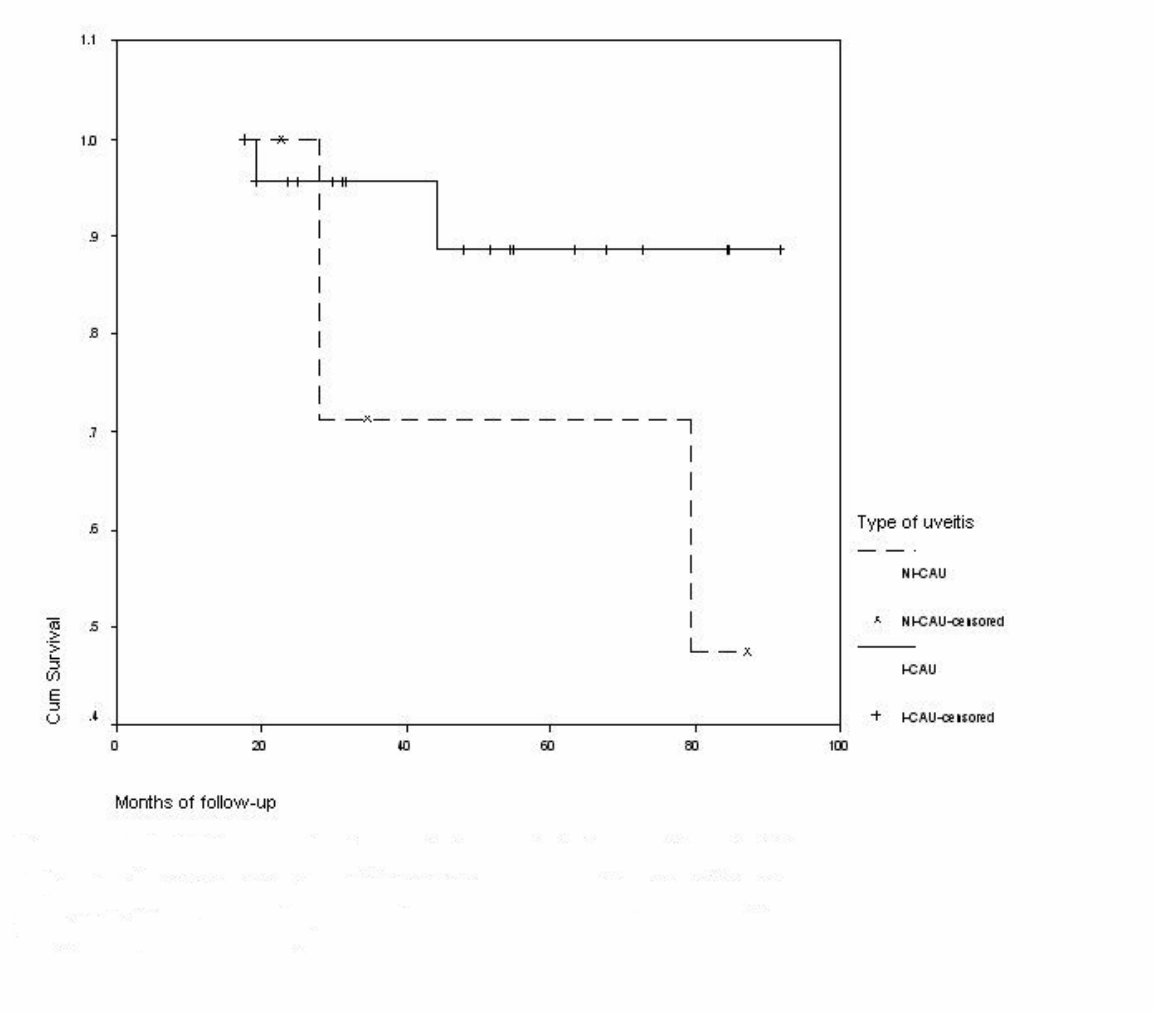


Figure 3.4. Kaplan-Meier curve showing cumulative survival of eyes with a potential for developing CMO in idiopathic CAU (I-CAU, solid line) versus non-idiopathic CAU (NI-CAU, dashed line) after 8 years of follow-up.



3.5. DISCUSSION

It is generally perceived that although any patient with AU may develop significant visual sequelae, patients with CAU do less well than those with acute recurrent disease. Patients with CAU often have no symptoms and minimal signs of inflammation and may be first diagnosed at a routine eye examination or when they present with visual loss. PS formation is much commoner feature in patients with chronic rather than acute inflammation. In addition to this, patients with CAU may need long term treatment, which is aimed at minimizing damage from the inflammation. Long term topical corticosteroids are the mainstay of treatment for CAU however, periocular corticosteroids can be given for macular edema but are contraindicated in steroid responders (Levin et al. 2002; Riordan-Eva et al. 1994). Systemic corticosteroids are indicated for bilateral macular edema in patients who cannot tolerate or do not respond to periocular corticosteroids. They may also be needed for the management of any underlying systemic disorder. Patients on chronic therapy are kept on the minimum possible medication to control the inflammation in order to minimize the side effects of corticosteroids such as cataract formation, especially the posterior subcapsular type, and raised intraocular pressure with or without optic disc damage and secondary visual field loss (Carnahan et al. 2000; Stanbury et al. 1998). In contrast, patients with FHC never develop PS and the inflammatory component of the disease does not respond to immunosuppression. Complications of this disease include cataract formation, glaucoma and vitritis (Jones 1993).

Our data shows that the overall outcome of patients with AU that persists more than 3 months is encouraging. Although there was a trend in patients with non-idiopathic CAU to develop CMO compared to these patients with idiopathic disease, no significant differences in visual outcome were found between any of the groups after long term follow up. As expected there was a significant difference in the formation of PS between patients with FHC and the overall group of patients with CAU, which appears to increase with time. Analysis of follow-ups longer than 8 years were not included, since the increased of censored observations resulted in a considerable reduced number of patients followed-up.

Posterior segment clinical manifestations in patients with acute/recurrent AU have been widely reported in the literature, however no definite conclusions as to what might be the cause other than the possible association with the presence of the HLA-B27 haplotype have been made. In these series of patients with chronic uveitis the two more common associated diseases were the group of seronegative arthropathies and sarcoidosis, and so the increased risk of CMO in non-idiopathic CAU patients might be related to systemic immune alterations of these systemic disorders. There are likely to be other genetic influences on disease outcome

in CAU. Different cytokine profiles have been found to correlate with the different clinical phenotypes, disease chronicity and response to treatment in the different types of AU (Muhaya et al. 1998; Muhaya et al. 1999). In addition to this, genotypic variations in cytokine promoter region may account not only for inter-individual variations in cytokine production but also susceptibility to the disease (Seki et al. 1999; Verity et al. 1999b). The correlation of certain SNPs in Caucasian patients with CAU was studied by Menezo et al. (abstract ARVO 2004). IL-1ra (+2018) T allele may predispose to the development of chronicity in patients with acute-recurrent AU, whereas the TNF (-238) GG genotype may contribute to the development of potentially sight threatening complications in British Caucasian patients with CAU. In theory, the identification of such variations of these SNPs could lead to more accurate ways of predicting outcome of disease and response to treatment in patient s with intraocular inflammation.

Despite the limitations that a retrospective study has, our results suggest that posterior segment complications in patients with CAU are more likely to occur in these patients with an underlying systemic disorder; however this does not appear to have a negative impact in the visual outcome of these patients.

CHAPTER FOUR

4. TNF-857T, A GENETIC RISK MARKER FOR ANTERIOR UVEITIS

4.1. INTRODUCTION

Uveitis, an umbrella term for intraocular inflammation, is of varying clinical phenotypes. It is largely T-cell mediated and many cytokines have been detected in uveitis patients (De Vos et al. 1992; Franks et al. 1992; Huhtinen et al. 2002; Muhaya et al. 1998; Ongkosuwito et al. 1998; Santos Lacomba et al. 2001b). Idiopathic AAU, in which there is often a severe inflammatory response in the anterior chamber or front of eye is the most common type seen in the general population. The disease severity and course varies between individuals and some patients, but not all, develop ocular complications that can threaten sight. Many different cytokines have been identified in the inflamed eye (De Vos et al. 1992; Franks et al. 1992; Huhtinen et al. 2002; Muhaya et al. 1998; Ongkosuwito et al. 1998; Santos Lacomba et al. 2001b; Verma et al. 1997), including TNF-α. High serum levels of TNF-α have been associated with a recurrent pattern of uveitis (Santos Lacomba et al. 2001b) and decreased inflammation was found in TNF receptor-deficient mice in immune complex-induced uveitis (Brito et al. 1999). TNF-α, produced predominantly by macrophages, plays a key role in many infectious and inflammatory diseases (Brennan et al. 1992; Grutters et al. 2002; Mannel et al. 2000; Taylor 2001). Lymphotoxin-α (LT-α; previously known as TNF-β) is produced mainly by lymphocytes and NK cells and displays a similar spectrum of biological activity in vitro, but is often less potent or displays apparent partial agonist activity (Bazzoni & Beutler 1996). TNF-α and LT-α both activate many genes involved in inflammatory and immunoregulatory responses and are structurally related molecules displaying 50% amino acid homology (Ware et al. 1995). TNF-α and LT-α compete for binding to the same receptors, TNFR1 and TNFR2. TNFR1 (55Kd) and TNFR2 (75Kd) (Aggarwal 1991; Bradley & Pober 2001; Inoue et al. 2000) are expressed on most nucleated cell surfaces and control the cascades of regulated signals. Binding of the trimeric ligands of TNF-α to the TNF receptor induces “molecular switching” of the receptor which then induces the signals required for immune function (Bazzoni & Beutler 1996). Excessive or inappropriate signaling through these receptors may cause severe inflammatory reactions and tissue injury (Bradley & Pober 2001; Hajeer et al. 2001).

Many SNPs exist in the TNF-α, LT-α, TNFR1 and TNFR2 genes (TNF, LTA, TNFRSF1A, and TNFRSF1B respectively) (Hajeer & Hutchinson 2001). It has been shown that differences in TNF-α production can be determined by genetic polymorphisms (Abraham et al. 1993; Grove et al. 1997; Higuchi et al. 1998; Jacob et al. 1990; Kroeger et al. 1997; Kroeger et al. 2000; Wilson et al. 1997). Meanwhile, a lot of evidences suggest that these polymorphisms may influence susceptibility and severity of inflammatory mediated disease

(Gonzalez et al. 2001; Grutters et al. 2002; Louis 2001; Negoro et al. 1999; Verity et al. 1999b; Waldron-Lynch et al. 2001). The aim of this study was to determine whether polymorphisms in genes critical to the inflammatory process have an effect in idiopathic AAU. Our objective was to investigate the association between published SNPs in TNF-α, LT-α, TNFR1, and TNFR2 genes and idiopathic AAU. As HLA-B27 is closely linked to TNF, the association between these SNPs and HLA-B27 was also studied. Furthermore, the role of these SNPs in determining the clinical phenotypes in AAU was evaluated.

4.2. MATERIALS AND METHODS

Subjects

Patients with idiopathic AAU, referred mainly from the southeast UK, were identified from the uveitis clinics (SL) of Moorfields Eye Hospital, London. AAU was defined as inflammation confined to the anterior chamber and/or anterior vitreous for less than 3 months. As uveitis is a clinically very heterogeneous disease, it would be impossible to look at these polymorphisms in a mixed group as the clinical outcomes would be so variable. Some AU may occur in patients with systemic disease and polymorphisms in the promoter region of TNF -1031T/C, -863C/A, and -857C/T have been reported to be associated with Crohn's disease and sarcoidosis (Grutters et al. 2002; Louis 2001). However the presence of systemic disease could generate confounding factors so we chose to limit the group studied still further to those with no detectable underlying disease, so a restricted clinical phenotype of idiopathic AU (IAU). Idiopathic disease was identified when there were no symptoms, signs or investigation results which suggested a relevant underlying disease process. Patients with any following situations were excluded from this study. 1) AU of infectious etiology (e.g. herpetic simplex or zoster uveitis, etc) 2) AU secondary to the inflammation from neighbouring tissues (e.g. scleritis, keratitis, post-operative uveitis, etc) 3) AU associated with known systemic diseases (e.g. AS, Reiter’s syndrome, sarcoidosis, Behcet’s disease, etc) 4) Acute uveitis secondary to other ocular diseases (e.g. FHC, Posner-Schlossman syndrome, etc)

A total of 98 patients were studied, including 24 patients with single episode IAU and 74 with recurrent IAU. All patients received a detailed ocular examination on several occasions, including corrected visual acuity, applanation tonometry for intraocular pressure, slit lamp examination, and fundus examination with 90-D diopter lens or indirect ophthalmoscope. Fluorescein angiography was performed if the vision had dropped and visually significant macular edema was considered possible. The following clinical parameters were also collected: age, sex, episodes of disease recurrence, HLA-B27, the presence or absence of PS, lens opacity and glaucoma. All patients were divided into either a inflammation-related complication group or a no complication group. The complication group contained the

patients with any of the following complications, which had been detected by the day of phenotyping and blood collection and included poor treatment response to topical steroids, secondary glaucoma, complicated cataract, cystoid macular edema, and requirement for cataract, glaucoma or retinal surgery. All these complications were related to inflammatory process.

Genetic analysis

Ten to 15 mLs of peripheral venous blood were collected for DNA extraction in all patients. Genomic DNA was extracted from EDTA-chelated peripheral whole blood, either with commercial kits (Qiagen UK Ltd, UK) according to manufacture's instructions or by a modified salting-out procedure (Bunce et al. 1995). Polymorphisms were determined using sequence-specific primers (SSPs). PCR-SSP utilizes SSPs with 3'-end mismatches and identifies the presence of specific allelic variants through PCR amplification. Seventeen SNPs were investigated in this study, including TNF -1031, -863, -857, -308, -238 (all promoter, NCBI RefSNP: rs1799964, rs1800630, rs1799724, rs361525, and rs1800629), LTA +720 (exon 3, NCBI RefSNP: rs1041981), +365 (intron 1, RefSNP: rs746868), +249 (intron 1, RefSNP: rs909253); TNFRSF1A -201, -230, -845, -839, -1135 (all promoter, RefSNP: rs4149570, rs4149621, rs767455, rs4149584, and rs1800692); TNFRSF1B +1663 (exon 10, RefSNP: rs1061624), +1668 (exon 10, RefSNP: rs5030792), +1690 (exon 10, RefSNP: rs3397), +676 (exon 6, RefSNP: rs1061622). For the polymorphisms in the TNF-α and LT-α genes, we used the primer sequences and primer mixtures previously described by Fanning et a.l (Fanning et al. 1997) and Grutters et al (Grutters et al. 2002). The primer sequences used for polymorphisms of TNFR1 gene are shown in table 2.5 (Table 2.5). For identifying the polymorphisms in the TNFR2 gene, we used the primer sequences and primer mixtures described by Pantelidis and colleagues (Table 2.6) (Pantelidis et al. 1999).

In all reactions, one of two different control primer pairs was used to generate a positive control band of 796 base pairs or 256 base pairs (Bunce et al. 1995), depending on the size of the target product. All PCR amplifications were carried out under identical conditions in PTC200 thermal cycler (MJ Research, GRI, UK) as previously described (Grutters et al. 2002). Briefly, each reaction contained 5 μL of the appropriate primer mix (allele-specific and control primers) and 8 μL of PCR reaction mixture, containing 1× PCR buffer (Bioline, London, UK), 300 μM of each deoxynucleotide triphosphate (Bioline), 2 mM $MgCl_2$, 0.32 U Taq polymerase (Bioline), and 0.01 to 0.1 μg DNA per well. The DNA mixtures were heated at 96°C for 1 min in the first round of denaturation and then subjected to 5 cycles of 25 sec at 96°C, 45 sec at 70°C, and 25sec at 72°C; then followed by 21 cycles of 25 sec at 96°C, 50 sec

at 65°C, 30 sec at 72°C; and 4 cycles of 30 sec at 96°C, 60 sec at 55°C, 90 sec at 72°C. The agarose gels were visualized over ultraviolet light (320nm) and photographed. A positive reaction was defined as the presence of an allele-specific band of the expected size, in conjunction with a control band. The absence of an allele-specific band in the presence of a control band was considered to be a negative reaction.

4.3. STATISTICAL ANALYSIS

All genotype frequencies in each population were tested for deviation from the Hardy-Weinberg equilibrium using the chi-square test. The genotype frequencies for each polymorphism were determined by direct counting and the allele and allele carriage frequencies calculated. Differences in genotype frequencies between patients and controls were analyzed with a 2x3 unisquare with 2 degrees of freedom. The allele and allele carriage frequency were compared using a 2x2 chi-squared test. The purpose of using three different comparisons in our analysis was to increase the power of our study to identify a significant susceptibility allele that the type 1 error was not much inflated (Ohashi et al. 2001). Statistical analysis was performed using chi-square contingency table analysis for all categorical data of clinical phenotypes and laboratory variables, depending on appropriate number of degrees of freedom. Fisher's Exact test was used if any expected frequency was lower than 5. The Student t test was used to compare the means of the continuous variables, such as age. A value of $p < 0.05$ was considered as significant.

Linkage is the association between alleles at linked loci which reflects, in part their proximity and the correspondingly low probability of recombination breaking the haplotype on which they are found (Collins A. Mol Biotechnol. 2009).

Two alleles are in LD if their co-occurrence in the population is higher than the product of their allele frequencies. It denotes the non-random association of alleles at two or more loci. If two loci have alleles A1, A2 and B1, B2 with frequencies of pA1, pA2, pB1, and pB2 respectively, there will be four possible haplotypes A1B1, A1B2, A2B1, A2B2 and their corresponding frequencies are pA1B1, pA1B2, pA2B1, pA2B2. In the event of no LD the frequency of the four haplotypes will be equal to the product of the frequencies of their alleles i.e., pA1B1 = pA1 x pB1.

However, LD is a non-quantitative phenomenon and among the measures that have been proposed for a two loci haplotype data the two most important are D' and r^2.

If the alleles at the two loci are not randomly associated, there will be a deviation (D) in the expected frequencies, which can be measured by:

D = pA1B1 x pA2B2 - pA1B2 x pA2B1, where A1B1 and A2B2 are referred as the coupling gametes and A1B2 and A2B1 the repulsion gametes. D is therefore the difference between these two gametes types.

As a measure of LD, the value of D will depend on the allele frequencies at the two loci, and Lewontin (1964) thus proposed standardizing D to the maximum possible value it can take: $D' = D/ D_{max}$, where D_{max} is the maximum value of D possible for the given allele frequencies. D' value will varies between 0 (no LD) and +/- 1 (complete association) and allows assessing the extent of LD relative to the maximum possible value it can take.

An alternative measure of LD proposed by Hill and Robertson (1964):

$r^2 = D^2 / pA1 \times pA2 \times pB1 \times pB2$. Where r^2 will equally varies between 0 and +/- 1.

r^2 reflects statistical power to detect LD.

nr^2 is the Pearson test statistic for independence in a 2 x 2 table of haplotype counts, therefore a low r^2 corresponds to a large sample size n that is required to detect the LD between the markers.

LD measuring r^2 was calculated for all pair wise combinations of SNPs within each gene. LD analysis was carried out in HelixTree™ (Golden Helix Inc, Bozeman). The role of SNPs in TNF is still controversial; therefore the analysis of haplotype in candidate area is important (Bayley et al. 2004). Haplotype frequency estimations were performed using the expectation-maximisation (EM) algorithm, which calculates maximum likelihood estimates of the possible haplotype frequencies, implemented in HelixTree™. To ensure the EM algorithm was producing robust estimates of haplotype frequencies, several random subsets of the data were selected and haplotype frequencies compared. There were no statistically significant differences between haplotype frequency estimations in random subsets of the data. Due to relatively strong LD it was possible to infer haplotypes by direct counting. Haplotype frequency comparisons were made by calculating the odds ratios for the frequency of each haplotype compared to the frequency of all other haplotypes. A correction using the Bonferroni method was used for multiple comparisons, using the formula of $p_c = p \times n$. (p_c represents the corrected value, p is the uncorrected value, and n is the numbers of comparisons performed in individual genes.)

The availability of high-throughput genotyping technologies and massive amounts of marker data for testing genetic associations is a dual-edged sword. On one side is the possibility that the causative gene will be found from among those tested for association, but on the other testing, many loci for association creates potential false positive results and the need to accommodate the multiple testing problem. One of the most widely used solutions involves correcting each test using adjustments such as a Bonferroni correction. Bonferroni adjustments are based on the reasoning that if a null hypothesis is true a significant difference ($P<0.05$) will be observed by chance once in 20 trials. This is the type I error, or alpha. When

20 independent tests are performed (for example, study groups are compared with regard to 20 unrelated variables) and the null hypothesis holds for all 20 comparisons, the chance of at least one test being significant is no longer 0.05, but 0.64. The formula for the error rate across the study is $1-(1-\text{alpha})^n$, where n is the number of tests performed.
The Bonferroni correction for multiple comparisons used the formula $p_c = p \times n$; where p_c represents the corrected value, p is the uncorrected value, and n is the numbers of comparisons performed in individual genes. We used an n value of 4 when comparing allele frequencies for each individual SNP, and a value of 6 when comparing genotype frequencies.

4.4. RESULTS

Forty-seven males and fifty-one females were included in this study (Mean age 46.6 years, SD 14.7 years). It has been demonstrated that there are ethnic variations in the frequency of TNF and LTα gene polymorphisms. Consequently, all the individuals (patients and controls) participating in this study were UK Caucasian in origin. The control population was collected mainly from the southeast of the UK as described before (Grutters et al. 2002; Pantelidis et al. 2001) and included 354 individuals for TNF-α and LT-α genes polymorphism, 132 individuals for TNFR1 gene polymorphism, and 100 individuals for TNFR2 gene polymorphism. The frequencies of TNF-α, LT-α and TNFR2 genes polymorphism have been previously reported (Grutters et al. 2002; Pantelidis et al. 2001). All genotype frequencies in case and control populations conformed to Hardy-Weinberg equilibrium.

Associations between SNPs and IAU

There was a significant increase in the allele frequency of TNF-857T in IAU patients as compared to controls (15.3% vs 7.3%, $p = 0.0006$, $p_c = 0.003$) (Table 4.1). In the patients with IAU, 28.6% carried the uncommon TNF-857T allele, compared with 14.4% in the control subjects ($p = 0.001$, $p_c = 0.005$). The pattern of LD between the 5 TNF SNPs is shown in figure 4.1 (Figure 4.1). The strongest evidence for LD between TNF-857 and the other SNPs tested was for TNF-307 (r correlation 0.19, Figure 4.1). Interestingly there was also a significant decrease in allele frequency of the uncommon TNFRSF1A-230G in the IAU group although this was not significant after correction with the Bonferroni method (1.0% vs 5.0%, $p = 0.02$, $p_c = 0.09$) (Table 4.2). Analysis of allele carriage frequency showed similar findings. There was no other significant difference of SNPs in allele, allele carriage or genotype frequency of LT-α and TNFR2 genes between IAU and controls (Table 4.3).

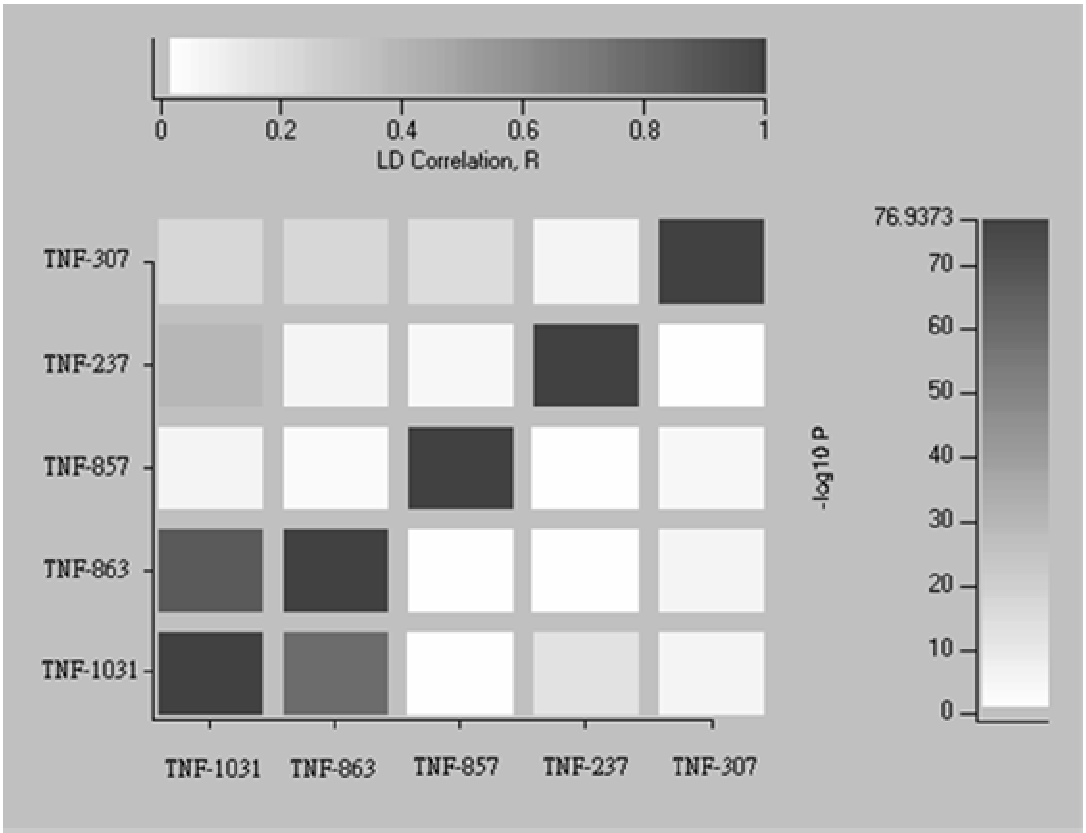
Table 4.1. Allele, genotype, and allele carriage frequencies of TNF-857 in IAU and control.

Polymorphism		**IAU (n = 98)**		**Control (n = 354)**			
		No.	%	No.	%	p value	p_c value
Allele Frequency							
TNF-857	C	166	(84.7)	656	(92.7)	0.0006*	0.003*
	T	30	(15.3)	52	(7.3)		
Genotype Frequency							
TNF-857	CC	70	(71.4)	303	(85.6)	0.002*	0.01*
	CT	26	(26.5)	50	(14.1)		
	TT	2	(2.0)	1	(0.3)		
Allele Carriage Frequency							
TNF-857	CC	70	(71.4)	303	(85.6)	0.001*	0.005*
	CT, TT	28	(28.6)	51	(14.4)		

IAU: idiopathic anterior uveitis

*Statistical significance

Figure 4.1. Linkage disequilibrium (LD) between TNF -1031, -863, -857, -308, -238.



LD is weak between the *TNF*-857 and the other SNPs in the gene. The strongest evidence for LD was between *TNF*-857 and *TNF*-307 (r correlation 0.19).

Table 4.2. Allele, genotype, and allele carriage frequencies of TNFRSF1A-230 in IAU and control.

Polymorphism		**IAU (n = 97)**		**Control (n = 119)**			
		No.	%	No.	%	p value	p_c value
Allele Frequency							
TNFRSF1A-230	A	192	(99.0)	226	(95.0)	0.02*	0.09
	G	2	(1.0)	12	(5.0)		
Genotype Frequency							
TNFRSF1A-230	AA	95	(98.0)	108	(90.8)	0.08	0.4
	AG	2	(2.0)	10	(8.4)		
	GG	0	(0.0)	1	(0.8)		
Allele Carriage Frequency							
TNFRSF1A-230	AA	95	(100.0)	108	(90.8)	0.03*	0.1
	AG, GG	2	(2.0)	11	(9.2)		

IAU: idiopathic anterior uveitis

* Statistical significance

Table 4.3. Genotype frequencies of LT-α and TNFR2 genes between IAU and controls.

		IAU		**Control**			
		(n = 98)	%	(n =349)	%	Chi2	*p* value
LT-α+720	CC	27	28.1	133	38.1	3.53	0.17130
	CA	51	53.1	166	47.6		
	AA	18	18.8	50	14.3		
LT-α+365	GG	36	37.5	128	36.7	0.10	0.94913
	GC	48	50.0	173	49.6		
	CC	12	12.5	48	13.8		
LT-α+249	GG	18	18.8	50	14.3	3.53	0.17130
	GA	51	53.1	166	47.6		
	AA	27	28.1	133	38.1		
TNFR2-663	AA	32	32.7	28	28.0	2.59	0.27327
	AG	48	49.0	44	44.0		
	GG	18	18.4	28	28.0		
TNFR2-1668	TT	89	90.8	86	86.0	1.12	0.29029
	TG	9	9.2	14	14.0		
	GG	0	0.0	0	0.0		
TNFR2-1690	CC	14	14.3	9	9.0	1.44	0.48670
	CT	49	50.0	51	51.0		
	TT	35	35.7	40	40.0		
TNFR2ex6-676	CT	64	65.3	34	54.0	5.94	0.05121
	TG	33	33.7	24	38.1		
	GG	1	1.0	5	7.9		

df = 2

^Caculated by 2x2, *df* = 1

From the results of the SNPs in the TNF promoter, we were able to construct the TNF haplotypes and calculate their frequencies (Table 4.4). The frequency of haplotype 4, containing the T allele at nucleotide position -857, was significantly increased in IAU as compared to controls either by direct counting (15.4% vs 7.1%, p = 0.0003, p_c = 0.002, odds ratio 2.4, 95% confidence interval 1.4-4.0) or by EM algorithm (15.3% vs 7.2%, p = 0.0004, p_c = 0.002, odds ratio 2.3, 95% confidence interval 1.4-3.9). No significant association was found in the frequencies of the other haplotypes.

Associations of SNPs and Clinical Phenotypes

The complication group contained 9 patients (9.2%) developing at least one type of ocular complication related to the inflammatory process, including 4 poor responses to topical steroid, 4 requiring surgery (cataract or glaucoma or retina surgery), and 1 cystoid macular edema. There was no association between the complication group and any of these SNPs. However, of the sixty-one HLA-B27 positive patients in the subgroup analysis, there were significant differences in the frequencies of the uncommon TNFRSF1A-201T and TNFRSF1A-1135T alleles between HLA-B27 positive patients with complications (n=5) and those without complications (80.0% vs 33.6%, p = 0.006, p_c = 0.03; 80.0% vs 36.6%, p = 0.01, p_c = 0.07, respectively). Analysis of the genotype and allele carriage frequencies showed similar significant findings in HLA-B27 positive patients (Table 4.5).

Higher allele carriage frequencies in TNFRSF1A-201T, TNFRSF1A-845A, and TNFRSF1A-1135T were found in recurrent IAU as compared to single episode IAU (71.2% vs 45.8%, p = 0.02, p_c = 0.1; 89.0%, 66.7%, p = 0.02, p_c = 0.1; and 75.7%, 45.8%, p = 0.006; p_c = 0.03 respectively) (Table 4.6). Differences in the genotype frequencies of TNFRSF1A-201, -845, -1135 were also found between recurrent and single episode IAU (p = 0.02, 0.004, and 0.008; p_c = 0.09, 0.02, and 0.04; respectively) (Table 4.7).

Table 4.4. Haplotype frequencies in IAU and control.

Haplotype	*TNF* Polymorphism					DC frequency		EM frequency	
	-1031	-863	-857	-308	-238	IAU	Control	IAU	Control
1	T	C	C	G	G	53.3%	54.4%	53.1%	54.0%
2	T	C	C	A	G	15.4%	18.3%	15.3%	18.2%
3	C	A	C	G	G	11.8%	14.7%	11.7%	14.0%
4	T	C	T	G	G	* 15.4%	7.1%	† 15.3%	7.2%
5	C	C	C	G	A	3.1%	4.9%	3.1%	4.8%
6	C	C	C	G	G	1.0%	0.7%	1.0%	0.7%

DC: direct counting; EM: expectation maximisation algorithm

IAU: idiopathic anterior uveitis, n=98; Control: n=354

* Odds ratio: 2.4, 95% confidence interval: 1.4-4.0; P = 0.0003, Pc = 0.002

† Odds ratio: 2.3, 95% confidence interval: 1.4-3.9; P = 0.0004, Pc = 0.002

Table 4.5. Allele, genotype, and allele carriage frequencies of TNFSF1A-201 & TNFSF1A-1135 in HLA-B27 positive patients with complication and without complication.

Polymorphism		**Comp (n = 5)**		**No Comp (n = 57)**			
		No.	%	No.	%	p value	p_c value
Allele Frequency							
TNFSF1A-201	G	2	(20.0)	73	(66.4)	0.006*	0.03*
	T	8	(80.0)	37	(33.6)		
TNFSF1A-1135	C	2	(20.0)	71	(63.4)	0.01*	0.07
	T	8	(80.0)	41	(36.6)		
Genotype Frequency							
TNFSF1A-201	GG	0	(0.0)	23	(41.8)	0.003*	0.02*
	GT	2	(40.0)	27	(49.1)		
	TT	3	(60.0)	5	(9.1)		
TNFSF1A-1135	CC	0	(0.0)	20	(35.7)	0.003*	0.02*
	CT	2	(40.0)	31	(55.4)		
	TT	3	(60.0)	5	(8.9)		
Allele Carriage Frequency							
TNFSF1A-1135	CC, CT	2	(40.0)	50	(91.4)	0.01*	0.07
	TT	3	(60.0)	5	(8.6)		
TNFSF1A-201	GG, GT	2	(40.0)	51	(91.2)	0.01*	0.07
	TT	3	(60.0)	5	(8.8)		

Comp: complication group; No Comp: no complication group; IAU: idiopathic anterior uveitis

* Statistical significance

Table 4.6. Allele carriage frequencies of TNFSF1A-201T, TNFSF1A-845A, and TNFSF1A-1135T were found in recurrent IAU as compared to single episode IAU.

		Recurrence (n = 74)				**Single episode** (n = 24)							
Polymorphism		Postive		Negative		Positive		Negative		Chi 2	*p* value		
TNFR1-201	T	52	71.2	21	28.8	11	45.8	13	54.2	5.12	0.02000	*	
TNFR1-230	A	73	100.0	0	0.0	24	100.0	0	0.0	0	1.00000		
TNFR1-845	A	65	89.0	8	11.0	16	66.7	8	33.3	Fisher	0.02000	*	***
TNFR1-839	A	4	5.4	70	94.6	1	4.2	23	95.8	0	1.00000		
TNFR1-1135	T	56	75.7	18	24.3	11	45.8	13	54.2	7.46	0.00600	*	

df=1

*Statistic significant

*** Expected cell frequency <5, suggest correction with Fisher's exact test

Table 15 Genotype frequencies of TNFR1polymorphisms between recurrent and single episode IAU.

		Recurrence (n = 74)		**Single episode** (n = 24)				
						Chi2	*p* value	
TNFRI-201	GG	21	28.8	13	54.2	8.03	0.02	*
	GT	39	53.4	5	20.8			
	TT	13	17.8	6	25.0			
TNFRI-230	AA	73	100.0	22	91.7	6.21	0.010	*
	AG	0	0.0	2	8.3			
	GG	0	0.0	0	0.0			
TNFRI-845	AA	21	28.8	10	41.7	10.78	0.004	*
	AG	44	60.3	6	25.0			
	GG	8	11.0	8	33.3			
TNFRI-839	GG	70	94.6	23	95.8	0.06	0.800	
	GA	4	5.4	1	4.2			
	AA	0	0.0	0	0.0			
TNFRI-1135	TT	12	16.2	5	20.8	9.54	0.008	*
	TC	44	59.5	6	25.0			
	CC	18	24.3	13	54.2			

df = 2

*Statistic significant

^Caculated by 2x2, *df* = 1

HLA-B27 and SNPs

Sixty-one of the 98 IAU patients (62.2%) were HLA-B27 positive. As expected, HLA-B27 was significantly associated with the recurrent form of IAU (χ^2 = 8.3, p = 0.004). The mean age of IAU patients with B27 positive was not significantly different from those with B27 negative (44.9 ± 13.0 yrs vs 49.3± 17.0 yrs, p = 0.16). Significant differences in the frequencies of the uncommon TNF-1031C and TNF-308A alleles between patients with B27 positive and B27 negative were found (11.5% vs 24.3%, p = 0.02, p_c = 0.09; 6.6% vs 31.1%, p = 0.000005, p_c = 0.00003, respectively) (Table 4.8). No significant difference in the allele and genotype frequency of TNF-857 was found between HLA-B27 positive patients and HLA-B27 negative patients. Of the haplotype analysis, the frequency of haplotype 1 of the TNF promoter, containing –1031T and –308G, was also significantly increased in IAU with B27 positive as compared to B27 negative either by direct counting (63.1% vs 37.0%, p = 0.0003, p_c = 0.002, odds ratio 3.0, 95% confidence interval 1.6-5.7) (Table 4.9) or EM algorithm (63.6% vs 36.5%, p = 0.0002, p_c = 0.001, odds ratio 2.8, 95% confidence interval 1.5-5.5). The frequency of haplotype 2, containing –1031T and –308A, was significantly decreased in IAU patients with B27 positive as compared to B27 negative by direct counting (6.6% vs 30.1%, p = 0.00001, p_c = 0.00006, odds ratio 0.17, 95% confidence interval 0.06-0.4) or by EM algorithm (6.6% vs 29.7%, p = 0.00001, p_c = 0.00006, odds ratio 0.17, 95% confidence interval 0.06-0.4).

Table 4.8. Allele frequencies of TNF promoter SNPs in HLA-B27 positive IAU and HLA-B27 negative IAU.

Polymorphism		B27(+) (n = 61)			B27(-) (n = 37)	
		No.	%		No.	%
TNF-1031 *	T	108	(88.5)		56	(75.7)
	C	14	(11.5)		18	(24.3)
TNF-863	C	111	(91.0)		62	(83.8)
	A	11	(9.0)		12	(16.2)
TNF-857	C	99	(81.1)		67	(90.5)
	T	23	(18.9)		7	(9.5)
TNF-307 †	G	114	(93.4)		51	(68.9)
	A	8	(6.6)		23	(31.1)
TNF-237	G	120	(98.4)		70	(94.6)
	A	2	(1.6)		4	(5.4)

* Statistical significance, $p = 0.02$, $p_c = 0.09$

† Statistical significance, $p = 0.000005$, $p_c = 0.00003$

Table 4.9.16 Haplotype frequencies of TNF promoter SNPs in HLA-B27 positive and HLA-B27negative IAU patients.

Haplotype	*TNF* Polymorphism						DC frequency			EM frequency	
	-1031	-863	-857	-307	-237		B27(+)	B27(-)		B27(+)	B27(-)
1	T	C	C	G	G		* **63.1%**	37.0%		**63.6%**	36.5%
2	T	C	C	**A**	G		6.6%	** **30.1%**		6.6%	†† **29.7%**
3	C	A	C	G	G		9.0%	16.4%		9.0%	16.2%
4	T	C	T	G	G		18.9%	9.6%		18.4%	9.4%
5	C	C	C	G	A		1.6%	5.5%		1.2%	5.4%
6	C	C	C	G	G		0.8%	1.4%		0.8%	1.4%

DC: direct counting; EM: expectation maximisation algorithm

HLA-B27(+): n=61; B27(-): n=37

* Odds ratio: 3.0, 95% confidence interval: 1.6-5.7; P = 0.0003, Pc = 0.002

† Odds ratio: 0.17, 95% confidence interval: 0.06-0.4; P = 0.00001, Pc = 0.0006

** Odds ratio: 2.8, 95% confidence interval: 1.5-5.5; P = 0.0002, Pc = 0.001

†† Odds ratio: 0.17, 95% confidence interval: 0.06-0.4; P = 0.0001, Pc = 0.0006

4.5. DISCUSSION

The association of SNPs of TNF-α, LT-α, TNF-R1 and TNF-R2 genes in patients with IAU has been investigated in this study. We have demonstrated that the frequency of the uncommon TNF-857T allele is significantly associated with IAU even after correction for multiple testing. Although a haplotype effect could also be demonstrated, haplotype 4 of TNF, containing the T allele at nucleotide position -857, was the only haplotype significantly associated with IAU. In addition, there was very little LD between TNF-857 and the other TNF SNPs, suggesting that the effect is largely attributable to TNF-857. Our results suggest that the uncommon TNF-857T allele is a susceptibility marker for IAU.

SNPs in the TNF promoter region are potentially functional sites influencing the expression of TNF-α, except TNF-α. -308G/A polymorphism (Bayley et al. 2004). The relationship between TNF-α expression and SNPs may be explained by the polymorphism TNF-863 (C/A). TNF-863 (C/A) polymorphism has been proposed to alter the binding affinity of the p50-p50 form of NFκ-B. The p50 subunit contains a DNA binding domain but no activation domain and the p50-p50 homodimer plays a role as a transcriptional repressor in the promoter region of the TNF gene. The affinity of the p50-p50 form NF-κB to its DNA binding site is significantly decreased in the TNF gene containing the uncommon -863A allele. Thus, the change from G to T at nucleotide position -863 results in increased TNF-α production (Udalova et al. 2000). The uncommon TNF-857T allele could have a similar function to –863A. Another hypothesis is that TNF-α is controlled through allele-specific binding of the transcription factor OCT-1. OCT-1 has been suggested to modulate TNF-α expression (Knight et al. 1999), and OCT-1 has been found to be associated with –857T in the promoter area, but not with –857G (Hohjoh & Tokunaga 2001).

Our results do not suggest that TNF-857T is the causal allele for IAU, but that it can be used as a marker for IAU. The same allele has been found to be associated with many other inflammatory diseases, such as Crohn's disease, sarcoidosis, and in RhA patients carrying the HLA-DR 'shared epitope' (Grutters et al. 2002; Negoro et al. 1999; Waldron-Lynch et al. 2001). The combination of the TNF -857T allele and DRB1*0405 yielded a significantly increased risk for developing systemic juvenile rheumatoid arthritis (Date et al. 1999). There may be other genes that affect susceptibility in linkage with TNF gene, such as the other HLA genes. TNF and HLA genes are located within the class III region of the highly polymorphic major histocompatibility complex (MHC) and have tight LD each other and so. It is therefore difficult to clarify the primary candidate marker for IAU and further study of the LD data for the haplotype containing TNF-857T is necessary for that. Herrmann et al reported the allele

frequency of TNF-857T were 13.7% and 17.8% from normal controls of Northern Ireland and France respectively (Herrmann et al. 1998). It is likely that these differences were due to differences in ethnicity since the TNF genes are situated within the MHC region, which is known to be both highly polymorphic and subject to ethnic variation. Ethnicity has been reported to be strongly associated with cytokine gene polymorphisms (Hoffmann et al. 2002). We suggest that differences between our control population and those reported by others are likely due to differences in ethnic origin. Repeating our study in other populations of different ethnicities is necessary to confirm the significance of our findings.

Fifty to 67% of patients with AAU are HLA-B27 positive (Derhaag et al. 1989; Mooller et al. 1980; Trevisani et al. 2001). Recurrent episodes are frequent in HLA-B27-associated AU (Mapstone et al. 1975; Power et al. 1998) and HLA-B27-associated AU tends to develop uveitis at a younger age (Rothova et al. 1987). Our results were similar. Some reports mention that the prognosis of the patients with HLA-B27-associated AU is similar to HLA-B27 negative ones (Linssen et al. 1995), but others have reported that patients who are HLA-B27 positive developed a more severe clinical course and a higher rate of ocular complication (Rothova et al. 1987), with a more severe outcome (Power et al. 1998). The classic role of MHC class I molecules is antigen presentation (Pamer et al. 1998) and recently, one study reported that HLA-B27 modulates NF-κβ activity in response to LPS in monocytic cells (Penttinen et al. 2002). NF-κβ is an important nuclear transcriptional factor in the expression of many genes which proteins are involved in the control of apoptosis and in the inflammatory processes, and NF-κβ can be activated by a variety of stimuli, including TNF-α (Pamer & Cresswell 1998; Penttinen et al. 2002). In this study, patients with inflammation-related complications (poor response to topical steroids, complicated cataract, cystoid macular edema, surgery) showed a trend of association with TNFRSF1A-201T and TNFRSF1A-1135T alleles in HLA-B27 positive patients. TNFR1, which is responsible for mediating most of the known TNF-α effects, contains two functional domains: a COOH-terminal region (death domain) and an NH_2-terminal region. TNFR1 initiates the pathway of NF-κβ activation through the death domain (Bradley & Pober 2001; Tkachuk et al. 1998; Vandevoorde et al. 1997). Binding of trimeric ligands of TNF-α to the TNFR1 induces signals required for the subsequent pathway activation (Bazzoni & Beutler 1996). The effect on gene expression of SNP in the promoter region of TNFR1 gene is still not clear. This might be associated with the reported increase in severity and complications in the HLA B27 positive group. But this association in this study should be treated as preliminary since the numbers of the patients with complications are small.

The uncommon TNF-238A allele has been reported to influence disease susceptibility in HLA-B27 negative primary AS but had no effect in B27 positive patients (Gonzalez et al. 2001). From the haplotype analysis of the TNF promoter in all IAU patients, haplotype 1, containing the common TNF-308G, is significantly associated with HLA B27 positive patients; and haplotype 2, containing the uncommon TNF-308A, is associated with HLA-B27 negative patients. The uncommon TNF-308A seems to be associated with HLA-B27 negative IAU patients. Both TNF-308A and TNF-238A alleles have been reported to be associated with a higher level of TNF-α production (Abraham et al. 1993; Grove et al. 1997; Higuchi et al. 1998; Jacob et al. 1990; Kroeger et al. 2000). We also found that HLA-B27 was associated with TNF-1031 and –308 in this study. These results strongly suggest there is LD between HLA-B27 and TNF-308. Therefore TNF-308A is unlikely to be an independent risk marker for HLA-B27 negative patients. Further work will be needed to clarify the role of the uncommon TNF-308A allele in the HLA-B27 negative patients.

In conclusion, this is the first study linking idiopathic AAU and its clinical manifestations to polymorphisms in TNF-α, LT-α, TNFR1 and TNFR2 genes. A significant difference in the frequency of TNF-857T allele was found in patients with IAU. There is a trend toward developing inflammation-related complications in IAU patients carrying TNFRSF1A-201 T and TNFRSF1A-1135 T allele. Genetic variations of these proinflammatory mediators and their receptors may influence the susceptibility and severity of the inflammatory response within the eye of idiopathic AAU and may provide useful prognostic information.

CHAPTER FIVE

5. CYTOKINE GENE POLYMORPHISMS INVOLVED IN CHRONICITY AND COMPLICATIONS OF ANTERIOR UVEITIS

5.1. INTRODUCTION

Uveitis is a generic term to describe intraocular inflammation, which has a variety of different clinical phenotypes and visual outcomes. AU, where there is no evidence of inflammation in the posterior segment, is considerably more common than posterior uveitis in which the inflammatory process occurs mainly in the posterior segment (McCannel et al. 1996). The explanation for why some patients develop chronic uveitis whereas others have a recurrent self-limiting type of uveitis is unknown. The development of chronicity is not due to inadequate treatment, is unrelated to HLA-B27 and it is likely that host factors are important determinants. Patients with CAU vary in their clinical phenotype. Some develop complications such as cataract, glaucoma and CMO, but others do not.

Cytokines are regulatory proteins produced by cells in different organs in response to a variety of inducing stimuli. They play an important role in controlling cell proliferation and cell differentiation phenotype. Th1-type cytokines such as interferon-γ mediate cellular immune responses and have an inflammatory action. Th2-type cytokines include interleukin-6 (IL-6) and interleukin-10 (IL-10) among others and inhibit Th1 cells suppressing inflammation as well as enhancing the humoral pathway of the immune response. The pro-inflammatory cytokines IL-1 and TNF are known to act as mediators of inflammation and even cause tissue damage in chronic inflammatory disease and they have been associated, together with other cytokines produced by T cells in the pathogenesis of uveitis (Sakaguchi et al. 1998). The clinical outcome of many autoimmune diseases including different forms of intraocular inflammation appears to be influenced by the balance between inflammatory and down-regulatory cytokines (Foxman et al. 2002; Muhaya et al. 1999; Ongkosuwito et al. 1998; Santos Lacomba et al. 2001a).

The level of cytokine production by cells in any individual has been associated with polymorphisms in cytokine gene promoters (Lazarus et al. 1997; Wilson et al. 1997). Since the levels of cytokine gene expression are partially genetically determined through common polymorphisms, these may influence the susceptibility to inflammatory disease or the severity of the disease (Lazarus et al. 1997). In addition to this, the cytokine and chemokine milieu of the aqueous has been shown to be associated with the outcome of uveitis (el-Shabrawi et al. 1998; Muhaya et al. 1998; Verma et al. 1997).

IL-1 is located on the long arm of chromosome 2 and contains three related genes that encode the pro-inflammatory cytokines IL-1α, IL-1β and their endogenous receptor antagonist IL-1ra. The ratio between IL1 and IL-1ra appears to be crucial in the outcome of inflammatory disease (Arend 2002). It has been shown in several animal models that intraocular injection of IL-1ra decreases the inflammatory response in AU caused by intravitreal injection of IL-1 and that seen after intraocular lens implantation (Nishi et al. 1994; Rosenbaum & Boney 1991). Some studies have linked IL-1ra gene polymorphisms with susceptibility or outcome of autoimmune disorders (Tountas et al. 1999; Vijgen et al. 2002; Watanabe et al. 2002).

IL-6 is a multifunctional cytokine that regulates the immune phase response, acute phase response and inflammation (Castell et al. 1989). Human IL-6 is synthesized as a precursor protein of 212 amino acids. Whereas the first 28 amino acids can be removed without affecting significantly the biological activity of IL-6, the removal of only 4 amino acids from the C-terminal of the IL-6 molecule may result in a complete loss of its biological activity (Kruttgen et al. 1990). It is associated with systemic-onset juvenile chronic arthritis (Fishman et al. 1998) and it also seems to contribute to the pathogenesis of RhA by influencing the age at disease onset (Pascual et al. 2000). This polymorphism was associated with different clinical and immunological features in Caucasian German patients with systemic lupus erythematosus, but it did not appear to affect disease susceptibility (Schotte et al. 2001). There is still some controversy between the IL-6 genotype and IL-6 levels. The -174 (G/C) polymorphism appears to affect IL-6 transcription and IL-6 levels and whereas some authors have found that the presence of allele C and CC genotype in patients with chronic inflammation is associated with higher levels of IL-6 (Jones et al. 2001), others found that in patients with primary Sjögren's syndrome those who were homozygous for allele G had increased plasma levels (Hulkkonen et al. 2001b). The same was true for HIV-infected male patients with Kaposi sarcoma (Foster et al. 2000) whereas other studies have not shown such association (Kilpinen et al. 2001; Rauramaa et al. 2000).

IL-10 is a regulatory cytokine with predominantly anti-inflammatory actions mediated inhibiting the production of pro-inflammatory cytokines. It also up-regulates TNFR1 and TNFR2, which in turns inhibits TNF-α response (Moore et al. 2001). Chronic enterocolitis that mimics IBD in humans has been induced in the IL-10 knock-out mice (Kuhn et al. 1993). Furthermore, intravenous injection of IL-10 in the animal model has shown reduction of the cellular infiltration in endotoxin-induced anterior uveitis (Hayashi et al. 1996; Rizzo et al. 1998; Rosenbaum et al. 1995).
The G/A polymorphism at position -1082 has been linked to high/low IL-10 producer status respectively (Lazarus et al. 1997; Tagore et al. 1999), and although the-1082 G/A

polymorphism appears to be in LD with other polymorphisms at -819, -592, it seems that the relationship between the IL-10 A allele and increased IL-10 gene transcription in B-cells is independent of these other polymorphisms (Rees et al. 2002). Tagore et al. reported a higher frequency of allele 1 in normal controls compared to patients with IBD (Tagore et al. 1999). IL-10 promoter polymorphisms have been found to be associated to different inflammatory diseases, such as RhA, primary Sjögren's syndrome, and SLE (Huizinga et al. 2000; Hulkkonen et al. 2001a; Lazarus et al. 1997).

The TNF cluster is located within the class III region of the MHC on chromosome 6p21 (Hajeer & Hutchinson 2000). TNF mediates its functions by binding to specific TNF receptors (Bazzoni & Beutler 1996). Signalling through these receptors influences T cell proliferation and proinflammatory responses (Hajeer & Hutchinson 2001). It has been shown that differences in TNF production can be determined by genetic polymorphisms (Higuchi et al. 1998; Kroeger et al. 1997; Kroeger et al. 2000; Wilson et al. 1997). As levels of cytokine gene expression are at least partially genetically determined through common polymorphisms, it is thought that these polymorphisms may influence susceptibility and severity of inflammatory disease. There are several SNPs within the promoter region of the TNF gene; among those the two most studied are the -308 G/A and the -238 G/A polymorphisms. In vitro stimulation of TNF-α production by cells and gene reporter assays have been used to investigate the relationship between circulating levels of TNF-α and their -308 and -238 polymorphisms with inconclusive results (Huizinga et al. 1997; Louis et al. 1996; Pociot et al. 1995).
The TNF–238 A allele has been associated with less severity in patients with multiple sclerosis (Huizinga et al. 1997). In a similar study, in patients with RhA the TNF-238 GA genotype was associated with decreased radiologically detectable progression of the disease (Brinkman et al. 1997). The TNF-α promoter-238 A polymorphism has been shown to influence disease susceptibility in patients with HLA-B27 negative AS (Gonzalez et al. 2001). McGarry et al. found a significant increase in the frequency of the common allele TNF-308 A in HLA-B27 positive patients with AS compared to HLA-B27 positive controls (McGarry et al. 1999). However other studies have shown no difference in the TNF-α allele frequency between patients with AS and controls (Huizinga et al. 1997; Kaijzel et al. 1999).

The aim of the study is to evaluate the role of specific SNPs of TNF, IL-1ra, IL-6 and IL-10 in influencing the development of chronicity and sight threatening complications in patients with AU.

5.2. MATERIALS AND METHODS

A total of 157 British Caucasian patients with AU were recruited from the uveitis clinics (SL) at Moorfields Eye Hospital between January 1999 and August 2003. Patients were divided into those with recurrent self-limiting AU (n = 118), those with CAU (n = 39). All patients had a complete ophthalmic examination including best Snellen visual acuity, biomicroscopy, aplanation tonometry and fundus examination. Fluorescein angiography, visual fields and ultrasonography were performed when indicated. Initial laboratory investigations included full blood cell count, renal and liver function, Treponema Pallidum serology, serum angiotensin-converting enzyme. HLA-B27 typing was not requested in all patients i.e., patients who already had a diagnosis other than the seronegative arthropathy or those with no complaints of seronegative related arthropathies. Chest or sacroiliac joint x-rays were requested for these patients suspected of having associated sarcoidosis or seronegative spondyloarthropathy respectively. Patients with clinical and laboratory evidence of associated systemic disease were referred to the appropriate medical team for further evaluation.

Controls

Peripheral blood was collected from 66 patients undergoing routine cataract surgery for age-related cataract and who had no previous ocular disease.

Ethical approval was obtained and informed consent given by all the patients and controls for venesection, and genetic analysis.

As many cytokine polymorphisms have different frequencies in different ethnic groups, all the individuals (patients and controls) in this study were British Caucasians.

DNA extraction

Between 10 and 20 ml of peripheral blood was obtained for DNA extraction in all patients and controls. Genomic DNA from all frozen bloods was extracted either using a salting out DNA extraction method or by using the commercial DNA extraction kit, QIAamp®DNA Blood Maxi Kit (QIAGEN Ltd., UK).

PCR analysis

Gene sequences from all individuals in this study were amplified by the TaqMan PCR (McGuigan et al. 2002).

The sequences of the probes and primers used as well as the PCR conditions have been previously described in the literature (Table 2.1) (Clay et al. 1996; D'Alfonso et al. 1994; Wilson et al. 1992).

5.3. STATISTICAL ANALYSIS

All genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium. Chi square contingency table analysis was used for all categorical data. Fisher's Exact test for 2 X 2 tables was used if any expected frequency was lower than 5. The Kruskal-Wallis test for independent samples was used to compare non-parametric data. Odds ratios (OR) were calculated for disease susceptibility or severity in patients carrying specific alleles. The 95% confidence intervals (95% CI) for the OR were also determined. Multiple significance tests i.e.; Bonferroni adjustment were applied to all results (Bland et al. 1995). All statistical calculations were performed using Statistical Package for the Social Sciences (SPSS) software (version 9.05 for Windows).

A sample size calculation was performed to calculate the power in order to detect two of the observed differences. Assuming a significant level of 5% we have 81% power to detect the difference in proportion of IL-1ra allele T in Caucasian patients with CAU compared to those with RAU and 69% power to detect the difference in proportion with TNF-α -308 allele G between patients with HLA-B27 negative versus HLA-B27 uveitis.

5.4. RESULTS

The different clinical entities and demographics of patients with AU in this study are summarised in table 5.1 (Table 5.1).

All genotype frequencies in both the case and control populations conformed to Hardy-Weinberg equilibrium (Salanti et al. 2005).

Table 5.1. Demographics and clinical characteristics of patients with anterior uveitis.

RAU	**N**	**%**
Male	63	53.4
Female	55	46.6
Mean age (+/-SD)	46.97 +/- 13.88	
Systemic Manifestation		
Idiopathic	88	74.6
Ankylosing Spondylitis	18	15.3
Reiter's Syndrome	3	2.5
Reactive Arthritis	2	1.7
Sarcoidosis	2	1.7
Psoriatic Arthropathy	1	0.8
Inflammatory bowel disease	1	0.8
Behçet's Syndrome	1	0.8
Other vasculitis	1	0.8
Multiple Sclerosis	1	0.8
CAU	**N**	**%**
Male	16	41
Female	23	59
Mean age (+/-SD)	53.97 +/- 16.16	
Systemic Manifestation		
Idiopathic	32	82.1
Sarcoidosis	2	5.1
Psoriatic Arthropathy	2	5.1
Ankylosing Spondylitis	1	2.6
Reiter's Syndrome	1	2.6
Reactive Arthritis	1	2.6

* All patients were of British Caucasian origin

Associations between SNPs and AU

No significant differences were found in either allele or genotype frequency in any of the SNPs when the total of patients with intraocular inflammation were compared to the normal controls (Tables 5.2 to 5.5)

Intra-group analysis showed a significant increase in the frequency of IL-1ra allele T *(83.3% vs 66.1% p_c = 0.004 OR 2.6 95% CI 1.3 – 4.9)* in Caucasian patients with CAU compared to those with recurrent disease (Table 5.6).

We also found a significant increase in the frequency of TNF-α 308 allele G *(91.8% vs 76.7% p_c = 0.02 OR 3.4 95% CI 1.4 – 8.5)* in Caucasian patients with HLA-B27 positive AU compared to those who tested negative for the HLA-B27 haplotype (Table 5.7).

Associations between SNPs and clinical phenotypes

To assess the severity of CAU, we analyzed data for patients who developed at least one of the complications described in the methods chapter, and not individually due to the relative small sample size. We did not find any significant associations between any of the SNPs and severity of the disease.

Table 5.2. Total uveitis vs Controls allele and genotype frequencies

IL1ra	**Total uveitis**	**Controls**		**%**	**%**
Homozygote A/A	79	36		50.32	54.55
Heterozygote A/a	63	27		40.13	40.91
Homozygote a/a	15	3		9.55	4.55
P Value =	**0.4466**		(*df*=2)		
Allele 1	221	99		70.38	75.00
Allele 2	93	33		29.62	25.00
P Value =	**0.3228**		(*df*=1)		
IL6	**Total uveitis**	**Control**		**%**	**%**
Homozygote A/A	58	27		37.91	40.91
Heterozygote A/a	65	30		42.48	45.45
Homozygote a/a	30	9		19.61	13.64
P Value =	**0.5701**		(*df*=2)		
Allele 1	181	84		59.15	63.64
Allele 2	125	48		40.85	36.36
P Value =	**0.3782**		(*df*=1)		
IL10	**Total uveitis**	**Control**		**%**	**%**
Homozygote A/A	41	22		26.11	33.33
Heterozygote A/a	75	27		47.77	40.91
Homozygote a/a	41	17		26.11	25.76
P Value =	**0.5123**		(*df*=2)		
Allele 1	157	71		50.00	53.79
Allele 2	157	61		50.00	46.21
P Value =	**0.4651**		(*df*=1)		
TNF-α (-238)	**Total uveitis**	**Control**		**%**	**%**
Homozygote A/A	147	59		93.63	89.39
Heterozygote A/a	10	7		6.37	10.61
Homozygote a/a	0	0		0.00	0.00
P Value =	**0.5531**		(*df*=2)		
Allele 1	304	125		96.82	94.70
Allele 2	10	7		3.18	5.30
P Value =	**0.2862**		(*df*=1)		
TNF-α (-308)	**Total uveitis**	**Control**		**%**	**%**
Homozygote A/A	115	45		73.25	68.18
Heterozygote A/a	37	19		23.57	28.79
Homozygote a/a	5	2		3.18	3.03
P Value =	**0.7139**		(*df*=2)		
Allele 1	267	109		85.03	82.58
Allele 2	47	23		14.97	17.42
P Value =	**0.5151**		(*df*=1)		

Table 5.3. RAU vs Controls allele and genotype frequencies

IL1ra		**RAU**	**Control**		%	%
Homozygote A/A		53	36		44.92	54.55
Heterozygote A/a		50	27		42.37	40.91
Homozygote a/a		15	3		12.71	4.55
P Value =		**0.1558**		(*df*=2)		
Allele 1		156	99		66.10	75.00
Allele 2		80	33		33.90	25.00
P Value =		**0.0759**		(*df*=1)		
IL6		**RAU**	**Control**		%	%
Homozygote A/A		48	27		42.11	40.91
Heterozygote A/a		45	30		39.47	45.45
Homozygote a/a		21	9		18.42	13.64
P Value =		**0.6227**		(*df*=2)		
Allele 1		141	84		61.84	63.64
Allele 2		87	48		38.16	36.36
P Value =		**0.7347**		(*df*=1)		
IL10		**RAU**	**Control**		%	%
Homozygote A/A		31	22		26.27	33.33
Heterozygote A/a		61	27		51.69	40.91
Homozygote a/a		26	17		22.03	25.76
P Value =		**0.3654**		(*df*=2)		
Allele 1		123	71		52.12	53.79
Allele 2		113	61		47.88	46.21
P Value =		**0.7584**		(*df*=1)		
TNF-α (-238)		**RAU**	**Control**		%	%
Homozygote A/A		110	59		93.22	89.39
Heterozygote A/a		8	7		6.78	10.61
Homozygote a/a		0	0		0.00	0.00
P Value =		**0.6611**		(*df*=2)		
Allele 1		228	125		96.61	94.70
Allele 2		8	7		3.39	5.30
P Value =		**0.3734**		(*df*=1)		
TNF-α (-308)		**RAU**	**Control**		%	%
Homozygote A/A		86	45		72.88	68.18
Heterozygote A/a		28	19		23.73	28.79
Homozygote a/a		4	2		3.39	3.03
P Value =		**0.7512**		(*df*=2)		
Allele 1		200	109		84.75	82.58
Allele 2		36	23		15.25	17.42
P Value =		**0.5863**		(*df*=1)		

Table 5.4. CAU vs Normal controls allele and genotype frequencies

IL1ra		**CAU**	**Control**		**%**	**%**
Homozygote A/A		26	36		66.67	54.55
Heterozygote A/a		13	27		33.33	40.91
Homozygote a/a		0	3		0.00	4.55
P Value =		**0.252**		(*df*=2)		
Allele 1		65	99		80.33	75.00
Allele 2		13	33		19.67	25.00
P Value =		**0.158**		(*df*=1)		
IL6		**CAU**	**Control**		**%**	**%**
Homozygote A/A		10	27		25.64	40.91
Heterozygote A/a		20	30		51.28	45.45
Homozygote a/a		9	9		23.08	13.64
P Value =		**0.215**		(*df*=2)		
Allele 1		40	84		51.28	63.64
Allele 2		38	48		48.72	36.36
P Value =		**0.078**		(*df*=1)		
IL10		**CAU**	Control		%	%
Homozygote A/A		10	22		25.64	33.33
Heterozygote A/a		14	27		35.90	40.91
Homozygote a/a		15	17		38.46	25.76
P Value =		**0.38**		(*df*=2)		
Allele 1		34	71		43.59	53.79
Allele 2		44	61		56.41	46.21
P Value =		**0.153**		(*df*=1)		
TNF-α (-238)		**CAU**	**Control**		**%**	**%**
Homozygote A/A		37	59		94.87	89.39
Heterozygote A/a		2	7		5.13	10.61
Homozygote a/a		0	0		0.00	0.00
P Value =		**0.625**		(*df*=2)		
Allele 1		76	125		97.44	94.70
Allele 2		2	7		2.56	5.30
P Value =		**0.343**		(*df*=1)		
TNF-α (-308)		**CAU**	**Control**		**%**	**%**
Homozygote A/A		29	45		74.36	68.18
Heterozygote A/a		9	19		23.08	28.79
Homozygote a/a		1	2		2.56	3.03
P Value =		**0.798**		(*df*=2)		
Allele 1		67	109		85.90	82.58
Allele 2		11	23		14.10	17.42
P Value =		**0.527**		(*df*=1)		

Table 5.5. CAU vs RAU allele and genotype frequencies

IL1ra		**CAU**	**RAU**		**%**	**%**
Homozygote A/A		26	53		66.67	44.92
Heterozygote A/a		13	50		33.33	42.37
Homozygote a/a		0	15		0	12.71
P Value =		**0.016**		(*df*=2)		
Allele 1		65	156		83.33	66.10
Allele 2		13	80		16.67	33.90
P Value =		**0.004**		(*df*=1)		
IL6		**CAU**	**RAU**		**%**	**%**
Homozygote A/A		10	48		25.64	42.11
Heterozygote A/a		20	21		51.28	18.42
Homozygote a/a		9	45		23.08	39.47
P Value =		**0.0003**		(*df*=2)		
Allele 1		40	117		51.28	51.32
Allele 2		38	111		48.72	48.68
P Value =				(*df*=1)		
IL10		**CAU**	**RAU**		**%**	**%**
Homozygote A/A		10	31		25.64	26.27
Heterozygote A/a		14	61		35.9	51.69
Homozygote a/a		15	26		38.46	22.03
P Value =		**0.102**		(*df*=2)		
Allele 1		34	123		43.59	52.12
Allele 2		44	113		56.41	47.88
P Value =		**0.191**		(*df*=1)		
TNF-α (-238)		**CAU**	**RAU**		**%**	**%**
Homozygote A/A		37	110		94.87	93.22
Heterozygote A/a		2	8		5.13	6.78
Homozygote a/a		0	0		0.00	0.00
P Value =		**0.935**		(*df*=2)		
Allele 1		76	228		97.44	96.61
Allele 2		2	8		2.56	3.39
P Value =		**0.718**		(*df*=1)		
TNF-α (-308)		**CAU**	**RAU**		**%**	**%**
Homozygote A/A		29	86		74.36	72.88
Heterozygote A/a		9	28		23.08	23.73
Homozygote a/a		1	4		2.56	3.39
P Value =		**0.962**		(*df*=2)		
Allele 1		67	200		85.9	84.75
Allele 2		11	36		14.1	15.25
P Value =		**0.804**		(*df*=1)		

Table 5.6. Frequency of IL-1ra allele T in Caucasian patients with chronic anterior uveitis compared to those with recurrent disease.

	CAU	**RAU**		Observed frequencies		
IL1ra				%		%
Homozygote TT	26	53		66.67		44.92
Heterozygote T/C	13	50		33.33		42.37
Homozygote C/C	0	15		0.00		12.71
	Pc value =0.09					
Allele T	65	156		83.33		66.10
Allele C	13	80		16.67		33.90
	Pc value=0.004 OR 2.6 95% CI 1.3 – 4.9					

Table 5.7. Frequency of TNF-α 308 allele G in patients with HLA-B27 positive anterior uveitis versus HLA-B27 negative anterior uveitis.

	HLA-B27 negative	**HLA-B27 positive**		Observed frequencies	
TNF-α -308				%	%
Allele G	46	101		76.7	91.8
Allele A	14	9		23.3	8.2
	Pc value=0.02 OR 3.4 95% CI 1.4 – 8.5				

5.5. DISCUSSION

We have investigated the association of different SNPs of IL-1ra, IL-6, IL-10 and, TNF genes in patients with CAU. The data of this study shows that the frequency of IL-1ra allele T is significantly associated with chronic disease in Caucasian patients with AU, even after correction for multiple testing. Although there was an association between homozygous patients for the IL-1ra (+2018) allele C and patients with recurrent inflammation, this was not significant after Bonferroni correction. HLA-B27 is known to be a risk factor for AU, and is closely linked to TNF. We found a significant association between TNF (-308) allele G and patients with AU who were HLA-B27 positive. However we did not find any associations between the TNF (-238)G/A in patients with CAU, suggesting that these polymorphisms are unlikely to be a major cause of disease susceptibility or development of chronicity in patients with AU. It is known that the TNF (-238) allele G is in LD with the HLA-B27 allele and that increased frequencies of the TNF (-308) allele A have been found in some infectious diseases (Cabrera et al. 1995; McGuire et al. 1994). But it will be of interest to know whether increased expression of TNF (-308) allele G is also found in the normal population. Although this might introduce a bias on the data, as some "normal" controls may go on to develop AU. McGarry et al. found an increased frequency of the TNF (-308) allele G in HLA-B27 positive patients with AS compared to HLA-B27 positive controls, suggesting that the -308 allele G may contribute to disease susceptibility independently of the HLA-B27(McGarry et al. 1999). Kaijzel et al. could not demonstrate whether TNF polymorphisms were associated with the presence of AU in a cohort of patients with AS, due to reduced sample size (Kaijzel et al. 1999). Our findings may correlate with those reported by Segundo-Gonzalez et al. where in a subgroup of HLA-B27 positive patients with AS the TNF (-308) allele G was found to be increased due to B27 LD. Therefore it still remains uncertain whether the low production of TNF-α associated with the -308 allele G could contribute to the clinical phenotype in patients with AU.

IL-1ra gene may have an important genetic contribution to AU. There are several reports on polymorphisms occurring within cytokine genes, some of which are related to disease (Bidwell et al. 2001). IL-1ra, the natural endogenous IL-1 antagonist binds to the receptor but does not initiate intracellular signalling. It acts as a competitive inhibitor and shows an anti-inflammatory effect in both in vivo and in vitro (Carter et al. 2001; Wilkinson et al. 1999). In the second intron of the IL-1ra gene there is a tandem repeat sequence 86-bp in length (Tarlow et al. 1993). Allele 1 is more common than allele 2 in most of the studied populations. Several studies have not been able to show a definite relationship between levels of IL-1ra and allele frequency (Mwantembe et al. 2001; Santtila et al. 1998; Tountas et al.

1999). Danis et al. observed that in healthy individuals allele 2 of IL-1ra was associated with an increased IL-1ra/IL-1α ratio (Danis et al. 1995). In addition to this, carriers of the rare IL-1ra allele 2 also show an increase in IL-1 beta circulating levels, which results in a lower IL-1ra/IL-1 beta ration and therefore increased inflammatory response (Hurme & Santtila 1998; Santtila et al. 1998). From these studies we can assume the pro-inflammatory effect of the rare IL-1ra allele 2. Also increased severity rather than predisposition to some inflammatory diseases has been associated with a higher frequency of the rare allele 2 of the IL-1ra (Arend 2002). We have analyzed the +2081 variation described by Clay et al. which is 100% in LD with the two most frequent alleles of IL-1ra (VNTR) (Clay et al. 1996). From our findings, patients with intraocular inflammation which persisted longer than 3 months had an increased frequency of the common IL-1ra (+2018) allele 1 ($p_c = 0.004$) compared to those with RAU. This was a surprising observation, and may look like conflicting evidence.

The exact functional consequences of these polymorphisms remain still unknown. One of the reasons being, that the VNTR in IL-1ra is in LD with several polymorphisms including this one at position +2018. It is also known the influence of IL-1β gene to regulate the production of IL-1ra (Hurme & Santtila 1998). Therefore, our findings may be explained by the influence of the decreased frequency of allele 2 on the IL-1ra /IL-1β ratio shifting the ration towards a more prolonged but milder inflammation in patients with uveitis.

We did not find any associations between the IL-6(-174)C/G, IL-10(-1082)G/A, or the TNF (-238)G/A in patients with AU, suggesting that these polymorphisms are unlikely to be a major cause of disease susceptibility or development of chronicity in patients with AU.

Not all our patients were genotyped for HLA-B27, especially these who had already been diagnosed with an associated systemic disease i.e., sarcoidosis, Behçet's syndrome. In our study we did not measure systemic or aqueous concentrations of any of these cytokines since it would be difficult to correlate their levels at any single time point of the disease due to the different disease duration, age, duration of the current episode, and interfering factors, such as the natural history of the disease and therapeutic interventions, and the heterogeneous associated group of diseases. One of possible reasons for the differences in these results may also be the relatively small sample size and therefore the lack of statistical power.

In conclusion, this study shows an interesting association between AU and IL-1ra gene polymorphism. There is however a need for larger groups, as well as measurement of cytokine levels in both peripheral and organ-specific levels.

CHAPTER SIX

6. CHEMOKINE GENE POLYMORPHISMS IN IDIOPATHIC ANTERIOR UVEITIS

6.1. INTRODUCTION

The pathogenesis of AAU is characterised by breakdown of the blood-aqueous barrier and acute inflammation of the ciliary body and iris and consequent leukocyte recruitment, a process that involves several proteins, including adhesion molecules, matrix metalloproteases, and chemotactic factors, which include the chemokine family. The successful trafficking of leukocytes to restricted places depends partly on the local secretion of chemokines and on the programmed expression of their chemokine receptors on cell surfaces.

The chemokine IL-8 is a pro-inflammatory chemokine that primarily mediates the activation and migration of neutrophils into tissue from peripheral blood. There is support in both animal and human model for the role of IL-8 in ocular inflammation (de Boer et al. 1993; Ferrick et al. 1991). Similarly, MCP-1 expression in the iris and ciliary body upregulates local T cell clones and monocytes during acute episodes of AU (Adamus et al. 2001).

There is evidence of certain polymorphisms of the human IL-8 gene have functional importance, and individuals homozygous for the -355A allele secrete more IL-8 than those homozygous for the T allele (Hull et al. 2000). Two SNPs affecting the distal regulatory region of the MCP-1 promoter; -2518A/G and -2076T/A transition have been reported (Rovin et al. 1999), and -2518A/G appears to influence transcriptional activity (Letendre et al. 2004; Rovin et al. 1999), and to determine the severity of organ inflammation in diseases where tissue leukocyte infiltration is MCP-1 dependent.

In the two previous chapters the association between different SNPs with susceptibility to develop AU, chronicity of the disease or worse outcome have been evaluated. In this study the role of polymorphisms in chemokines and their receptors as potential pathogenic or protective factors in the disease, as well as, their possible role in disease phenotype prediction are evaluated.

6.2. MATERIALS AND METHODS

This study was approved by the Ethics Committee of Moorfields Eye Hospital and was conducted according to the Tenents of the Declaration of Helsinky. Sixty Caucasian patients with idiopathic AAU were recruited from the Uveitis Clinic at Moorfields Eye Hospital, London for this study. The clinical phenotype of the patients was recorded at the time of blood donation. After verbal and written informed consent was obtained, demographic details, detailed medical and ocular history were recorded for each of the patients. All patients had detailed ocular examination including best corrected visual acuity, slit lamp examination, applanation tonometry, and dilated fundus examination with 90-D diopter lens or indirect ophthalmoscope. Fluorescein angiography or Optical Coherent tomography (OCT) was

performed if macular edema was considered likely on clinical examination. The following clinical parameters were particularly noted; pattern of the AU (single episode versus recurrent), laterality, response to topical steroids, complications (high IOP, permanent PS, cataract and macular edema) need for systemic/periocular steroids and other immunosuppressants. One hundred and twenty healthy Caucasian control subjects were recruited from blood and organ donors from Southern England. DNA was extracted either using a modified salting out DNA extraction method or with a commercial DNA extraction kit, QIAamp® DNA Blood Midi Kit (QIAGEN Ltd., UK).

To locate potential SNPs, multiple sequences for IL-8, CXCR1, MCP-1 and CCR2 deposited in GenBank were compared. Where differences between sequences were identified, SNPs located in promoter regions and exon were preferentially selected for analysis. To confirm the presence of each putative polymorphism, the SNPs were determined on a series of pooled Caucasian DNA samples using sequence-specific primers and PCR. This technique identifies the presence of specific allelic variants through PCR amplification, using sequence-specific primers with 3'end mismatches. All PCRs were run under identical conditions as previously described (Hizawa et al. 1999). The presence of an allele-specific band of the expected size, in conjunction with a control band, was considered positive evidence for each particular allele. The absence of an allele-specific band and the presence of a control band were considered evidence for the absence of an allele.

This resulted in the identification of 15 SNPs of the 2 chemokine genes and 2 chemokine receptor genes as shown in table 6.1, which were then determined in our IAU group and controls (Table 6.1).

Table 6.1. List of single nucleotide polymorphisms investigated.

Gene	Polymorphism site	Locus	NCBI dbSNP ID	Gene Bank Accession No.
IL-8	IL-8 37511 C/T	Intron	rs2227306	AC112519
	IL-8 36849 C/T	Exon	rs1803205	AC112520
IL-8RA	IL-8RA 4205 G/A	3' UTR	rs1567868	AC097483
	IL-8RA 6694 C/G	Exon	rs2234671	AC097484
	IL-8RA 10188 T/C	Promoter	rs2671222	AC097485
MCP-1	MCP-1 62534 A/T	Promoter	rs2867655	AC005549
	MCP-1 63997 T/C *	Promoter	rs1024611	AC005550
	MCP-1 63555 A/T †	Promoter	rs1024610	AC005551
CCR2	CCR2 50490 T/A	3' UTR	rs1034382	U95626
	CCR2 49776 C/G	3' UTR	rs762790	U95627
	CCR2 49715 A/G	3' UTR	rs762789	U95628
	CCR2 49652 T/C	3' UTR	rs762788	U95629
	CCR2 49105 A/G	Exon	rs743660	U95630
	CCR2 46295 G/A ‡	Exon	rs1799864	U95631
	CCR2 39353 A/G	Promoter	rs768539	U95632

6.3. STATISTICAL ANALYSIS

The genotypes were determined by direct counting and the allele and allele carriage frequencies calculated. Conformation of the Hardy-Weinberg equilibrium was tested by using a Chi-square goodness-of fit test. Haplotypes and LD (D'= LD value) were identified using the estimate haplotype frequencies program ARLEQUIN (http://lgb.unige.ch/arlequin/). Data mining for significant associations was performed using Knowledge Seeker® (Angoss Software, UK), and Chi-square or Fisher's exact test was used to test for differences in allele distribution between the patient and control groups, as well as within the patient group itself. Confidence intervals were calculated at the 95% level. A two-tailed P-value of <0.05 was considered significant. A correction using the Bonferroni method was used for multiple comparison, using the formula of $p_c = P \times n$. (p_c represents the corrected value, P is the uncorrected value, and n is the numbers of comparisons performed in individual genes). QUANTO© software (http://hydra.usc.edu/gxe) was used to compute the power of study using the sample of 60 subjects and the power to detect the allelic frequency difference was more than 80% for this sample size.

6.4. RESULTS

The allele frequencies of the investigated MCP-1, CCR2, IL-8 and IL-8RA polymorphisms for patients and control subjects were found to fit with Hardy-Weinberg equilibrium. The allelic frequencies of all SNPs analysed are shown in table 6.1 (Table 6.2).

IL-8 38366 C/T and IL-8 37511 C/T were found to be in strong LD with a D' value of 0.99. All the subjects homozygous for IL-8 38366 C were also homozygous for IL-8 37511 C and subjects with IL-8 38366 TT genotype were never homozygous for IL-8 37511 C. Similarly, T allele of CR2 50490 T/A and T allele of CCR2 49652 T/C were in complete LD, as were A allele of 49105 A/G and A allele of 39352 A/G with a D' value of 1.00.

As shown in table 6.1 there was a significant difference in the genotype frequencies of MCP-1 63555 A/T between IAU patients and controls. The frequency of the T allele of MCP-1 63555 polymorphism was significantly higher in the control group (26.25%) compared to the patient group (15%) (P=0.0160, p_c=0.032, OR 0.49, 95% CI: 0.27-0.88). The allelic frequencies and the allele carriage frequencies are shown in detail in table 6.2 (Table 6.3).

As expected the frequency of HLA-B27 positive subjects was significantly higher in the patient group than the control group (63.3% versus 15.8%; P<0.0001, OR: 9.18, 95% CI: 4.47-18.8). Thirty-eight out of 60 patients and 19 out of 120 controls were HLA-B27 positive (Figure 6.1).

The association of the MCP-1 63555 polymorphism with IAU was independent of the HLA-B27 gene and similarly no linkage was noted between HLA-B27 and other polymorphisms.

With the three SNPs of the MCP-1 promoter region, we were able to construct three common haplotypes. As shown in table 6.3, haplotype ATT, having the mutation T at 63555 and ancestral allele T at position 63997 was significantly higher in the control group (P=0.01, p_c=0.03, OR: 0.47, 95% CI: 0.25-0.88). Also the ancestral haplotype AAT was more common in the IAU group (57.7% versus 47.05 in controls) although it was not statistically significant (Table 6.3).

As shown in table 6.2, no other polymorphism showed any association with IAU including MCP-1 63997 T/C (published as MCP-1 -2518A/G) and CCR2 46295 (published as CCR2 V64I). Also no effect of any of the SNPs was noted on the risk of developing complication and visual outcome in IAU.

Table 6.2. Genotype frequencies of chemokine SNPs in AU.

		AU		Control		
		(n = 60)	%	(n = 120)	%	*p value*
IL-8 37511 C/T	CC	19	31.7	45	37.5	0.50539
	CT	30	50.0	49	40.8	
	TT	11	18.3	26	21.7	
IL-8 36849 C/T	CC	59	98.3	119	99.2	0.61510
	CT	1	1.7	1	0.8	
	TT	0	0.0	0	0.0	
IL-8RA 4205 G/A	GG	0	0.0	1	0.8	0.60678
	GA	5	8.3	14	11.7	
	AA	55	91.7	105	87.5	
IL-8RA 6694 C/G	CC	55	91.7	107	89.2	0.59816
	CG	5	8.3	13	10.8	
	GG	0	0.0	0	0.0	
IL-8RA 10188 T/C	TT	0	0.0	0	0.0	0.25256
	TC	3	5.0	12	10.0	
	CC	57	95.0	108	90.0	
MCP-1 62534 A/T	AA	55	91.7	114	95.0	0.37879
	AT	5	8.3	6	5.0	
	TT	0	0.0	0	0.0	
MCP-1 63997 T/C *	TT	31	51.7	63	52.5	0.96504
	TC	25	41.7	48	40.0	
	CC	4	6.7	9	7.5	
MCP-1 63555 A/T †	AA	44	73.3	65	54.2	0.04537
	AT	14	23.3	47	39.2	
	TT	2	3.3	8	6.7	
CCR2 50490 T/A	TT	3	5.0	6	5.0	0.94502
	TA	20	33.3	43	35.8	
	AA	37	61.7	71	59.2	
CCR2 49776 C/G	CC	43	71.7	87	72.5	0.76160
	CG	17	28.3	32	26.7	
	GG	0	0.0	1	0.8	
CCR2 49715 A/G	CA	8	13.3	11	9.2	0.67232
	AG	19	31.7	42	35.0	
	GG	33	55.0	67	55.8	
CCR2 49652 T/C	TT	3	5.0	6	5.0	0.94502
	TC	20	33.3	43	35.8	
	CC	37	61.7	71	59.2	
CCR2 49105 A/G	AA	3	5.0	6	5.0	0.94502
	AG	20	33.3	43	35.8	
	GG	37	61.7	71	59.2	
CCR2 46295 G/A ‡	GG	52	86.7	107	89.2	0.82549
	GA	7	11.7	12	10.0	
	AA	1	1.7	1	0.8	
CCR2 39353 A/G	AA	3	5.0	6	5.0	0.97501
	AG	20	33.3	42	35.0	
	GG	37	61.7	72	60.0	
HLA B-27	positive	38	63.3	19	15.8	<0.0001
HLA B-27	negative	22	36.7	101	84.2	

* Published as MCP-1 -2518A/G polymorphism by Rovin et al. (Rovin et al. 1999).

† Published as MCP-1 -2076A/T polymorphism by Rovin et al. (Rovin et al. 1999).

‡ Published as CCR2 V64I by Keino et al. (Keino et al. 2003).

Table 6.3. Allele, genotype, and allele carriage frequencies of MCP-1 63555 in IAU and control.

Polymorphism		IAU (n = 60)		Control (n = 120)			
		No.	%	No.	%	p value	p_c value
Allele Frequency							
MCP-1 63555	T	18	15.0)	63	(26.3)	0.016*	0.048*
	A	102	(85.0)	177	(73.8)		
Genotype Frequency							
MCP-1 63555	TT	2	(3.3)	8	(6.7)	0.045*	0.14
	AT	14	(23.3)	47	(39.2)		
	AA	44	(73.3)	65	(54.2)		
Allele Carriage Frequency							
MCP-1 63555	TT,AT	16	(26.7)	56	(45.8)	0.013*	0.039*
	AA	44	(73.3)	65	(54.2)		

IAU: idiopathic anterior uveitis

* Statistical significance

Figure 6.13 Relationship between HLA-B27 and IAU (p<0.0001).

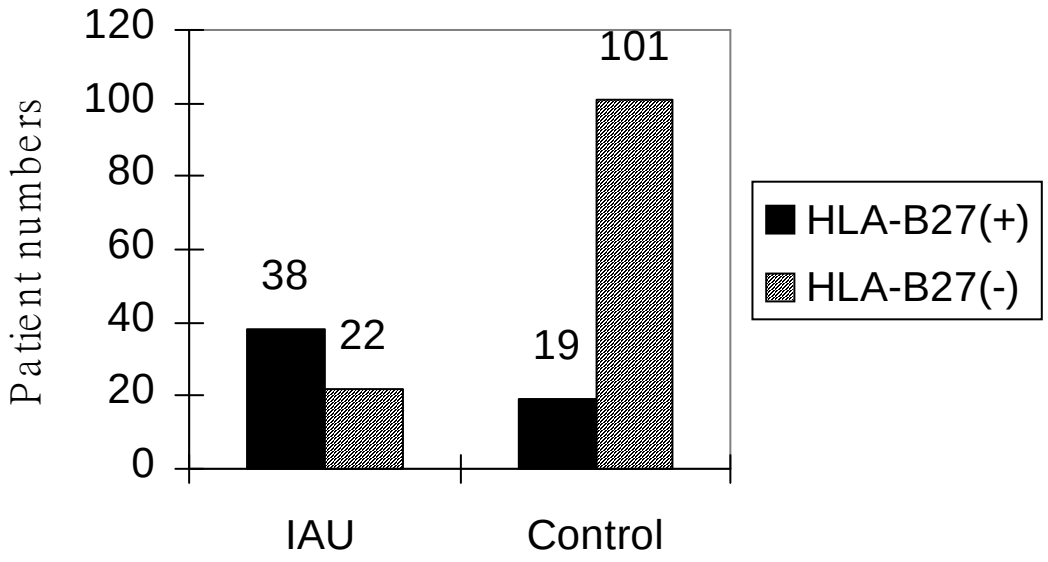


Table 6.4. Haplotype frequencies in IAU and control.

Haplotype	MCP-1 Polymorphism				IAU	Control				
	62534	63997	63555		(n = 60)	(n = 120)	Odds Ratio[†]		p value	p_c value
1	A	T	A		57.7%	47.0%	-		0.055	ns
2	A	T	T		13.8%	25.5%	0.47	(0.25-0.88)	0.011	0.033
3	A	C	A		24.3%	24.3%	-		ns	ns
Others					4.2%	3.2%	-		ns	ns

IAU: idiopathic anterior uveitis; ns: not significant

[†] 95% confidence interval

6.5. DISCUSSION

The association of chemokine gene polymorphisms in patients with AAU of who 63% of them were HLA-B27 positive, has been investigated in this study. Except for MCP-1 63555 A/T polymorphism there was no correlation noted between IAU and SNPs in IL-8, CCR2 and CXCR1 genes. MCP-1 is now thought to play an important role in AU (Mo et al. 1999; Tuaillon et al. 2002), and animal studies have demonstrated that MCP-1 is involved in induction of AU (Adamus et al. 1997).

However, like other studies on immune mediated diseases (Gonzalez-Escribano et al. 2003; Hwang et al. 2002; Kroner et al. 2004; Liao et al. 2004; Steinmetz et al. 2004) we did not observe any difference in allelic frequencies of MCP-1 63997 T/C alleles (-2518 A/G) between patients and controls.

Although the C allele is known to increase the expression of MCP-1 (Hizawa et al. 1999; Rovin et al. 1999), and many studies have shown relation between this C allele (alternatively designated as G allele at -2518) and MCP-1 level (Letendre et al. 2004; Tabara et al. 2003), other studies however failed to show any significant increase in the frequency of C allele in their experimental samples. In our study, the frequency of the T allele of MCP-1 63555 was found to be significantly higher in the control group compared to the patient group (Pearson's P=0.0160, p_c=0.048). The allelic frequencies in our control population were similar to those of Rovin et al. (Rovin et al. 1999) denoting that this allele plays some sort of protective role against AU. However most autoimmune mediated diseases are complex multigenetic disorders and it is hard to point at a single gene as causative factor. Most of the time more than one gene polymorphism has been implicated and haplotypes rather than single SNPs determine the nature and course of these complex diseases. In our study haplotype ATT was more prevalent in controls as compared to IAU (P=0.011, p_c=0.033). This haplotype contains T allele at 63555 (noted to be protective in our study) and a T allele at 63977 (known to be associated with decreased MCP-1 production). It appears that the presence of these two alleles together on the same gene exert a combined protective effect against IAU. How this mutation confers this protection is not known. Although Rovin et al. (Rovin et al. 1999) showed that polymorphism at site 63555 did not effect MCP-1 expression; it is worth mentioning that in their study the maximum transcriptional activity of MCP-1 promoter region was noted in subjects with the AC haplotype (A at -2076 and G at -2518 in their study). In another study, the same AC haplotype was associated with accelerated progress to AIDS and death and a high risk of HIV associated dementia in HIV positive Caucasian Americans (Gonzalez et al. 2002). In our study instead of the AC haplotype being associated with the disease it was the TA haplotype with both protective alleles, which was more common in the control group. Alternatively, it is quite possible that the protective effective of t allele at 63555 is independent of the 63977 polymorphism, as also noted by Modi et al. in AIDS patients (Modi

et al. 2003), were the T allele frequency was significantly higher in high risk exposes uninfected subjects as compared to sero-converters.

Although the association between IAU and MCP-1 63555 is established, whether this SNP is functional or not in not known. In the medical literature as mentioned above there is only one study that has studied functional effects of this polymorphism. Rovin et al. showed with electro mobility shift assay technique that there was significant difference in amount of nuclear protein complexes formed between the two alleles, and at last with the T allele sequence nuclear protein complex contains GATA (Rovin et al. 1999). By using online bioinformatics tool TRANFEC to study putative bindings sites for transcription factors (Schug 2008), not only confirms that the more GATA sites become available in the presence of T allele; but it also shows that the presence of the presence of A allele enables the c-MYB transcription factor to bind with MCP-1 promoter site. This transcription factor is believed to be involved in increasing the expression of its receptor gene (MCP-1 in this case) through DNA α polymerase activation (Venturelli et al. 1990).

Given the lack of in vivo or in vitro functional associations thee more plausible theory of the role of this SNP is through linkage with other SNPs in the same gene. To the best of our knowledge only another study on MCP-1 63555 polymorphism (CCL2 -2076A/T) and outcome to autoimmune disorder has been published to date (Ambruzova et al. 2009). Interestingly the authors concluded that the presence of the CCL2 -2076TT genotype was associated with decreased organ survival following hallogenic stem cell transplantation, although they considered these results to be preliminary due to the wide heterogeneity of their clinical data and lack of correction for multiple comparisons. .

In a similar study to ours, Wegscheider et al. showed that MCP-1 -2518G allele was associated with HLA-B27 associated AU (Wegscheider et al. 2005). Although we did not notice this association these studies together point towards the presence of genetic susceptibility locus for IAU in MCP-1 gene.

In conclusion, our study has demonstrated the association of MCP-1 promoter haplotypes with IAU. This is most likely due to the presence of the T allele of MCP-1 63555 which seems to be protective against the development of IAU. While MCP-1 63555 is not the only pathogenic factor it may still be a crucial key in deciphering the pathogenesis of AU. This in future could be a useful tool for clinicians in determining the possibility of disease development and prognosis in AAU.

CHAPTER SEVEN

7. DISCUSSION

The research carried out in this thesis has shown the following major findings. (1) The presence of PS in patients with CAU is not associated with worse clinical outcome; (2) patients with CAU associated with a systemic autoimmune disorder were more likely to suffer CMO; (3) however the overall long-term visual prognosis in patients with CAU was good. This research also identified (4) TNF-α -857T as a marker of susceptibility in Caucasian patients to develop AAU; (5) TNFRSF1A -201G/T and TNFRSF1A -1135C/T as potential contributors to the development of complications in patients with HLA-B27 associated AU. Furthermore, (6) IL-1ra +2081T was identified as a predictor of chronicity in Caucasian patients with AU. In addition (7) the presence of the allele T of the chemokine MCP-1 -2076 has a protective role against the development of idiopathic AAU.

The work in this thesis showed that the presence of PS was not significantly associated with an increased rate of complications leading to poorer visual outcomes. The same outcome was observed for the remaining complications, which included, cataract formation, CMO, or raised intraocular pressure. Interestingly this was true not only when the total group of patients with CAU was compared to those with FHC, but also after intra-group analysis of patients with idiopathic and non-idiopathic CAU, since it would be expected that patients with FHC do not develop PS, at least pre-operatively and therefore they would be more likely to have an overall better visual outcome.

Nevertheless, I showed a trend in patients with CAU associated to an inflammatory systemic disorder to develop CMO, however there was no difference in the long-term visual outcome compared to patients with idiopathic disease.

Similarly, although FHC is typically associated with raised IOP in 15 to 59% of patients, I found a trend in terms of increased IOP in the overall group of patients with CAU compared to those with FHC; however this may only reflect a bias from the number of patients with sarcoidosis-associated uveitis in whom the presence of glaucoma is not uncommon or from the population studied from a tertiary referral centre.

In the same way that posterior segment complications in patients with AAU have been widely reported in the literature, no definite conclusions as to what might be the cause can be extrapolated from this study. A possible explanation for the increased risk of CMO in the sub-group of patients with non-idiopathic CAU could be related to systemic immune alterations associated with sarcoidosis or SpA, or other genetic factors related to variations in the promoter region of cytokine genes affecting cytokine production or disease susceptibility.

I then investigated the association of different SNPs of IL-1ra, IL-6, IL-10 and, TNF-α genes in patients with CAU.
I analyzed the IL-1ra +2081 variation described by Clay et al. (Clay et al. 1996). The findings revealed an increased frequency of the more common IL-1ra +2018T allele in patients with CAU compared to those with recurrent AU. This is an interesting observation since the less common IL-1ra +2018C allele is linked with increased IL-1ra production in healthy individuals, and because IL-1ra prevents IL-1 signalling through its receptor, the presence of this allele would limit IL-1 mediated signals. However, the presence of the rare C allele has also been linked with increased levels of IL-1β and assuming its pro-inflammatory effect. These findings would be supported by the association between increased severity rather than predisposition to some inflammatory diseases and higher frequency of the rare allele C (Arend 2002), and would correlate with our results where the influence of the decreased frequency of allele C on the IL-1ra /IL-1β ratio shifts towards a more prolonged but milder inflammation.

A second association in this group of patients was observed between TNF-α -308G allele in HLA-B27 positive patients with AU. However, not all our patients in this study were genotyped for HLA-B27, in particular these who had already been diagnosed with non-related HLA-B27 diseases, such as sarcoidosis or Behçet's disease. Nevertheless this finding could explained by LD, given the short distance between the TNF-α gene and HLA-B locus in chromosome 6. For this reason a group of healthy HLA-B27 individuals could be introduced, although this could have introduced a bias on the results, since 1% of HLA-B27 positive normal controls may go on to develop AU at some stage in their lifetime.

Consequently, we looked at the associations of different SNPs of the TNF-α gene, including -308 in patients with idiopathic AAU. Interestingly we found from haplotype analysis of the TNF promoter in patients with IAU, that haplotype 1 containing the common TNF-α -308G allele is significantly associated with HLA-B27 positivity and haplotype 2 containing TNF-α -308A is associated with HLA-B27 negativity. However we also found that TNF-α -1031T allele was also associated with HLA-B27 positivity, suggesting that there is LD between HLA-B27 and TNF-α. More importantly these results have been replicated in a separate population by different authors, where they found lower A allele frequencies at position -308 of the TNF-α gene in patients with HLA-B27 positive associated AAU than those in HLA-B27 negative control subjects (El-Shabrawi et al. 2006). However, They also included a second control group of HLA-B27 positive individuals in order to assess LD and similar to the findings in the HLA-B27 negative control group, a lower frequency of the A allele was

observed among patients, suggesting that the protective role of the TNF-α -308A allele is due to LD to HLA-B27 is unlikely.
Another association seen in this cohort of patients was the increased frequency of TNF-α -857T allele in patients with IAU compared to control subjects. This allele has been linked with other autoimmune disorders associated with HLA-DR, conferring an increased risk of developing juvenile rheumatoid arthritis (Date et al. 1999), and hence, there might be other genes in the highly polymorphic MHC in LD. Therefore these results suggest that the uncommon TNF-α -857T allele may not be the causal allele for IAU, but is an independent susceptibility marker for IAU, and TNF-α -308A is unlikely to be an independent risk marker for HLA-B27 negative patients.

A further interesting finding from this study is the association between the uncommon TNFSF1A-1135T allele and the presence of recurrences of AU. Although many of these patients with a single episode of inflammation will develop at least one more if followed for a longer period of time (Levinson et al. 2002).

Finally we investigated the associations of chemokine polymorphisms in patients with AAU. We found the frequency of MCP-1 -2076T allele was found to be significantly higher in the control group compared to the patient group. As expected the frequency of HLA-B27 positive individuals was higher in the uveitis group, however the association between the MCP-1 -2076 polymorphism with IAU was independent of HLA-B27 gene and there was no evidence of LD. In addition to this, the allelic frequencies in our control population were similar to those of Rovin et al. (Rovin et al. 1999) suggesting that this allele may have a protective role against AU.

These findings support the principal hypothesis of this thesis that proposes the association of disease susceptibility and certain cytokine polymorphisms, as well as the role of certain SNPs in the development of chronicity in patients with AAU.

Advances in immunology continue to provide new insights about the ocular immunological response. Despite these insights, the clinical outcome of the different uveitis entities and the reasons for the diverse responses to treatment remains unclear.
AU is a term that encompasses several clinical entities. It may occur in isolation, or be associated with systemic disease. It can present suddenly or have an insidious onset, with patients being asymptomatic until the onset of complications such as cataract or macular oedema. In addition, in some cases the inflammatory process is self-limited lasting a few

weeks and in other cases it persists, either in a relapsing fashion with long periods of inactivity in between attacks or it may persist, relapsing soon after discontinuing therapy. Often there are no differentiating clinical features that can help in predicting the clinical pattern of uveitis and ultimately the course and prognosis. This is typified by the clinical entities HLA-B27-associated AAU and Behçet's disease-associated AU, which frequently share a similar form of presentation with sudden onset of symptoms, severe anterior segment inflammation and characteristic presence of hypopyon, yet have different outcomes. On the other hand, sarcoidosis-associated uveitis typically manifests with "mutton-fat" KPs, severe inflammatory cellular response in the anterior chamber with PS, and involvement of the posterior segment, but it can also present as a mild localised AU with a chronic course. Additionally there are entities, such as FHC where the inflammation is typically associated with specific complications such as cataract formation or raised intraocular pressure, but it is not associated with the presence of PS or CMO, and typically exhibits a lack of response to steroid therapy.

Thought the precise pathogenesis of AU remains largely unknown, in this thesis I have described the current evidence showing that both innate and adaptive immune responses regulate different mechanisms involved in the development of uveitis and how different genetic markers can influence the development of AU and ultimately determine the course of the inflammatory process and different phenotypic manifestations of this disease.
One of the critical links between the two arms of the immune response is a group of pattern recognition receptors located on the cell membrane called TLRs. They are expressed in a variety of cells, mostly macrophages and dendritic cells, and are known to be expressed in the human uveal tissue. In addition, it is known that they have a unique specificity for highly conserved ligands, and for instance TLR4 recognizes LPS of gram negative bacteria which is implicated in the pathogenesis of uveitis.
LPS activates monocytes, macrophages, epithelial and endothelial cells to produce several pro-inflammatory cytokines such as TNF-α, IL-1, or IL-6, which are commonly found in the AqH in patients with AAU, as well as in experimental animals with EIU. In healthy individuals a single exposure to LPS induces a status of functional endotoxin tolerance, with significant reduction of TNF-α and IL-6 production following a second LPS stimulation results. Interestingly in patients with AAU TLR4 activation is followed by a significant reduction of IL-6 and IFN-γ production, whereas TLR2 stimulation results in a significant increase of IL-1β and TNF-α.
Genotype analysis of TLR2 and TLR4 gene polymorphisms have been found to be associated with other autoimmune disorders such as RhA; however, to date no associations have been detected in patients with AAU, suggesting that mutations in the TLRs genes might not have a

role in the pathogenesis of AU, but they provide strong evidence of the role of microbial organisms in the pathogenesis of AAU through TLRs.

Genes involved in the adaptive immune response include HLA class I molecules. They play an important role in the pathogenesis of immune-mediated disorders. The expression of HLA antigens is upregulated in uveal tissue in patients with uveitis and correlates with levels of IFN-γ in AqH. The strong association between the HLA-B27 antigen and inflammatory conditions such as AS, and AAU is well known; additionally the discovery of different HLA-B27 subtypes determined by alterations in the antigen-binding region supports the hypothesis that AU may be a consequence of self-reactive cytotoxic T lymphocytes activated via molecular mimicry against local peptides. However despite 45 to 70% of patients with AAU being HLA-B27 positive compared to approximately 5% of the general Caucasian population, the lifetime cumulative incidence in healthy individuals to develop HLA-B27 associated AAU is only of 1% (Chang et al. 2005), implying that other genetic or environmental factors are involved in the development of AAU.

Furthermore, there is also evidence from family studies and genome wide screening that other genes besides the HLA-B27 within and outside the MHC that play a role in disease susceptibility. One of them is the MHC class I chain-related gene A (MICA). Despite MICA allele 4 being in LD with HLA-B27, its frequency is significantly higher in HLAB27 negative patients with AAU suggesting that the MICA itself or another nearby gene to be involved in the development of AAU.

Cytokines are known to regulate cell proliferation and cell differentiation phenotype. They are both pleiotropic and redundant, working in a network that initiates gene activation and suppression. They have also shown to be highly polymorphic, and these polymorphisms can be represented by SNPs, VNTRs, and microsatellites. The majority of them are found in the promoter, intronic or 3' untranslated regions, and although the role of polymorphisms may vary, it is through functional SNPs in the promoter regions that ultimately transcription regulatory molecules such as NFκβ and other genes involved in signal transduction pathways may be disrupted leading to disease associations or disease outcomes.

The cytokine and chemokine genes chosen in this thesis have been implicated in the development of uveitis, or have been used as marker of intraocular inflammation. They also have been shown to influence disease susceptibility or play a role in the development of the various clinical outcomes in different autoimmune disorders, some of them closely related to uveitis.

In order to determine whether specific cytokine gene polymorphisms were associated with the development of uveitis and different clinical phenotypes we investigated SNPs in the promoter region since they are potentially functional sites influencing gene expression.

7.1. LIMITATIONS

Even when genetic analysis reveals a possible association between a cytokine gene polymorphism and a disease, it may still be difficult to fully establish its relationship with the pathogenesis of such condition. Establishing the relationship between the disease associated genotype and cytokine gene expression both at the mRNA using quantitative PCR technology or protein levels might be useful, although these methods are limited by small sample size, especially for rare cytokine genotypes. Furthermore the genotype might not correlate with cytokine levels due to other confounding factors.

In our studies we did not measure systemic or aqueous concentrations of any of these cytokines since it would be difficult to correlate their levels at any single time point of the disease due to the different disease duration, age, duration of the current episode, and interfering factors, such as the natural history of the disease and therapeutic interventions, and the heterogeneous associated group of diseases.

It might be too simplistic to try to consider the level of expression of a cytokine in isolation and they should be analysed in the context of their network and activation pathway. Similarly cytokine genes are up and down-regulated by their interaction with transcription factors and other regulatory molecules, many of which can be altered by other environmental interactions, such as infection.

The findings presented here demonstrate that cytokine gene polymorphisms have an unquestionable role in the orchestration of the immune response, which ultimately influence individual disease susceptibility and outcome.

Even though associations between cytokine and chemokine polymorphisms and different subtypes of AU in a Caucasian cohort of patients have been performed, it is clear that in order to obtain a better understanding of the genetic mechanisms involved in the pathogenesis it is necessary to consider that heterogeneity of this group of disorders, the occurrence of specific combinations of functional polymorphisms, the presence of LD with other biologically significant polymorphisms and considering the complex nature of the pathogenesis of uveitis, it is unlikely that only genetic aspects will influence the establishment or progression of intraocular inflammation.

However the identification of a clear genetic susceptibility profile is a powerful tool for diagnostic and therapeutic intervention.

7.2. FUTURE DIRECTIONS

It appears clear from the literature presented above that although there is a great variability in the efficacy of TNF-α blockage in the treatment of inflammatory disease, anti-TNF drugs have a role in controlling intraocular inflammation. This may be in acute disease such as the severe relapsing episodes in Behçet's eye disease, in chronic disease in which macular oedema may improve even after several months, or as a steroid sparer. However, patients show a great variability in their response to TNF-α blockage. Infliximab appears to be the more effective than etanercept, although most series are small and the dose regimen that is best for uveitis may be different from the very regular infusions required for the management of other systemic chronic diseases such as RhA. This is likely to be related to different pathogenic mechanisms involved in intraocular inflammation compared with inflammation elsewhere.

In addition to this, and although serious side effects of these drugs such as reactivation of latent mycobacterium tuberculosis are reported to be infrequent, other major side effects like exacerbation of demyelinating disorders or induction of neutropenia and thrombocytopenia have been described. The development of autoantibodies that are associated with SLE have been observed at different rates depending on the underlying disease (Charles et al. 2000; Ferraro-Peyret et al. 2004; Vermeire et al. 2003), but only in a few cases SLE-like syndrome have been described (Antoni et al. 2002; De Bandt et al. 2005).

Since up to 40% of patients with RhA treated with anti-TNF-α agents are labelled as non-responders (Lipsky et al. 2000), and given the marked difference in efficacy in controlling intraocular inflammation compared to systemic inflammation in patients with JIA (Schmeling & Horneff 2005), it would be useful to identify responders and non-responders to TNF-α inhibitor agents.

Several SNP have been identified in the promoter region of the TNF-α gene (Allen 1999), and these polymorphisms may have a functional significance in modulating the magnitude of the secretory response of this cytokine (Bouma et al. 1996). The effect of TNF-α promoter -308 A/G polymorphism on TNF-α production, disease susceptibility severity in patients with RhA has been extensively studied. Although still controversial, most studies have found that the less common allele A is associated with greater levels of TNF transcription (Bouma et al. 1996; Braun et al. 1996; de Jong et al. 2002; Louis et al. 1998; Rudwaleit et al. 2001)

Several groups have therefore, investigated the relationship between TNF-α -308 genotype and therapeutic response to TNF-α blockers. The first report to test this hypothesis was in patients with RhA and their response to infliximab (Mugnier et al. 2003). They found that patients with the -308 G/G genotype were more likely to respond to infliximab therapy that

these patients with the -308 A/G or A/A genotypes. This could be due to the higher production of TNF-α or increased severity in RhA patients associated with the presence of the -308 A/G or A/A genotypes. However, in their study they could not confirm any of these possibilities.

These results were supported by another study looking at the therapeutic response of etanercept, infliximab and adalimumab in patients with RhA, psoriatic arthropathy and AS. A positive clinical outcome of anti-TNF-α treatment was significantly associated with the -308G/G diplotype irrespective of their disease; whereas patients with RhA carrying the -308 A/A diplotype did not respond to anti-TNF-α treatment, those carrying the G allele showed a moderate or good response. Similarly, patients with psoriatic arthropathy and AS with the -308 G/G genotype were more likely to respond to anti-TNF-α treatment (Seitz et al. 2007).

Louis et al. analysed the value of CRP and TNF-α serum levels before treatment with infliximab, as well as the TNF -308 gene polymorphism in the prediction of response to infliximab treatment in Crohn's disease (Louis et al. 2002). They found that CRP levels before treatment were significantly higher in responders than in non-responders. Furthermore, response rate was significantly higher in patients with elevated CRP (>5 mg/l) than in patients with a normal CRP value before treatment. A subgroup analysis in their study found a significant association in patients with luminal disease who did not respond to treatment and a higher frequency of the rare allele A. Allelic and genotype frequencies, however for the -308 TNF gene polymorphism were not significantly different between responders and non-responders in either luminal or fistulizing Crohn's disease.

Similarly, Cimaz et al. investigated the genetic contributions of IL-1 and TNF-α gene polymorphisms and the response to treatment with anti-TNF-α inhibitors in patients with JIA (Cimaz et al. 2007). They also failed to find any significant differences for both allelic and genotypic frequencies of IL-1ra (+2018) and TNF-α (-238 and -308) between responders and non-responders in this cohort of patients with JIA.

However, the methodology of these studies is limited by to the study of one or two cytokine, and the complex interaction between different genetic polymorphisms and their clinical response to treatment.

These shortcomings may be minimized by looking at other polymorphic sites within the TNF locus that may predict clinical response to TNF-α blockage. Among 13 genotyped SNPs in a Korean study, seven SNPs, which included -1031T/C, -863C/A, -857C/T, -308G/A, and -238G/A in the TNF-α gene and +177A/G, and +319C/A in the lymphotoxin-α gene were polymorphic and within the Hardy-Weinberg equilibrium. Of these seven TNF SNPs, only the -857C/T SNP in the TNF-α promoter gene was associated with the etanercept response in patients with RhA, according to the American College of Rheumatology (ACR) criteria. More

so, patients who carried the T allele were more likely to respond than those who were homozygous for the C allele (Kang et al. 2005). This may be explained by the differences in the TNF-α gene expression due to allele-specific bindings, such as the transcription factor OCT-1 to the -857T allele, inhibiting the TNF-α gene activity, which in turn may result in less TNF-α production and better response to etanercept therapy (Hohjoh & Tokunaga 2001). These findings correlate to those by van Heel et al., who reported a higher TNF-α production in healthy -857C allele homozygous that in -857T allele carriers (van Heel et al. 2002). But controversy still remains as a Japanese study observed an increased TNF-α production in the carriers of the -857T allele (Soga et al. 2003).

Therefore, another possible way to try to elucidate this controversy would be to combine different SNPs rather than one SNP as a more useful way of predicting responsiveness to anti-TNF-α therapy. In a cohort of 58 patients with RhA looking at TNF and TNF receptor polymorphisms and their response to anti-TNF-α therapy, no independent polymorphism was associated with the patient's response to treatment, but the polymorphisms -857C/T and 489G/A were found to be in LD. In addition, the combination of both TNFR2 polymorphism allele 676T, together with the genotypic association of -857C/489G was associated with good response to infliximab therapy (Chatzikyriakidou et al. 2007).

The methodology used in all the above studies analyzes complex interactions between genetic polymorphisms and clinical response to TNF blockade, however most of these studies have an already selected population, in which patients were receiving treatment, and therefore already had severe disease. In addition to this, it is not always possible to reach universal agreement in defining clinical response depending on the condition treated. Although most previous studies have focused on SNPs in the promoter regions, it is also known that LD is an important factor for the interpretation of genetic data. The TNF-α gene is located within the MHC class III region on chromosome 6. The class III region lies between the class I and class II regions, and it would be therefore possible that other genes within the MHC may contribute towards not only susceptibility, but also response to treatment. Few studies have looked at MHC polymorphisms in relation to anti-TNF-α therapy, but due to the small sample size, the authors did not extrapolate any meaningful conclusion from their study (Martinez et al. 2003). Genetic analysis performed in the majority of the studies published, are performed for a limited number of alleles, mainly those that are considered functionally relevant. Therefore, the cytokine gene polymorphisms investigated consist of only a minority of the existing polymorphisms, and are chosen on the basis of previous reports in their functional and, or clinical significance. However, the associations found in these studies might be a useful tool

for predicting the efficacy of the different biologic agents in refractory intraocular inflammation, and allowing for a targeted choice of agent and treatment regime.

8. REFERENCES

Abi-Hanna D, McCluskey P, Wakefield D. HLA antigens in the iris and aqueous humor gamma interferon levels in anterior uveitis. Invest Ophthalmol Vis Sci 1989; 30 (5):990-4.

Abraham LJ, French MA, Dawkins RL. Polymorphic MHC ancestral haplotypes affect the activity of tumour necrosis factor-alpha. Clin Exp Immunol 1993; 92 (1):14-8.

Adamus G, Machnicki M, Amundson D et al. Similar pattern of MCP-1 expression in spinal cords and eyes of Lewis rats with experimental autoimmune encephalomyelitis associated anterior uveitis. J Neurosci Res 1997; 50 (4):531-8.

Adamus G, Manczak M, Machnicki M. Expression of CC chemokines and their receptors in the eye in autoimmune anterior uveitis associated with EAE. Invest Ophthalmol Vis Sci 2001; 42 (12):2894-903.

Afzali B, Lombardi G, Lechler RI et al. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 2007; 148 (1):32-46.

Agarwal RK, Horai R, Viley AM et al. Abrogation of Anti-Retinal Autoimmunity in IL-10 Transgenic Mice Due to Reduced T Cell Priming and Inhibition of Disease Effector Mechanisms. J Immunol 2008; 180 (8):5423-9.

Aggarwal BB. Structure of tumor necrosis factor and its receptor. Biotherapy 1991; 3 (2):113-20.

Aguilar F, Gonzalez-Escribano MF, Sanchez-Roman J et al. MCP-1 promoter polymorphism in Spanish patients with systemic lupus erythematosus. Tissue Antigens 2001; 58 (5):335-8.

Ahn JK, Park YG, Park SW et al. Combined low dose cyclosporine and prednisone down-regulate natural killer cell-like effector functions of CD8brightCD56+ T cells in patients with active Behcet uveitis. Ocul Immunol Inflamm 2006a; 14 (5):267-75.

Ahn JK, Yu HG, Chung H et al. Intraocular cytokine environment in active Behcet uveitis. Am J Ophthalmol 2006b; 142 (3):429-34.

Akkoc N. Are spondyloarthropathies as common as rheumatoid arthritis worldwide? A review. Curr Rheumatol Rep 2008; 10 (5):371-8.

Allen RD. Polymorphism of the human TNF-alpha promoter--random variation or functional diversity? Mol Immunol 1999; 36 (15-16):1017-27.

Al-Mansour YS, Al-Rajhi AA, Al-Dhibi H et al. Clinical features and prognostic factors in Fuchs' uveitis. Int Ophthalmol.

Amadi-Obi A, Yu C-R, Liu X et al. TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. 2007; 13 (6):711-8.

Ambruzova Z, Mrazek F, Raida L et al. Association of IL6 and CCL2 gene polymorphisms with the outcome of allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant 2009; 44 (4):227-35.

Amor B. Reiter's syndrome. Diagnosis and clinical features. Rheum Dis Clin North Am 1998; 24 (4):677-95, vii.

Amor B, Dougados M, Mijiyawa M. [Criteria of the classification of spondylarthropathies]. Rev Rhum Mal Osteoartic 1990; 57 (2):85-9.

Andus T, Daig R, Vogl D et al. Imbalance of the interleukin 1 system in colonic mucosa--association with intestinal inflammation and interleukin 1 receptor antagonist [corrected] genotype 2. Gut 1997; 41 (5):651-7.

Annunziato F, Cosmi L, Santarlasci V et al. Phenotypic and functional features of human Th17 cells. J Exp Med 2007; 204 (8):1849-61.

Anthony RM, Rutitzky LI, Urban JF et al. Protective immune mechanisms in helminth infection. 2007; 7 (12):975-87.

Antoni C, Braun J. Side effects of anti-TNF therapy: current knowledge. Clin Exp Rheumatol 2002; 20 (6 Suppl 28):S152-7.

Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev 2002; 13 (4-5):323-40.

Armitage RJ. Tumor necrosis factor receptor superfamily members and their ligands. Curr Opin Immunol 1994; 6 (3):407-13.

Balamugesh T, Behera D, Bhatnagar A et al. Inflammatory cytokine levels in induced sputum and bronchoalveolar lavage fluid in pulmonary sarcoidosis. Indian J Chest Dis Allied Sci 2006; 48 (3):177-81.

Barton GM. A calculated response: control of inflammation by the innate immune system. The Journal of Clinical Investigation 2008; 118 (2):413-20.

Bastiaensen LA, Verpalen MC, Pijpers PM et al. Conjunctival sarcoidosis. Doc Ophthalmol 1985; 59 (1):5-9.

Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet 2003; 361 (9363):1111-8.

Bayley JP, de Rooij H, van den Elsen PJ et al. Functional analysis of linker-scan mutants spanning the -376, -308, -244, and -238 polymorphic sites of the TNF-alpha promoter. Cytokine 2001; 14 (6):316-23.

Bayley JP, Ottenhoff TH, Verweij CL. Is there a future for TNF promoter polymorphisms? Genes Immun 2004; 5 (5):315-29.

Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. N Engl J Med 1996; 334 (26):1717-25.

Benitez-del-Castillo JM, Martinez-de-la-Casa JM, Pato-Cour E et al. Long-term treatment of refractory posterior uveitis with anti-TNFalpha (infliximab). Eye 2005; 19 (8):841-5.

Beutler E, Gelbart T, Kuhl W. Interference of heparin with the polymerase chain reaction. Biotechniques 1990; 9 (2):166.

Bhattacherjee P, Henderson B. Inflammatory responses to intraocularly injected interleukin 1. Curr Eye Res 1987; 6 (7):929-34.

Bidwell J, Keen L, Gallagher G et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 1. Genes Immun 2001; 2 (2):61-70.

Biester S, Deuter C, Michels H et al. Adalimumab in the therapy of uveitis in childhood. Br J Ophthalmol 2007; 91 (3):319-24.

Bioque G, Crusius JB, Koutroubakis I et al. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. Clin Exp Immunol 1995; 102 (2):379-83.

Birnbaum AD, Tessler HH, Schultz KL et al. Epidemiologic relationship between fuchs heterochromic iridocyclitis and the United States rubella vaccination program. Am J Ophthalmol 2007; 144 (3):424-8.

Bjorkman PJ, Parham P. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu Rev Biochem 1990; 59:253-88.

Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. Bmj 1995; 310 (6973):170.

Bloch-Michel E, Nussenblatt RB. International Uveitis Study Group recommendations for the evaluation of intraocular inflammatory disease. Am J Ophthalmol 1987; 103 (2):234-5.

Bonfioli AA, Curi AL, Orefice F. Fuchs' heterochromic cyclitis. Semin Ophthalmol 2005; 20 (3):143-6.

Bouma G, Crusius JB, Oudkerk Pool M et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scand J Immunol 1996; 43 (4):456-63.

Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). Oncogene 2001; 20 (44):6482-91.

Brand S, Staudinger T, Schnitzler F et al. The role of Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype of Crohn's disease. Inflamm Bowel Dis 2005; 11 (7):645-52.

Braun J, Baraliakos X, Listing J et al. Decreased incidence of anterior uveitis in patients with ankylosing spondylitis treated with the anti-tumor necrosis factor agents infliximab and etanercept. Arthritis Rheum 2005; 52 (8):2447-51.

Braun N, Michel U, Ernst BP et al. Gene polymorphism at position -308 of the tumor-necrosis-factor-alpha (TNF-alpha) in multiple sclerosis and it's influence on the regulation of TNF-alpha production. Neurosci Lett 1996; 215 (2):75-8.

Brennan FM, Maini RN, Feldmann M. TNF alpha--a pivotal role in rheumatoid arthritis? Br J Rheumatol 1992; 31 (5):293-8.

Bresnihan B, Alvaro-Gracia JM, Cobby M et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998; 41 (12):2196-204.

Brewerton DA, Caffrey M, Nicholls A et al. Acute anterior uveitis and HL-A 27. Lancet 1973; 2 (7836):994-6.

Brinkman BM, Huizinga TW, Kurban SS et al. Tumour necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? Br J Rheumatol 1997; 36 (5):516-21.

Brito BE, O'Rourke LM, Pan Y et al. IL-1 and TNF receptor-deficient mice show decreased inflammation in an immune complex model of uveitis. Invest Ophthalmol Vis Sci 1999; 40 (11):2583-9.

Brito BE, Zamora DO, Bonnah RA et al. Toll-like receptor 4 and CD14 expression in human ciliary body and TLR-4 in human iris endothelial cells. Exp Eye Res 2004; 79 (2):203-8.

Brockmeyer NH, Willers CP, Anders S et al. Cytokine profile of HIV-positive Kaposi's sarcoma derived cells in vitro. Eur J Med Res 1999; 4 (3):95-100.

Brown KD, Claudio E, Siebenlist U. The roles of the classical and alternative nuclear factor-kappaB pathways: potential implications for autoimmunity and rheumatoid arthritis. Arthritis Res Ther 2008; 10 (4):212.

Brown KS, Nackos E, Morthala S et al. Monocyte chemoattractant protein-1: plasma concentrations and A(-2518)G promoter polymorphism of its gene in systemic lupus erythematosus. J Rheumatol 2007; 34 (4):740-6.

Brull DJ, Montgomery HE, Sanders J et al. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001; 21 (9):1458-63.

Brusko TM, Bluestone JA. Regulatory T cells directed to the site of the action. Proc Natl Acad Sci U S A 2009.

Bunce M, O'Neill CM, Barnardo MC et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 1995; 46 (5):355-67.

Cabrera M, Shaw MA, Sharples C et al. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J Exp Med 1995; 182 (5):1259-64.

Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68 (12):7010-7.

Carnahan MC, Goldstein DA. Ocular complications of topical, peri-ocular, and systemic corticosteroids. Curr Opin Ophthalmol 2000; 11 (6):478-83.

Carter MJ, di Giovine FS, Jones S et al. Association of the interleukin 1 receptor antagonist gene with ulcerative colitis in Northern European Caucasians. Gut 2001; 48 (4):461-7.

Castell JV, Gomez-Lechon MJ, David M et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 1989; 242 (2):237-9.

Castro-Santos P, Suarez A, Lopez-Rivas L et al. TNFalpha and IL-10 gene polymorphisms in inflammatory bowel disease. Association of -1082 AA low producer IL-10 genotype with steroid dependency. Am J Gastroenterol 2006; 101 (5):1039-47.

Chan CC, Detrick B, Nussenblatt RB et al. HLA-DR antigens on retinal pigment epithelial cells from patients with uveitis. Arch Ophthalmol 1986; 104 (5):725-9.

Chang JH, Hampartzoumian T, Everett B et al. Changes in Toll-like receptor (TLR)-2 and TLR4 expression and function but not polymorphisms are associated with acute anterior uveitis. Invest Ophthalmol Vis Sci 2007; 48 (4):1711-7.

Chang JH, McCluskey P, Wakefield D. Expression of toll-like receptor 4 and its associated lipopolysaccharide receptor complex by resident antigen-presenting cells in the human uvea. Invest Ophthalmol Vis Sci 2004; 45 (6):1871-8.

Chang JH, McCluskey PJ, Wakefield D. Acute anterior uveitis and HLA-B27. Surv Ophthalmol 2005; 50 (4):364-88.

Charles PJ, Smeenk RJ, De Jong J et al. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum 2000; 43 (11):2383-90.

Charo IF, Myers SJ, Herman A et al. Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. Proc Natl Acad Sci U S A 1994; 91 (7):2752-6.

Chatzikyriakidou A, Georgiou I, Voulgari PV et al. Combined tumour necrosis factor-alpha and tumour necrosis factor receptor genotypes could predict rheumatoid arthritis patients' response to anti-TNF-alpha therapy and explain controversies of studies based on a single polymorphism. Rheumatology (Oxford) 2007; 46 (6):1034-5.

Chen L, Yang P, Zhou H et al. Diminished frequency and function of CD4+CD25high regulatory T cells associated with active uveitis in Vogt-Koyanagi-Harada syndrome. Invest Ophthalmol Vis Sci 2008; 49 (8):3475-82.

Chen Y, Vaughan RW, Kondeatis E et al. Chemokine gene polymorphisms associate with gender in patients with uveitis. Tissue Antigens 2004; 63 (1):41-5.

Cho ML, Kim JY, Ko HJ et al. The MCP-1 promoter -2518 polymorphism in Behcet's disease: correlation between allele types, MCP-1 production and clinical symptoms among Korean patients. Autoimmunity 2004; 37 (1):77-80.

Cimaz R, Cazalis MA, Reynaud C et al. IL1 and TNF gene polymorphisms in patients with juvenile idiopathic arthritis treated with TNF inhibitors. Ann Rheum Dis 2007; 66 (7):900-4.

Clay FE, Tarlow JK, Cork MJ et al. Novel interleukin-1 receptor antagonist exon polymorphisms and their use in allele-specific mRNA assessment. Hum Genet 1996; 97 (6):723-6.

Collantes E, Zarco P, Munoz E et al. Disease pattern of spondyloarthropathies in Spain: description of the first national registry (REGISPONSER) extended report. Rheumatology (Oxford) 2007; 46 (8):1309-15.

Colobran R, Pujol-Borrell R, Armengol MP et al. The chemokine network. II. On how polymorphisms and alternative splicing increase the number of molecular species and configure intricate patterns of disease susceptibility. Clin Exp Immunol 2007; 150 (1):1-12.

Colotta F, Dower SK, Sims JE et al. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol Today 1994; 15 (12):562-6.

Combadiere C, Ahuja SK, Van Damme J et al. Monocyte chemoattractant protein-3 is a functional ligand for CC chemokine receptors 1 and 2B. J Biol Chem 1995; 270 (50):29671-5.

Conlon K, Lloyd A, Chattopadhyay U et al. CD8+ and CD45RA+ human peripheral blood lymphocytes are potent sources of macrophage inflammatory protein 1 alpha, interleukin-8 and RANTES. Eur J Immunol 1995; 25 (3):751-6.

Cox A, Camp NJ, Nicklin MJ et al. An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. Am J Hum Genet 1998; 62 (5):1180-8.

Crawley E, Kay R, Sillibourne J et al. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999; 42 (6):1101-8.

Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease. Lancet 1990; 335 (8697):1078-80.

Cross DS, Ivacic LC, Stefanski EL et al. Population based allele frequencies of disease associated polymorphisms in the Personalized Medicine Research Project. BMC Genet 2010; 11 (1):51.

Curnow SJ, Falciani F, Durrani OM et al. Multiplex bead immunoassay analysis of aqueous humor reveals distinct cytokine profiles in uveitis. Invest Ophthalmol Vis Sci 2005; 46 (11):4251-9.

D'Alfonso S, Richiardi PM. A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics 1994; 39 (2):150-4.

D'Ambrosio D, Mariani M, Panina-Bordignon P et al. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am J Respir Crit Care Med 2001; 164 (7):1266-75.

Danis VA, Millington M, Hyland VJ et al. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995; 99 (2):303-10.

Date Y, Seki N, Kamizono S et al. Identification of a genetic risk factor for systemic juvenile rheumatoid arthritis in the 5'-flanking region of the TNFalpha gene and HLA genes. Arthritis Rheum 1999; 42 (12):2577-82.

De Bandt M, Sibilia J, Le Loet X et al. Systemic lupus erythematosus induced by anti-tumour necrosis factor alpha therapy: a French national survey. Arthritis Res Ther 2005; 7 (3):R545-51.

de Boer JH, Hack CE, Verhoeven AJ et al. Chemoattractant and neutrophil degranulation activities related to interleukin-8 in vitreous fluid in uveitis and vitreoretinal disorders. Invest Ophthalmol Vis Sci 1993; 34 (12):3376-85.

de Jong BA, Westendorp RG, Bakker AM et al. Polymorphisms in or near tumour necrosis factor (TNF)-gene do not determine levels of endotoxin-induced TNF production. Genes Immun 2002; 3 (1):25-9.

De Vos AF, Hoekzema R, Kijlstra A. Cytokines and uveitis, a review. Curr Eye Res 1992; 11 (6):581-97.

Derhaag PJ, de Waal LP, Linssen A et al. Acute anterior uveitis and HLA-B27 subtypes. Invest Ophthalmol Vis Sci 1988; 29 (7):1137-40.

Derhaag PJ, van der Horst AR, de Waal LP et al. HLA-B27+ acute anterior uveitis and other antigens of the major histocompatibility complex. Invest Ophthalmol Vis Sci 1989; 30 (10):2160-4.

Deschenes J, Murray PI, Rao NA et al. International Uveitis Study Group (IUSG): clinical classification of uveitis. Ocul Immunol Inflamm 2008; 16 (1):1-2.

Deshmane SL, Kremlev S, Amini S et al. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009; 29 (6):313-26.

Deuter CM, Kotter I, Wallace GR et al. Behcet's disease: ocular effects and treatment. Prog Retin Eye Res 2008; 27 (1):111-36.

Dick AD, Duncan L, Hale G et al. Neutralizing TNF-alpha activity modulates T-cell phenotype and function in experimental autoimmune uveoretinitis. J Autoimmun 1998; 11 (3):255-64.

Dinarello CA. Blocking IL-1 in systemic inflammation. J Exp Med 2005; 201 (9):1355-9.

Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991; 77 (8):1627-52.

Dinarello CA, Wolff SM. The role of interleukin-1 in disease. N Engl J Med 1993; 328 (2):106-13.

Dohlman HG, Thorner J, Caron MG et al. Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 1991; 60:653-88.

Doranz BJ, Rucker J, Yi Y et al. A Dual-Tropic Primary HIV-1 Isolate That Uses Fusin and the ²-Chemokine Receptors CKR-5, CKR-3, and CKR-2b as Fusion Cofactors. 1996; 85 (7):1149-58.

Dougados M, van der Linden S, Juhlin R et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991; 34 (10):1218-27.

Dripps DJ, Brandhuber BJ, Thompson RC et al. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J Biol Chem 1991; 266 (16):10331-6.

Du L, Kijlstra A, Yang P. Immune response genes in uveitis. Ocul Immunol Inflamm 2009; 17 (4):249-56.

Dumoutier L, Lejeune D, Colau D et al. Cloning and Characterization of IL-22 Binding Protein, a Natural Antagonist of IL-10-Related T Cell-Derived Inducible Factor/IL-22. J Immunol 2001; 166 (12):7090-5.

Durmazlar SPK, Ulkar GB, Eskioglu F et al. Significance of serum interleukin-8 levels in patients with Behcet's disease: high levels may indicate vascular involvement. International Journal of Dermatology 2009; 48 (3):259-64.

Durrani K, Foster CS. Psoriatic uveitis: a distinct clinical entity? Am J Ophthalmol 2005; 139 (1):106-11.

Economou M, Trikalinos TA, Loizou KT et al. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. Am J Gastroenterol 2004; 99 (12):2393-404.

Eisenberg SP, Evans RJ, Arend WP et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 1990; 343 (6256):341-6.

Elahi MM, Asotra K, Matata BM et al. Tumor necrosis factor alpha - 308 gene locus promoter polymorphism: An analysis of association with health and disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 2009; 1792 (3):163-72.

El-Shabrawi Y, Hermann J. Anti-tumor necrosis factor-alpha therapy with infliximab as an alternative to corticosteroids in the treatment of human leukocyte antigen B27-associated acute anterior uveitis. Ophthalmology 2002; 109 (12):2342-6.

el-Shabrawi Y, Livir-Rallatos C, Christen W et al. High levels of interleukin-12 in the aqueous humor and vitreous of patients with uveitis. Ophthalmology 1998; 105 (9):1659-63.

El-Shabrawi Y, Wegscheider BJ, Weger M et al. Polymorphisms within the tumor necrosis factor-alpha promoter region in patients with HLA-B27-associated uveitis: association with susceptibility and clinical manifestations. Ophthalmology 2006; 113 (4):695-700.

El-Shabrawi YG, Christen WG, Foster SC. Correlation of metalloproteinase-2 and -9 with proinflammatory cytokines interleukin-1b, interleukin-12 and the interleukin-1 receptor antagonist in patients with chronic uveitis. Curr Eye Res 2000; 20 (3):211-4.

Eskdale J, Gallagher G. A polymorphic dinucleotide repeat in the human IL-10 promoter. Immunogenetics 1995; 42 (5):444-5.

Eskdale J, Kube D, Gallagher G. A second polymorphic dinucleotide repeat in the 5' flanking region of the human IL10 gene. Immunogenetics 1996; 45 (1):82-3.

Eugenin EA, Berman JW. Chemokine-dependent mechanisms of leukocyte trafficking across a model of the blood-brain barrier. Methods 2003; 29 (4):351-61.

Fang IM, Yang CH, Lin CP et al. Expression of chemokine and receptors in Lewis rats with experimental autoimmune anterior uveitis. Exp Eye Res 2004; 78 (6):1043-55.

Fanning GC, Bunce M, Black CM et al. Polymerase chain reaction haplotyping using 3' mismatches in the forward and reverse primers: application to the biallelic polymorphisms of tumor necrosis factor and lymphotoxin alpha. Tissue Antigens 1997; 50 (1):23-31.

Fantini MC, Rizzo A, Fina D et al. IL-21 regulates experimental colitis by modulating the balance between T and Th17 cells. European Journal of Immunology 2007; 37 (11):3155-63.

Feltkamp TE, Ringrose JH. Acute anterior uveitis and spondyloarthropathies. Curr Opin Rheumatol 1998; 10 (4):314-8.

Ferraro-Peyret C, Coury F, Tebib JG et al. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two-year prospective study. Arthritis Res Ther 2004; 6 (6):R535-43.

Ferrick MR, Thurau SR, Oppenheim MH et al. Ocular inflammation stimulated by intravitreal interleukin-8 and interleukin-1. Invest Ophthalmol Vis Sci 1991; 32 (5):1534-9.

Fishman D, Faulds G, Jeffery R et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998; 102 (7):1369-76.

Fong CH, Bebien M, Didierlaurent A et al. An antiinflammatory role for IKKbeta through the inhibition of "classical" macrophage activation. J Exp Med 2008; 205 (6):1269-76.

Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; 4 (4):330-6.

Foster CB, Lehrnbecher T, Samuels S et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 2000; 96 (7):2562-7.

Foxman EF, Zhang M, Hurst SD et al. Inflammatory mediators in uveitis: differential induction of cytokines and chemokines in Th1- versus Th2-mediated ocular inflammation. J Immunol 2002; 168 (5):2483-92.

Frade J, Mellado M, del Real G et al. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. J Immunol 1997; 159 (11):5576-84.

Fraile A, Nieto A, Beraun Y et al. Tumor necrosis factor gene polymorphisms in ankylosing spondylitis. Tissue Antigens 1998; 51 (4 Pt 1):386-90.

Franci C, Wong LM, Van Damme J et al. Monocyte chemoattractant protein-3, but not monocyte chemoattractant protein-2, is a functional ligand of the human monocyte chemoattractant protein-1 receptor. J Immunol 1995; 154 (12):6511-7.

Franks WA, Limb GA, Stanford MR et al. Cytokines in human intraocular inflammation. Curr Eye Res 1992; 11 Suppl:187-91.

Fujihara J, Shiwaku K, Yasuda T et al. Variation of interleukin 8 -251 A>T polymorphism in worldwide populations and intra-ethnic differences in Japanese populations. Clin Chim Acta 2007; 377 (1-2):79-82.

Fujino S, Andoh A, Bamba S et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003; 52 (1):65-70.

Gazouli M, Mantzaris G, Kotsinas A et al. Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. World J Gastroenterol 2005; 11 (5):681-5.

Gladman DD, Antoni C, Mease P et al. Psoriatic arthritis: epidemiology, clinical features, course, and outcome. Ann Rheum Dis 2005; 64 Suppl 2:ii14-7.

Gladman DD, Farewell VT. HLA studies in psoriatic arthritis: current situation and future needs. J Rheumatol 2003; 30 (1):4-6.

Gonzalez E, Rovin BH, Sen L et al. HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. Proc Natl Acad Sci U S A 2002; 99 (21):13795-800.

Gonzalez S, Torre-Alonso JC, Martinez-Borra J et al. TNF-238A promoter polymorphism contributes to susceptibility to ankylosing spondylitis in HLA-B27 negative patients. J Rheumatol 2001; 28 (6):1288-93.

Gonzalez-Escribano MF, Torres B, Aguilar F et al. MCP-1 promoter polymorphism in Spanish patients with rheumatoid arthritis. Hum Immunol 2003; 64 (7):741-4.

Graziano F, Ruzzo A, Canestrari E et al. Variations in the interleukin-1 receptor antagonist gene impact on survival of patients with advanced colorectal cancer. Pharmacogenomics J 2009; 9 (1):78-84.

Grimm MC, Elsbury SK, Pavli P et al. Enhanced expression and production of monocyte chemoattractant protein-1 in inflammatory bowel disease mucosa. J Leukoc Biol 1996; 59 (6):804-12.

Grove J, Daly AK, Bassendine MF et al. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology 1997; 26 (1):143-6.

Grutters JC, Sato H, Pantelidis P et al. Increased frequency of the uncommon tumor necrosis factor -857T allele in British and Dutch patients with sarcoidosis. Am J Respir Crit Care Med 2002; 165 (8):1119-24.

Guignard S, Gossec L, Salliot C et al. Efficacy of tumour necrosis factor blockers in reducing uveitis flares in patients with spondylarthropathy: a retrospective study. Ann Rheum Dis 2006; 65 (12):1631-4.

Hacking D, Knight JC, Rockett K et al. Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility. Genes Immun 2004; 5 (4):274-82.

Hajeer AH, Hutchinson IV. Influence of TNFalpha gene polymorphisms on TNFalpha production and disease. Hum Immunol 2001; 62 (11):1191-9.

Hajeer AH, Hutchinson IV. TNF-alpha gene polymorphism: clinical and biological implications. Microsc Res Tech 2000; 50 (3):216-28.

Hamideh F, Prete PE. Ophthalmologic manifestations of rheumatic diseases. Semin Arthritis Rheum 2001; 30 (4):217-41.

Hamzaoui K, Hamzaoui A, Guemira F et al. Cytokine profile in Behcet's disease patients. Relationship with disease activity. Scand J Rheumatol 2002; 31 (4):205-10.

Hamzaoui K, Hamzaoui A, Houman H. CD4+CD25+ regulatory T cells in patients with Behcet's disease. Clin Exp Rheumatol 2006; 24 (5 Suppl 42):S71-8.

Hannum CH, Wilcox CJ, Arend WP et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990; 343 (6256):336-40.

Haribabu B, Zhelev DV, Pridgen BC et al. Chemoattractant receptors activate distinct pathways for chemotaxis and secretion. Role of G-protein usage. J Biol Chem 1999; 274 (52):37087-92.

Hayashi S, Guex-Crosier Y, Delvaux A et al. Interleukin 10 inhibits inflammatory cells infiltration in endotoxin-induced uveitis. Graefes Arch Clin Exp Ophthalmol 1996; 234 (10):633-6.

Heid CA, Stevens J, Livak KJ et al. Real time quantitative PCR. Genome Res 1996; 6 (10):986-94.

Heinzmann A, Ahlert I, Kurz T et al. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. J Allergy Clin Immunol 2004; 114 (3):671-6.

Herbort CP, Rao NA, Mochizuki M et al. International Criteria for the Diagnosis of Ocular Sarcoidosis: Results of the First International Workshop on Ocular Sarcoidosis (IWOS). Ocular Immunology and Inflammation 2009; 17 (3):160-9.

Herrmann, Ricard, Nicaud et al. Polymorphisms of the tumour necrosis factor-alpha gene, coronary heart disease and obesity. European Journal of Clinical Investigation 1998; 28 (1):59-66.

Higuchi T, Seki N, Kamizono S et al. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 1998; 51 (6):605-12.

Hill T, Galatowicz G, Akerele T et al. Intracellular T lymphocyte cytokine profiles in the aqueous humour of patients with uveitis and correlation with clinical phenotype. Clinical & Experimental Immunology 2005; 139 (1):132-7.

Hirano T, Matsuda T, Turner M et al. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol 1988; 18 (11):1797-801.

Hirschfeld M, Weis JJ, Toshchakov V et al. Signaling by Toll-Like Receptor 2 and 4 Agonists Results in Differential Gene Expression in Murine Macrophages. Infect. Immun. 2001; 69 (3):1477-82.

Hizawa N, Yamaguchi E, Furuya K et al. The role of the C-C chemokine receptor 2 gene polymorphism V64I (CCR2-64I) in sarcoidosis in a Japanese population. Am J Respir Crit Care Med 1999; 159 (6):2021-3.

Hoffmann SC, Stanley EM, Cox ED et al. Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant 2002; 2 (6):560-7.

Hohjoh H, Tokunaga K. Allele-specific binding of the ubiquitous transcription factor OCT-1 to the functional single nucleotide polymorphism (SNP) sites in the tumor necrosis factor-alpha gene (TNFA) promoter. Genes Immun 2001; 2 (2):105-9.

Horiuchi T, Mitoma H, Harashima S et al. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. Rheumatology (Oxford) 2010; 49 (7):1215-28.

Huang H-Y, Thuita L, Strickland P et al. Frequencies of single nucleotide polymorphisms in genes regulating inflammatory responses in a community-based population. BMC Genetics 2007; 8 (1):7.

Huhtinen M, Karma A. HLA-B27 typing in the categorisation of uveitis in a HLA-B27 rich population. Br J Ophthalmol 2000; 84 (4):413-6.

Huhtinen M, Repo H, Laasila K et al. Systemic inflammation and innate immune response in patients with previous anterior uveitis. Br J Ophthalmol 2002; 86 (4):412-7.

Hui L, DelMonte T, Ranade K. Genotyping using the TaqMan assay. Curr Protoc Hum Genet 2008; Chapter 2:Unit 2 10.

Huizinga TW, Keijsers V, Yanni G et al. Are differences in interleukin 10 production associated with joint damage? Rheumatology (Oxford) 2000; 39 (11):1180-8.

Huizinga TW, Westendorp RG, Bollen EL et al. TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. J Neuroimmunol 1997; 72 (2):149-53.

Hulkkonen J, Pertovaara M, Antonen J et al. Genetic association between interleukin-10 promoter region polymorphisms and primary Sjogren's syndrome. Arthritis Rheum 2001a; 44 (1):176-9.

Hulkkonen J, Pertovaara M, Antonen J et al. Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. Rheumatology (Oxford) 2001b; 40 (6):656-61.

Hull J, Ackerman H, Isles K et al. Unusual Haplotypic Structure of IL8, a Susceptibility Locus for a Common Respiratory Virus. 2001; 69 (2):413-9.

Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000; 55 (12):1023-7.

Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 1998; 28 (8):2598-602.

Huth C, Illig T, Herder C et al. Joint analysis of individual participants' data from 17 studies on the association of the IL6 variant -174G>C with circulating glucose levels, interleukin-6 levels, and body mass index. Ann Med 2009; 41 (2):128-38.

Hwang SY, Cho ML, Park B et al. Allelic frequency of the MCP-1 promoter -2518 polymorphism in the Korean population and in Korean patients with rheumatoid arthritis, systemic lupus erythematosus and adult-onset Still's disease. Eur J Immunogenet 2002; 29 (5):413-6.

Ikeuchi H, Kuroiwa T, Hiramatsu N et al. Expression of interleukin-22 in rheumatoid arthritis: Potential role as a proinflammatory cytokine. Arthritis & Rheumatism 2005; 52 (4):1037-46.

Inoue J, Ishida T, Tsukamoto N et al. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. Exp Cell Res 2000; 254 (1):14-24.

Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002a; 13 (4-5):357-68.

Ishihara K, Hirano T. Molecular basis of the cell specificity of cytokine action. Biochim Biophys Acta 2002b; 1592 (3):281-96.

Ishizu T, Osoegawa M, Mei FJ et al. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain 2005; 128 (Pt 5):988-1002.

Jabs DA, Johns CJ. Ocular involvement in chronic sarcoidosis. Am J Ophthalmol 1986; 102 (3):297-301.

Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol 2005; 140 (3):509-16.

Jacob CO, Fronek Z, Lewis GD et al. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to systemic lupus erythematosus. Proc Natl Acad Sci U S A 1990; 87 (3):1233-7.

Jahromi MM, Millward BA, Demaine AG. A polymorphism in the promoter region of the gene for interleukin-6 is associated with susceptibility to type 1 diabetes mellitus. J Interferon Cytokine Res 2000; 20 (10):885-8.

Jakeman CM, Jordan K, Keast-Butler J et al. Cataract surgery with intraocular lens implantation in Fuchs' heterochromic cyclitis. Eye 1990; 4 (Pt 4):543-7.

James DG. Epidemiology of sarcoidosis. Sarcoidosis 1992; 9 (2):79-87.

Jang WC, Nam YH, Ahn YC et al. Interleukin-17F gene polymorphisms in Korean patients with Behcet's disease. Rheumatol Int 2008; 29 (2):173-8.

Jee Y, Yoon WK, Okura Y et al. Upregulation of monocyte chemotactic protein-1 and CC chemokine receptor 2 in the central nervous system is closely associated with relapse of autoimmune encephalomyelitis in Lewis rats. J Neuroimmunol 2002; 128 (1-2):49-57.

Jiang Y, Genant HK, Watt I et al. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. Arthritis Rheum 2000; 43 (5):1001-9.

Jiang Y, Salafranca MN, Adhikari S et al. Chemokine receptor expression in cultured glia and rat experimental allergic encephalomyelitis. Journal of neuroimmunology 1998; 86 (1):1-12.

Jie Y, Zhang WH, Pan ZQ et al. Interleukin-1 receptor antagonist eye drops promoting high-risk corneal allografts survival in rats. Chin Med J (Engl) 2004; 117 (5):711-6.

Joffre O, Nolte MA, Spörri R et al. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunological Reviews 2009; 227 (1):234-47.

Jones KG, Brull DJ, Brown LC et al. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 2001; 103 (18):2260-5.

Jones NP. Cataract surgery in Fuchs' heterochromic uveitis: past, present, and future. J Cataract Refract Surg 1996; 22 (2):261-8.

Jones NP. Fuchs' Heterochromic Uveitis: a reappraisal of the clinical spectrum. Eye 1991a; 5 (Pt 6):649-61.

Jones NP. Fuchs' heterochromic uveitis: an update. Surv Ophthalmol 1993; 37 (4):253-72.

Jones NP. Glaucoma in Fuchs' Heterochromic Uveitis: aetiology, management and outcome. Eye 1991b; 5 (Pt 6):662-7.

Joseph A, Raj D, Dua HS et al. Infliximab in the treatment of refractory posterior uveitis. Ophthalmology 2003; 110 (7):1449-53.

Kahn P, Weiss M, Imundo LF et al. Favorable response to high-dose infliximab for refractory childhood uveitis. Ophthalmology 2006; 113 (5):860-4 e2.

Kaijzel EL, Bayley JP, van Krugten MV et al. Allele-specific quantification of tumor necrosis factor alpha (TNF) transcription and the role of promoter polymorphisms in rheumatoid arthritis patients and healthy individuals. Genes Immun 2001; 2 (3):135-44.

Kaijzel EL, Brinkman BM, van Krugten MV et al. Polymorphism within the tumor necrosis factor alpha (TNF) promoter region in patients with ankylosing spondylitis. Hum Immunol 1999; 60 (2):140-4.

Kaiser PK, Lowder CY, Sullivan P et al. Chest computerized tomography in the evaluation of uveitis in elderly women. American journal of ophthalmology 2002; 133 (4):499-505.

Kaluza W, Reuss E, Grossmann S et al. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. J Invest Dermatol 2000; 114 (6):1180-3.

Kamizato M, Nishida K, Masuda K et al. Interleukin 10 inhibits interferon gamma- and tumor necrosis factor alpha-stimulated activation of NADPH oxidase 1 in human colonic epithelial cells and the mouse colon. J Gastroenterol 2009; 44 (12):1172-84.

Kang CP, Lee KW, Yoo DH et al. The influence of a polymorphism at position -857 of the tumour necrosis factor alpha gene on clinical response to etanercept therapy in rheumatoid arthritis. Rheumatology (Oxford) 2005; 44 (4):547-52.

Kaplanski G, Marin V, Montero-Julian F et al. IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. Trends Immunol 2003; 24 (1):25-9.

Karin M, Yamamoto Y, Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004; 3 (1):17-26.

Kasner L, Chan C, Whitcup S et al. The paradoxical effect of tumor necrosis factor alpha (TNF-alpha) in endotoxin-induced uveitis. Invest. Ophthalmol. Vis. Sci. 1993; 34 (10):2911-7.

Kataria RK, Brent LH. Spondyloarthropathies. Am Fam Physician 2004; 69 (12):2853-60.

Kavanaugh A, St Clair EW, McCune WJ et al. Chimeric anti-tumor necrosis factor-alpha monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. J Rheumatol 2000; 27 (4):841-50.

Kawaguchi M, Adachi M, Oda N et al. IL-17 cytokine family. The Journal of allergy and clinical immunology 2004; 114 (6):1265-73.

Kawaguchi T, Hanada A, Horie S et al. Evaluation of Characteristic Ocular Signs and Systemic Investigations in Ocular Sarcoidosis Patients. Japanese Journal of Ophthalmology 2007; 51 (2):121-6.

Keino H, Takeuchi M, Kezuka T et al. Chemokine and chemokine receptor expression during experimental autoimmune uveoretinitis in mice. Graefes Arch Clin Exp Ophthalmol 2003; 241 (2):111-5.

Kempeni J. Preliminary results of early clinical trials with the fully human anti-TNFalpha monoclonal antibody D2E7. Ann Rheum Dis 1999; 58 Suppl 1:I70-2.

Kempeni J. Update on D2E7: a fully human anti-tumour necrosis factor alpha monoclonal antibody. Ann Rheum Dis 2000; 59 Suppl 1:i44-5.

Khan MA. Update: the twenty subtypes of HLA-B27. Curr Opin Rheumatol 2000; 12 (4):235-8.

Kilpinen S, Hulkkonen J, Wang XY et al. The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults. Eur Cytokine Netw 2001; 12 (1):62-8.

Kim HA, Choi KW, Song YW. Arthropathy in Behcet's disease. Scand J Rheumatol 1997; 26 (2):125-9.

Kim JM, Brannan CI, Copeland NG et al. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J Immunol 1992; 148 (11):3618-23.

Kimura A, Naka T, Kishimoto T. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. Proc Natl Acad Sci U S A 2007; 104 (29):12099-104.

Kirkham BW, Lassere MN, Edmonds JP et al. Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: A two-year prospective study (the DAMAGE study cohort). Arthritis & Rheumatism 2006; 54 (4):1122-31.

Kishimoto T. Interleukin-6: discovery of a pleiotropic cytokine. Arthritis Res Ther 2006; 8 Suppl 2:S2.

Kiss S, Letko E, Qamruddin S et al. Long-term progression, prognosis, and treatment of patients with recurrent ocular manifestations of Reiter's syndrome. Ophthalmology 2003; 110 (9):1764-9.

Klein J, Sato A. The HLA system. First of two parts. N Engl J Med 2000; 343 (10):702-9.

Klein W, Tromm A, Griga T et al. The polymorphism at position -174 of the IL-6 gene is not associated with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2001; 13 (1):45-7.

Knight JC, Udalova I, Hill AV et al. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. Nat Genet 1999; 22 (2):145-50.

Koerner SK, Sakowitz AJ, Appelman RI et al. Transbronchinal lung biopsy for the diagnosis of sarcoidosis. N Engl J Med 1975; 293 (6):268-70.

Kontogiannis V, Powell RJ. Behcet's disease. Postgrad Med J 2000; 76 (900):629-37.

Korendowych E, McHugh N. Genetic factors in psoriatic arthritis. Curr Rheumatol Rep 2005; 7 (4):306-12.

Korn T, Bettelli E, Gao W et al. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. 2007; 448 (7152):484-7.

Kramer M, Monselise Y, Bahar I et al. Serum cytokine levels in active uveitis and remission. Curr Eye Res 2007; 32 (7-8):669-75.

Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997; 34 (5):391-9.

Kroeger KM, Steer JH, Joyce DA et al. Effects of stimulus and cell type on the expression of the -308 tumour necrosis factor promoter polymorphism. Cytokine 2000; 12 (2):110-9.

Kroner A, Maurer M, Loserth S et al. Analysis of the monocyte chemoattractant protein 1 -2518 promoter polymorphism in patients with multiple sclerosis. Tissue Antigens 2004; 64 (1):70-3.

Kruttgen A, Rose-John S, Dufhues G et al. The three carboxy-terminal amino acids of human interleukin-6 are essential for its biological activity. FEBS Lett 1990; 273 (1-2):95-8.

Kuhn R, Lohler J, Rennick D et al. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993; 75 (2):263-74.

Kuwano Y, Tominaga K, Kawahara T et al. Tumor necrosis factor [alpha] activates transcription of the NADPH oxidase organizer 1 (NOXO1) gene and upregulates superoxide production in colon epithelial cells. Free Radical Biology and Medicine 2008; 45 (12):1642-52.

La Hey E, Rothova A, Baarsma GS et al. Fuchs' heterochromic iridocyclitis is not associated with ocular toxoplasmosis. Arch Ophthalmol 1992; 110 (6):806-11.

Lahdenne P, Vahasalo P, Honkanen V. Infliximab or etanercept in the treatment of children with refractory juvenile idiopathic arthritis: an open label study. Ann Rheum Dis 2003; 62 (3):245-7.

Lawrence T, Fong C. The resolution of inflammation: anti-inflammatory roles for NF-kappaB. Int J Biochem Cell Biol; 42 (4):519-23.

Lazarus M, Hajeer AH, Turner D et al. Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. J Rheumatol 1997; 24 (12):2314-7.

Le Y, Zhou Y, Iribarren P et al. Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell Mol Immunol 2004; 1 (2):95-104.

Leavitt JA, Campbell RJ. Cost-effectiveness in the diagnosis of sarcoidosis: the conjunctival biopsy. Eye 1998; 12 (Pt 6):959-62.

Lee DA, Barker SM, Su WP et al. The clinical diagnosis of Reiter's syndrome. Ophthalmic and nonophthalmic aspects. Ophthalmology 1986; 93 (3):350-6.

Lee EB, Kim JY, Zhao J et al. Haplotype association of IL-8 gene with Behcet's disease. Tissue Antigens 2007; 69 (2):128-32.

Lee WP, Tai DI, Lan KH et al. The -251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in Chinese population. Clin Cancer Res 2005; 11 (18):6431-41.

Lee YH, Harley JB, Nath SK. Meta-analysis of TNF-[alpha] promoter -308 A//G polymorphism and SLE susceptibility. 2006; 14 (3):364-71.

Lee YH, Kim HJ, Rho YH et al. Functional polymorphisms in matrix metalloproteinase-1 and monocyte chemoattractant protein-1 and rheumatoid arthritis. Scand J Rheumatol 2003; 32 (4):235-9.

Lee YH, Song GG. Lack of association of TNF-alpha promoter polymorphisms with ankylosing spondylitis: a meta-analysis. Rheumatology (Oxford) 2009; 48 (11):1359-62.

Leonard WJ, Zeng R, Spolski R. Interleukin 21: a cytokine/cytokine receptor system that has come of age. J Leukoc Biol 2008; 84 (2):348-56.

Leppkes M, Becker C, Ivanov, II et al. RORgamma-expressing Th17 cells induce murine chronic intestinal inflammation via redundant effects of IL-17A and IL-17F. Gastroenterology 2009; 136 (1):257-67.

Letendre S, Marquie-Beck J, Singh KK et al. The monocyte chemotactic protein-1 -2578G allele is associated with elevated MCP-1 concentrations in cerebrospinal fluid. J Neuroimmunol 2004; 157 (1-2):193-6.

Levin DS, Han DP, Dev S et al. Subtenon's depot corticosteroid injections in patients with a history of corticosteroid-induced intraocular pressure elevation. Am J Ophthalmol 2002; 133 (2):196-202.

Levinson RD, Greenhill LH. The monthly variation in acute anterior uveitis in a community-based ophthalmology practice. Ocul Immunol Inflamm 2002; 10 (2):133-9.

Li Z, Liu B, Maminishkis A et al. Gene Expression Profiling in Autoimmune Noninfectious Uveitis Disease. J Immunol 2008; 181 (7):5147-57.

Liang SC, Tan XY, Luxenberg DP et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 2006; 203 (10):2271-9.

Liao CH, Yao TC, Chung HT et al. Polymorphisms in the promoter region of RANTES and the regulatory region of monocyte chemoattractant protein-1 among Chinese children with systemic lupus erythematosus. J Rheumatol 2004; 31 (10):2062-7.

Lim WK, Fujimoto C, Ursea R et al. Suppression of immune-mediated ocular inflammation in mice by interleukin 1 receptor antagonist administration. Arch Ophthalmol 2005; 123 (7):957-63.

Lin J, Ziring D, Desai S et al. TNFalpha blockade in human diseases: an overview of efficacy and safety. Clin Immunol 2008; 126 (1):13-30.

Linker-Israeli M, Wallace DJ, Prehn J et al. Association of IL-6 gene alleles with systemic lupus erythematosus (SLE) and with elevated IL-6 expression. Genes Immun 1999; 1 (1):45-52.

Linssen A, Meenken C. Outcomes of HLA-B27-positive and HLA-B27-negative acute anterior uveitis. Am J Ophthalmol 1995; 120 (3):351-61.

Lipsky PE, van der Heijde DM, St Clair EW et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; 343 (22):1594-602.

Lit LC, Wong CK, Tam LS et al. Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. Ann Rheum Dis 2006; 65 (2):209-15.

Liu L, Xu Y, Wang J et al. Upregulated IL-21 and IL-21 receptor expression is involved in experimental autoimmune uveitis (EAU). Mol Vis 2009; 15:2938-44.

Liu X, Lee YS, Yu CR et al. Loss of STAT3 in CD4+ T cells prevents development of experimental autoimmune diseases. J Immunol 2008; 180 (9):6070-6.

Liversidge JM, Sewell HF, Forrester JV. Human retinal pigment epithelial cells differentially express MHC class II (HLA, DP, DR and DQ) antigens in response to in vitro stimulation with lymphokine or purified IFN-gamma. Clin Exp Immunol 1988; 73 (3):489-94.

Lobo A, Barton K, Minassian D et al. Visual loss in sarcoid-related uveitis. Clinical & Experimental Ophthalmology 2003; 31 (4):310-6.

Lotz M, Jirik F, Kabouridis P et al. B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. J Exp Med 1988; 167 (3):1253-8.

Louis E. The immuno-inflammatory reaction in Crohn's disease and ulcerative colitis: characterisation, genetics and clinical application. Focus on TNF alpha. Acta Gastroenterol Belg 2001; 64 (1):1-5.

Louis E, Franchimont D, Piron A et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998; 113 (3):401-6.

Louis E, Satsangi J, Roussomoustakaki M et al. Cytokine gene polymorphisms in inflammatory bowel disease. Gut 1996; 39 (5):705-10.

Louis E, Vermeire S, Rutgeerts P et al. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. Scand J Gastroenterol 2002; 37 (7):818-24.

Lovell DJ, Giannini EH, Reiff A et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. N Engl J Med 2000; 342 (11):763-9.

Lu MC, Yang KL, Tung CH et al. Higher LPS-stimulated TNF-alpha mRNA levels in peripheral blood mononuclear cells from Chinese ankylosing spondylitis patients with -

308G/A polymorphism in promoter region of tumor necrosis factor: association with distinct A33/B58/Cw10 haplotypes. Rheumatol Int 2008a; 29 (2):189-95.

Lu Z, Chen L, Li H et al. Effect of the polymorphism of tumor necrosis factor-alpha-308 G/A gene promoter on the susceptibility to ulcerative colitis: a meta-analysis. Digestion 2008b; 78 (1):44-51.

Ludwiczek O, Vannier E, Borggraefe I et al. Imbalance between interleukin-1 agonists and antagonists: relationship to severity of inflammatory bowel disease. Clin Exp Immunol 2004; 138 (2):323-9.

Lyons JL, Rosenbaum JT. Uveitis associated with inflammatory bowel disease compared with uveitis associated with spondyloarthropathy. Arch Ophthalmol 1997; 115 (1):61-4.

Mackay CR. Chemokines: immunology's high impact factors. Nat Immunol 2001; 2 (2):95-101.

Maksymowych WP, Reeve JP, Reveille JD et al. High-throughput single-nucleotide polymorphism analysis of the IL1RN locus in patients with ankylosing spondylitis by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Arthritis Rheum 2003; 48 (7):2011-8.

Mannel DN, Echtenacher B. TNF in the inflammatory response. Chem Immunol 2000; 74:141-61.

Mapstone R, Woodrow JC. HL-A 27 and acute anterior uveitis. Br J Ophthalmol 1975; 59 (5):270-5.

Markomichelakis NN, Theodossiadis PG, Pantelia E et al. Infliximab for chronic cystoid macular edema associated with uveitis. Am J Ophthalmol 2004; 138 (4):648-50.

Martin TM, Smith JR, Rosenbaum JT. Anterior uveitis: current concepts of pathogenesis and interactions with the spondyloarthropathies. Curr Opin Rheumatol 2002; 14 (4):337-41.

Martin TM, Zhang G, Luo J et al. A locus on chromosome 9p predisposes to a specific disease manifestation, acute anterior uveitis, in ankylosing spondylitis, a genetically complex, multisystem, inflammatory disease. Arthritis Rheum 2005; 52 (1):269-74.

Martinez A, Pascual M, Pascual-Salcedo D et al. Genetic polymorphisms in Spanish rheumatoid arthritis patients: an association and linkage study. Genes Immun 2003; 4 (2):117-21.

Martinez-Borra J, Gonzalez S, López-Vazquez A et al. HLA-B27 alone rather than B27-related class I haplotypes contributes to ankylosing spondylitis susceptibility. Human Immunology 2000; 61 (2):131-9.

Mathew A, Pandey M, Murthy NS. Survival analysis: caveats and pitfalls. Eur J Surg Oncol 1999; 25 (3):321-9.

Matsubayashi T, Sugiura H, Arai T et al. Anakinra therapy for CINCA syndrome with a novel mutation in exon 4 of the CIAS1 gene. Acta Paediatr 2006; 95 (2):246-9.

Matsuda R, Koide T, Tokoro C et al. Quantitive cytokine mRNA expression profiles in the colonic mucosa of patients with steroid naïve ulcerative colitis during active and quiescent disease. Inflammatory Bowel Diseases 2009; 15 (3):328-34.

Matsuki T, Nakae S, Sudo K et al. Abnormal T cell activation caused by the imbalance of the IL-1/IL-1R antagonist system is responsible for the development of experimental autoimmune encephalomyelitis. Int Immunol 2006; 18 (2):399-407.

Matsumoto T, Tsurumoto T, Shindo H. Interleukin-6 levels in synovial fluids of patients with rheumatoid arthritis correlated with the infiltration of inflammatory cells in synovial membrane. Rheumatol Int 2006; 26 (12):1096-100.

McCannel CA, Holland GN, Helm CJ et al. Causes of uveitis in the general practice of ophthalmology. UCLA Community-Based Uveitis Study Group. Am J Ophthalmol 1996; 121 (1):35-46.

McGarry F, Neilly J, Anderson N et al. A polymorphism within the interleukin 1 receptor antagonist (IL-1Ra) gene is associated with ankylosing spondylitis. Rheumatology (Oxford) 2001; 40 (12):1359-64.

McGarry F, Walker R, Sturrock R et al. The -308.1 polymorphism in the promoter region of the tumor necrosis factor gene is associated with ankylosing spondylitis independent of HLA-B27. J Rheumatol 1999; 26 (5):1110-6.

McGeachy MJ, Bak-Jensen KS, Chen Y et al. TGF-[beta] and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell-mediated pathology. 2007; 8 (12):1390-7.

McGuigan FE, Ralston SH. Single nucleotide polymorphism detection: allelic discrimination using TaqMan. Psychiatr Genet 2002; 12 (3):133-6.

McGuire W, Hill AV, Allsopp CE et al. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature 1994; 371 (6497):508-10.

Medawar PB. Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 1948; 29 (1):58-69.

Medzhitov R, Janeway C, Jr. Innate immunity. N Engl J Med 2000; 343 (5):338-44.

Medzhitov R, Janeway CA, Jr. Decoding the patterns of self and nonself by the innate immune system. Science 2002; 296 (5566):298-300.

Meloni F, Caporali R, Marone Bianco A et al. Cytokine profile of bronchoalveolar lavage in systemic sclerosis with interstitial lung disease: comparison with usual interstitial pneumonia. Ann Rheum Dis 2004; 63:892 - 4.

Menezo V, Lobo A, Yeo TK et al. Ocular features in neurosarcoidosis. Ocul Immunol Inflamm 2009; 17 (3):170-8.

Merhi-Soussi F, Berti M, Wehrle-Haller B et al. Intracellular interleukin-1 receptor antagonist type 1 antagonizes the stimulatory effect of interleukin-1 alpha precursor on cell motility. Cytokine 2005; 32 (3-4):163-70.

Michou L, Croiseau P, Petit-Teixeira E et al. Validation of the reshaped shared epitope HLA-DRB1 classification in rheumatoid arthritis. Arthritis Research & Therapy 2006; 8 (3):R79.

Mikuniya T, Nagai S, Takeuchi M et al. Significance of the interleukin-1 receptor antagonist/interleukin-1 beta ratio as a prognostic factor in patients with pulmonary sarcoidosis. Respiration 2000; 67 (4):389-96.

Miller BH, Rosado-de-Christenson ML, McAdams HP et al. Thoracic sarcoidosis: radiologic-pathologic correlation. Radiographics 1995; 15 (2):421-37.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16 (3):1215.

Mintz R, Feller ER, Bahr RL et al. Ocular manifestations of inflammatory bowel disease. Inflamm Bowel Dis 2004; 10 (2):135-9.

Mira JP, Cariou A, Grall F et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. Jama 1999; 282 (6):561-8.

Mitoma H, Horiuchi T, Tsukamoto H et al. Mechanisms for cytotoxic effects of anti-tumor necrosis factor agents on transmembrane tumor necrosis factor alpha-expressing cells: comparison among infliximab, etanercept, and adalimumab. Arthritis Rheum 2008; 58 (5):1248-57.

Mitsuyama K, Sasaki E, Toyonaga A et al. Colonic mucosal interleukin-6 in inflammatory bowel disease. Digestion 1991; 50 (2):104-11.

Mizuno K, Takahashi J. Sarcoid cyclitis. Ophthalmology 1986; 93 (4):511-7.

Mo JS, Matsukawa A, Ohkawara S et al. Role and regulation of IL-8 and MCP-1 in LPS-induced uveitis in rabbits. Exp Eye Res 1999; 68 (3):333-40.

Modi WS, Goedert JJ, Strathdee S et al. MCP-1-MCP-3-Eotaxin gene cluster influences HIV-1 transmission. Aids 2003; 17 (16):2357-65.

Mohler KM, Torrance DS, Smith CA et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993; 151 (3):1548-61.

Mooller P, Vinje O, Olsen EG. HLA B27, sacro-iliitis and peripheral arthropathy in acute anterior uveitis. Scand J Rheumatol 1980; 9 (4):234-6.

Moore KW, de Waal Malefyt R, Coffman RL et al. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001; 19:683-765.

Morelli S, Perrone C, Ferrante L et al. Cardiac involvement in Behcet's disease. Cardiology 1997; 88 (6):513-7.

Mosmann T, Cherwinski H, Bond M et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986; 136 (7):2348-57.

Mugnier B, Balandraud N, Darque A et al. Polymorphism at position -308 of the tumor necrosis factor alpha gene influences outcome of infliximab therapy in rheumatoid arthritis. Arthritis Rheum 2003; 48 (7):1849-52.

Muhaya M, Calder V, Towler HM et al. Characterization of T cells and cytokines in the aqueous humour (AH) in patients with Fuchs' heterochromic cyclitis (FHC) and idiopathic anterior uveitis (IAU). Clin Exp Immunol 1998; 111 (1):123-8.

Muhaya M, Calder VL, Towler HM et al. Characterization of phenotype and cytokine profiles of T cell lines derived from vitreous humour in ocular inflammation in man. Clin Exp Immunol 1999; 116 (3):410-4.

Mukaida N, Shiroo M, Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 1989; 143 (4):1366-71.

Murphy CC, Ayliffe WH, Booth A et al. Tumor necrosis factor alpha blockade with infliximab for refractory uveitis and scleritis. Ophthalmology 2004; 111 (2):352-6.

Murphy PM. The molecular biology of leukocyte chemoattractant receptors. Annu Rev Immunol 1994; 12:593-633.

Murphy PM, Baggiolini M, Charo IF et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev 2000; 52 (1):145-76.

Mwantembe O, Gaillard MC, Barkhuizen M et al. Ethnic differences in allelic associations of the interleukin-1 gene cluster in South African patients with inflammatory bowel disease (IBD) and in control individuals. Immunogenetics 2001; 52 (3-4):249-54.

Nanke Y, Kotake S, Goto M et al. Decreased percentages of regulatory T cells in peripheral blood of patients with Behcet's disease before ocular attack: a possible predictive marker of ocular attack. Mod Rheumatol 2008; 18 (4):354-8.

Negoro K, Kinouchi Y, Hiwatashi N et al. Crohn's disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. Gastroenterology 1999; 117 (5):1062-8.

Nicklin MJH, Barton JL, Nguyen M et al. A Sequence-Based Map of the Nine Genes of the Human Interleukin-1 Cluster. Genomics 2002; 79 (5):718-25.

Nishi O, Nishi K, Ohmoto Y. Effect of interleukin 1 receptor antagonist on the blood-aqueous barrier after intraocular lens implantation. Br J Ophthalmol 1994; 78 (12):917-20.

Nograles KE, Brasington RD, Bowcock AM. New insights into the pathogenesis and genetics of psoriatic arthritis. Nat Clin Pract Rheumatol 2009; 5 (2):83-91.

Nyquist PA, Winkler CA, McKenzie LM et al. Single nucleotide polymorphisms in monocyte chemoattractant protein-1 and its receptor act synergistically to increase the risk of carotid atherosclerosis. Cerebrovasc Dis 2009; 28 (2):124-30.

Ogilvie EM, Khan A, Hubank M et al. Specific gene expression profiles in systemic juvenile idiopathic arthritis. Arthritis Rheum 2007; 56 (6):1954-65.

Ohashi J, Yamamoto S, Tsuchiya N et al. Comparison of statistical power between 2 * 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. Ann Hum Genet 2001; 65 (Pt 2):197-206.

Ohta K, Wiggert B, Yamagami S et al. Analysis of immunomodulatory activities of aqueous humor from eyes of mice with experimental autoimmune uveitis. J Immunol 2000; 164 (3):1185-92.

Okhravi N, Lightman SL, Towler HM. Assessment of visual outcome after cataract surgery in patients with uveitis. Ophthalmology 1999; 106 (4):710-22.

Olivieri I, Barozzi L, Padula A et al. Clinical manifestations of seronegative spondylarthropathies. Eur J Radiol 1998; 27 (Suppl 1):S3-6.

Ollier WE. Cytokine genes and disease susceptibility. Cytokine 2004; 28 (4-5):174-8.

Ongkosuwito JV, Feron EJ, van Doornik CE et al. Analysis of immunoregulatory cytokines in ocular fluid samples from patients with uveitis. Invest Ophthalmol Vis Sci 1998; 39 (13):2659-65.

Ooi KG, Galatowicz G, Towler HM et al. Multiplex cytokine detection versus ELISA for aqueous humor: IL-5, IL-10, and IFNgamma profiles in uveitis. Invest Ophthalmol Vis Sci 2006; 47 (1):272-7.

Ozaki K, Kikly K, Michalovich D et al. Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. Proc Natl Acad Sci U S A 2000; 97 (21):11439-44.

Ozen S, Alikasifoglu M, Bakkaloglu A et al. Tumour necrosis factor alpha G-->A -238 and G-->A -308 polymorphisms in juvenile idiopathic arthritis. Rheumatology (Oxford) 2002; 41 (2):223-7.

Paiva ES, Macaluso DC, Edwards A et al. Characterisation of uveitis in patients with psoriatic arthritis. Ann Rheum Dis 2000; 59 (1):67-70.

Pamer E, Cresswell P. Mechanisms of MHC class I--restricted antigen processing. Annu Rev Immunol 1998; 16:323-58.

Pan RY, Chen SL, Xiao X et al. Therapy and prevention of arthritis by recombinant adeno-associated virus vector with delivery of interleukin-1 receptor antagonist. Arthritis Rheum 2000; 43 (2):289-97.

Pandey JP, Frederick M. TNF-alpha, IL1-beta, and immunoglobulin (GM and KM) gene polymorphisms in sarcoidosis. Hum Immunol 2002; 63 (6):485-91.

Pantelidis P, Fanning GC, Wells AU et al. Analysis of tumor necrosis factor-alpha, lymphotoxin-alpha, tumor necrosis factor receptor II, and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2001; 163 (6):1432-6.

Pantelidis P, Lympany PA, Foley PJ et al. Polymorphic analysis of the high-affinity tumor necrosis factor receptor 2. Tissue Antigens 1999; 54 (6):585-91.

Pappu R, Ramirez-Carrozzi V, Ota N et al. The IL-17 Family Cytokines in Immunity and Disease. Journal of Clinical Immunology 2010; 30 (2):185-95.

Parrish-Novak J, Dillon SR, Nelson A et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. 2000; 408 (6808):57-63.

Pascual M, Nieto A, Mataran L et al. IL-6 promoter polymorphisms in rheumatoid arthritis. Genes Immun 2000; 1 (5):338-40.

Pascual V, Allantaz F, Arce E et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005; 201 (9):1479-86.

Pene J, Chevalier S, Preisser L et al. Chronically Inflamed Human Tissues Are Infiltrated by Highly Differentiated Th17 Lymphocytes. J Immunol 2008; 180 (11):7423-30.

Penttinen MA, Holmberg CI, Sistonen L et al. HLA-B27 modulates nuclear factor kappaB activation in human monocytic cells exposed to lipopolysaccharide. Arthritis Rheum 2002; 46 (8):2172-80.

Peschon JJ, Torrance DS, Stocking KL et al. TNF Receptor-Deficient Mice Reveal Divergent Roles for p55 and p75 in Several Models of Inflammation. J Immunol 1998; 160 (2):943-52.

Pignatti P, Moscato G, Casarini S et al. Downmodulation of CXCL8/IL-8 receptors on neutrophils after recruitment in the airways. J Allergy Clin Immunol 2005; 115 (1):88-94.

Pociot F, D'Alfonso S, Compasso S et al. Functional analysis of a new polymorphism in the human TNF alpha gene promoter. Scand J Immunol 1995; 42 (4):501-4.

Poltorak A, He X, Smirnova I et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998; 282 (5396):2085-8.

Power WJ, Rodriguez A, Pedroza-Seres M et al. Outcomes in anterior uveitis associated with the HLA-B27 haplotype. Ophthalmology 1998; 105 (9):1646-51.

Powrie F, Mauze S, Coffman RL. CD4+ T-cells in the regulation of inflammatory responses in the intestine. Res Immunol 1997; 148 (8-9):576-81.

Prasse A, Georges CG, Biller H et al. Th1 cytokine pattern in sarcoidosis is expressed by bronchoalveolar CD4+ and CD8+ T cells. Clin Exp Immunol 2000; 122 (2):241-8.

Rafiq S, Stevens K, Hurst AJ et al. Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. Genes Immun 2007; 8 (4):344-51.

Ramsay A, Lightman S. Hypopyon uveitis. Surv Ophthalmol 2001; 46 (1):1-18.

Rantapaa-Dahlqvist S, Boman K, Tarkowski A et al. Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. Ann Rheum Dis 2007; 66 (1):121-3.

Rauramaa R, Vaisanen SB, Luong LA et al. Stromelysin-1 and interleukin-6 gene promoter polymorphisms are determinants of asymptomatic carotid artery atherosclerosis. Arterioscler Thromb Vasc Biol 2000; 20 (12):2657-62.

Razzak A, al Samarrai A. Intraocular lens implantation following cataract extraction in Fuchs' heterochromic uveitis. Ophthalmic Res 1990; 22 (2):134-6.

Rees LE, Wood NA, Gillespie KM et al. The interleukin-10-1082 G/A polymorphism: allele frequency in different populations and functional significance. Cell Mol Life Sci 2002; 59 (3):560-9.

Reich K, Huffmeier U, Konig IR et al. TNF polymorphisms in psoriasis: association of psoriatic arthritis with the promoter polymorphism TNF*-857 independent of the PSORS1 risk allele. Arthritis Rheum 2007; 56 (6):2056-64.

Reiff A. Long-term outcome of etanercept therapy in children with treatment-refractory uveitis. Arthritis Rheum 2003; 48 (7):2079-80.

Reiff A, Takei S, Sadeghi S et al. Etanercept therapy in children with treatment-resistant uveitis. Arthritis Rheum 2001; 44 (6):1411-5.

Reinisch W, Gasche C, Tillinger W et al. Clinical relevance of serum interleukin-6 in Crohn's disease: single point measurements, therapy monitoring, and prediction of clinical relapse. Am J Gastroenterol 1999; 94 (8):2156-64.

Reveille JD, Maganti RM. Subtypes of HLA-B27: history and implications in the pathogenesis of ankylosing spondylitis. Adv Exp Med Biol 2009; 649:159-76.

Richards JC, Tay-Kearney ML, Murray K et al. Infliximab for juvenile idiopathic arthritis-associated uveitis. Clin Experiment Ophthalmol 2005; 33 (5):461-8.

Riordan-Eva P, Lightman S. Orbital floor steroid injections in the treatment of uveitis. Eye 1994; 8 (Pt 1):66-9.

Rizzo LV, Xu H, Chan CC et al. IL-10 has a protective role in experimental autoimmune uveoretinitis. Int Immunol 1998; 10 (6):807-14.

Robertson M, Liversidge J, Forrester JV et al. Neutralizing tumor necrosis factor-alpha activity suppresses activation of infiltrating macrophages in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 2003; 44 (7):3034-41.

Robinson J, Waller MJ, Fail SC et al. The IMGT/HLA and IPD databases. Human Mutation 2006; 27 (12):1192-9.

Rodriguez A, Akova YA, Pedroza-Seres M et al. Posterior segment ocular manifestations in patients with HLA-B27-associated uveitis. Ophthalmology 1994; 101 (7):1267-74.

Rodriguez A, Calonge M, Pedroza-Seres M et al. Referral patterns of uveitis in a tertiary eye care center. Arch Ophthalmol 1996; 114 (5):593-9.

Rollins BJ. Chemokines. Blood 1997; 90 (3):909-28.

Rosenbaum JT, Angell E. Paradoxical effects of IL-10 in endotoxin-induced uveitis. J Immunol 1995; 155 (8):4090-4.

Rosenbaum JT, Boney RS. Activity of an interleukin 1 receptor antagonist in rabbit models of uveitis. Arch Ophthalmol 1992; 110 (4):547-9.

Rosenbaum JT, Boney RS. Use of a soluble interleukin-1 receptor to inhibit ocular inflammation. Curr Eye Res 1991; 10 (12):1137-9.

Rosenbaum JT, Samples JR, Hefeneider SH et al. Ocular inflammatory effects of intravitreal interleukin 1. Arch Ophthalmol 1987; 105 (8):1117-20.

Rothova A. Ocular involvement in sarcoidosis. Br J Ophthalmol 2000; 84 (1):110-6.

Rothova A, La Hey E, Baarsma GS et al. Iris nodules in Fuchs' heterochromic uveitis. Am J Ophthalmol 1994; 118 (3):338-42.

Rothova A, Suttorp-van Schulten MS, Frits Treffers W et al. Causes and frequency of blindness in patients with intraocular inflammatory disease. Br J Ophthalmol 1996; 80 (4):332-6.

Rothova A, van Veenedaal WG, Linssen A et al. Clinical features of acute anterior uveitis. Am J Ophthalmol 1987; 103 (2):137-45.

Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem Biophys Res Commun 1999; 259 (2):344-8.

Rovin BH, Lu L, Zhang X. A novel interleukin-8 polymorphism is associated with severe systemic lupus erythematosus nephritis. Kidney Int 2002; 62 (1):261-5.

Ruddy MJ, Wong GC, Liu XK et al. Functional cooperation between interleukin-17 and tumor necrosis factor-alpha is mediated by CCAAT/enhancer-binding protein family members. J Biol Chem 2004; 279 (4):2559-67.

Rudwaleit M, Siegert S, Yin Z et al. Low T cell production of TNFalpha and IFNgamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. Ann Rheum Dis 2001; 60 (1):36-42.

Rybicki BA, Major M, Popovich J, Jr. et al. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. Am J Epidemiol 1997; 145 (3):234-41.

Rybicki BA, Maliarik MJ, Poisson LM et al. Sarcoidosis and granuloma genes: a family-based study in African-Americans. Eur Respir J 2004; 24 (2):251-7.

Ryffel B, Mihatsch MJ. TNF receptor distribution in human tissues. Int Rev Exp Pathol 1993; 34 Pt B:149-56.

Sacca S, Pascotto A, Siniscalchi C et al. Ocular complications of latanoprost in uveitic glaucoma: three case reports. J Ocul Pharmacol Ther 2001; 17 (2):107-13.

Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to Leishmania major in mice. 2002; 2 (11):845-58.

Sakaguchi M, Sugita S, Sagawa K et al. Cytokine production by T cells infiltrating in the eye of uveitis patients. Jpn J Ophthalmol 1998; 42 (4):262-8.

Salanti G, Amountza G, Ntzani EE et al. Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. Eur J Hum Genet 2005.

Salmon JF, Wright JP, Murray AD. Ocular inflammation in Crohn's disease. Ophthalmology 1991; 98 (4):480-4.

Salvarani C, Vlachonikolis IG, van der Heijde DM et al. Musculoskeletal manifestations in a population-based cohort of inflammatory bowel disease patients. Scand J Gastroenterol 2001; 36 (12):1307-13.

Santos Lacomba M, Marcos Martin C, Gallardo Galera JM et al. [Aqueous humor and serum interleukin-6 in patients with uveitis]. Arch Soc Esp Oftalmol 2001a; 76 (6):345-50.

Santos Lacomba M, Marcos Martin C, Gallardo Galera JM et al. Aqueous humor and serum tumor necrosis factor-alpha in clinical uveitis. Ophthalmic Res 2001b; 33 (5):251-5.

Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998; 47 (3):195-8.

Sartani G, Silver PB, Rizzo LV et al. Anti-tumor necrosis factor alpha therapy suppresses the induction of experimental autoimmune uveoretinitis in mice by inhibiting antigen priming. Invest Ophthalmol Vis Sci 1996; 37 (11):2211-8.

Saruhan-Direskeneli G, Yentur SP, Akman-Demir G et al. Cytokines and chemokines in neuro-Behcet's disease compared to multiple sclerosis and other neurological diseases. J Neuroimmunol 2003; 145 (1-2):127-34.

Sather BD, Treuting P, Perdue N et al. Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. J Exp Med 2007; 204 (6):1335-47.

Sato W, Aranami T, Yamamura T. Cutting Edge: Human Th17 Cells Are Identified as Bearing CCR2+CCR5- Phenotype. J Immunol 2007; 178 (12):7525-9.

Schena FP, Cerullo G, Torres DD et al. Role of interferon-gamma gene polymorphisms in susceptibility to IgA nephropathy: a family-based association study. Eur J Hum Genet 2006; 14 (4):488-96.

Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. J Biol Chem 2007; 282 (28):20059-63.

Schmeling H, Horneff G. Etanercept and uveitis in patients with juvenile idiopathic arthritis. Rheumatology (Oxford) 2005; 44 (8):1008-11.

Schmeling H, Wagner U, Peterson A et al. Tumor necrosis factor alpha promoter polymorphisms in patients with juvenile idiopathic arthritis. Clin Exp Rheumatol 2006; 24 (1):103-8.

Schmidt K, Martinez-Gamboa L, Meier S et al. Bronchoalveoloar lavage fluid cytokines and chemokines as markers and predictors for the outcome of interstitial lung disease in systemic sclerosis patients. Arthritis Research & Therapy 2009; 11 (4):R111.

Schotte H, Schluter B, Rust S et al. Interleukin-6 promoter polymorphism (--174 G/C) in Caucasian German patients with systemic lupus erythematosus. Rheumatology (Oxford) 2001; 40 (4):393-400.

Schug J. Using TESS to predict transcription factor binding sites in DNA sequence. Curr Protoc Bioinformatics 2008; Chapter 2:Unit 2 6.

Scott RA, Sullivan PM, Aylward GW et al. The effect of pars plana vitrectomy in the management of Fuchs heterochromic cyclitis. Retina 2001; 21 (4):312-6.

Seckinger P, Williamson K, Balavoine JF et al. A urine inhibitor of interleukin 1 activity affects both interleukin 1 alpha and 1 beta but not tumor necrosis factor alpha. J Immunol 1987; 139 (5):1541-5.

Seitz M, Wirthmuller U, Moller B et al. The -308 tumour necrosis factor-alpha gene polymorphism predicts therapeutic response to TNFalpha-blockers in rheumatoid arthritis and spondyloarthritis patients. Rheumatology (Oxford) 2007; 46 (1):93-6.

Seki N, Yamaguchi K, Yamada A et al. Polymorphism of the 5'-flanking region of the tumor necrosis factor (TNF)-alpha gene and susceptibility to human T-cell lymphotropic virus type I (HTLV-I) uveitis. J Infect Dis 1999; 180 (3):880-3.

Sfikakis PP, Theodossiadis PG, Katsiari CG et al. Effect of infliximab on sight-threatening panuveitis in Behcet's disease. Lancet 2001; 358 (9278):295-6.

Sharma S, Ghosh B, Sharma SK. Association of TNF polymorphisms with sarcoidosis, its prognosis and tumour necrosis factor (TNF)-alpha levels in Asian Indians. Clin Exp Immunol 2008; 151 (2):251-9.

Sharma SK, Balamurugan A, Pandey RM et al. Human leukocyte antigen-DR alleles influence the clinical course of pulmonary sarcoidosis in Asian Indians. Am J Respir Cell Mol Biol 2003; 29 (2):225-31.

Sherwood DR, Rosenthal AR. Cataract surgery in Fuchs' heterochromic iridocyclitis. Br J Ophthalmol 1992; 76 (4):238-40.

Shevach EM, Davidson TS, Huter EN et al. Role of TGF-Beta in the induction of Foxp3 expression and T regulatory cell function. J Clin Immunol 2008; 28 (6):640-6.

Shulman JP, Latkany P, Chin KJ et al. Whole-body 18FDG PET-CT imaging of systemic sarcoidosis: ophthalmic oncology and uveitis. Ocul Immunol Inflamm 2009; 17 (2):95-100.

Smith AJP, Humphries SE. Cytokine and cytokine receptor gene polymorphisms and their functionality. Cytokine & Growth Factor Reviews 2009; 20 (1):43-59.

Smith JR, Levinson RD, Holland GN et al. Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. Arthritis Rheum 2001; 45 (3):252-7.

Society AT. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am Rev Respir Dis 1990; 142:481 - 6.

Soga Y, Nishimura F, Ohyama H et al. Tumor necrosis factor-alpha gene (TNF-alpha) -1031/-863, -857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. J Clin Periodontol 2003; 30 (6):524-31.

Song PI, Abraham TA, Park Y et al. The expression of functional LPS receptor proteins CD14 and toll-like receptor 4 in human corneal cells. Invest Ophthalmol Vis Sci 2001; 42 (12):2867-77.

Spaide RF, Ward DL. Conjunctival biopsy in the diagnosis of sarcoidosis. Br J Ophthalmol 1990; 74 (8):469-71.

Spies T, Blanck G, Bresnahan M et al. A new cluster of genes within the human major histocompatibility complex. Science 1989; 243 (4888):214-7.

Spriggs DR, Deutsch S, Kufe DW. Genomic structure, induction, and production of TNF-alpha. Immunol Ser 1992; 56:3-34.

Stanbury RM, Graham EM. Systemic corticosteroid therapy--side effects and their management. Br J Ophthalmol 1998; 82 (6):704-8.

Steinmetz OM, Panzer U, Harendza S et al. No association of the -2518 MCP-1 A/G promoter polymorphism with incidence and clinical course of IgA nephropathy. Nephrol Dial Transplant 2004; 19 (3):596-601.

Stevens C, Walz G, Singaram C et al. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 expression in inflammatory bowel disease. Dig Dis Sci 1992; 37 (6):818-26.

Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J Leukoc Biol 2003; 74 (2):179-85.

Streilein JW, Wilbanks GA, Cousins SW. Immunoregulatory mechanisms of the eye. J Neuroimmunol 1992; 39 (3):185-200.

Suarez A, Castro P, Alonso R et al. Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. Transplantation 2003; 75 (5):711-7.

Sugita S, Taguchi C, Takase H et al. Soluble Fas ligand and soluble Fas in ocular fluid of patients with uveitis. Br J Ophthalmol 2000; 84 (10):1130-4.

Suzuki Kurokawa M, Suzuki N. Behcet's disease. Clin Exp Med 2004; 4 (1):10-20.

Swider C, Schnittger L, Bogunia-Kubik K et al. TNF-alpha and HLA-DR genotyping as potential prognostic markers in pulmonary sarcoidosis. Eur Cytokine Netw 1999; 10 (2):143-6.

Szekanecz Z, Koch AE. Chemokines and angiogenesis. Curr Opin Rheumatol 2001; 13 (3):202-8.

Tabara Y, Kohara K, Yamamoto Y et al. Polymorphism of the monocyte chemoattractant protein (MCP-1) gene is associated with the plasma level of MCP-1 but not with carotid intima-media thickness. Hypertens Res 2003; 26 (9):677-83.

Tagore A, Gonsalkorale WM, Pravica V et al. Interleukin-10 (IL-10) genotypes in inflammatory bowel disease. Tissue Antigens 1999; 54 (4):386-90.

Takase H, Futagami Y, Yoshida T et al. Cytokine profile in aqueous humor and sera of patients with infectious or noninfectious uveitis. Invest Ophthalmol Vis Sci 2006; 47 (4):1557-61.

Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005; 17 (1):1-14.

Tanuma N, Sakuma H, Sasaki A et al. Chemokine expression by astrocytes plays a role in microglia/macrophage activation and subsequent neurodegeneration in secondary progressive multiple sclerosis. Acta Neuropathol 2006; 112 (2):195-204.

Tarlow JK, Blakemore AI, Lennard A et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993; 91 (4):403-4.

Tarrant TK, Silver PB, Wahlsten JL et al. Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. J Exp Med 1999; 189 (2):219-30.

Taylor PC. Anti-TNF therapy for rheumatoid arthritis and other inflammatory diseases. Mol Biotechnol 2001; 19 (2):153-68.

Taylor PC, Peters AM, Paleolog E et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. Arthritis Rheum 2000; 43 (1):38-47.

Tejwani S, Murthy S, Sangwan VS. Cataract extraction outcomes in patients with Fuchs' heterochromic cyclitis. J Cataract Refract Surg 2006; 32 (10):1678-82.

ten Hove T, van Montfrans C, Peppelenbosch MP et al. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut 2002; 50 (2):206-11.

Teoh SC, Sharma S, Hogan A et al. Tailoring biological treatment: anakinra treatment of posterior uveitis associated with the CINCA syndrome. Br J Ophthalmol 2007; 91 (2):263-4.

Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000; 275 (24):18138-44.

Thelier N, Assous N, Job-Deslandre C et al. Osteoarticular involvement in a series of 100 patients with sarcoidosis referred to rheumatology departments. J Rheumatol 2008; 35 (8):1622-8.

Theodossiadis PG, Markomichelakis NN, Sfikakis PP. Tumor necrosis factor antagonists: preliminary evidence for an emerging approach in the treatment of ocular inflammation. Retina 2007; 27 (4):399-413.

Thorburn E, Kolesar L, Brabcova E et al. CXC and CC chemokines induced in human renal epithelial cells by inflammatory cytokines. Apmis 2009; 117 (7):477-87.

Tkachuk M, Bolliger S, Ryffel B et al. Crucial role of tumor necrosis factor receptor 1 expression on nonhematopoietic cells for B cell localization within the splenic white pulp. J Exp Med 1998; 187 (4):469-77.

Toledo de Abreu M, Belfort R, Jr., Hirata PS. Fuchs' heterochromic cyclitis and ocular toxoplasmosis. Am J Ophthalmol 1982; 93 (6):739-44.

Tountas NA, Casini-Raggi V, Yang H et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. Gastroenterology 1999; 117 (4):806-13.

Trevisani VF, Mattos KT, Esteves RF et al. Autoantibodies specificity in acute anterior uveitis according to the presence of the HLA-B27 allele. Ocul Immunol Inflamm 2001; 9 (4):231-42.

Tsukamoto K, Ohta N, Shirai Y et al. A highly polymorphic CA repeat marker at the human tumor necrosis factor alpha (TNFA alpha) locus. J Hum Genet 1998; 43 (4):278-9.

Tuaillon N, Shen DF, Berger RB et al. MCP-1 expression in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 2002; 43 (5):1493-8.

Tucci M, Barnes EV, Sobel ES et al. Strong association of a functional polymorphism in the monocyte chemoattractant protein 1 promoter gene with lupus nephritis. Arthritis Rheum 2004; 50 (6):1842-9.

Tynjala P, Kotaniemi K, Lindahl P et al. Adalimumab in juvenile idiopathic arthritis-associated chronic anterior uveitis. Rheumatology (Oxford) 2008; 47 (3):339-44.

Udalova IA, Richardson A, Denys A et al. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promoter region. Mol Cell Biol 2000; 20 (24):9113-9.

Underhill DM, Ozinsky A, Smith KD et al. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci U S A 1999; 96 (25):14459-63.

Vallee D, Muller P, de Wall N et al. [Value of bronchoalveolar lavage and findings of cytologic studies in the diagnosis of interstitial lung diseases]. Pneumologie 1990; 44 Suppl 1:301-2.

van der Linden SM, Valkenburg HA, de Jongh BM et al. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. Arthritis Rheum 1984; 27 (3):241-9.

van der Paardt M, Crusius JB, Garcia-Gonzalez MA et al. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms in ankylosing spondylitis. Rheumatology (Oxford) 2002; 41 (12):1419-23.

van Heel DA, Udalova IA, De Silva AP et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF(-kappa)B transcription factors. Hum Mol Genet 2002; 11 (11):1281-9.

Vandevoorde V, Haegeman G, Fiers W. Induced expression of trimerized intracellular domains of the human tumor necrosis factor (TNF) p55 receptor elicits TNF effects. J Cell Biol 1997; 137 (7):1627-38.

Vargas-Alarcon G, Casasola-Vargas J, Rodriguez-Perez JM et al. Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with spondyloarthritis. Hum Immunol 2006; 67 (10):826-32.

Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992; 10:411-52.

Vazquez-Cobian LB, Flynn T, Lehman TJ. Adalimumab therapy for childhood uveitis. J Pediatr 2006; 149 (4):572-5.

Veal CD, Capon F, Allen MH et al. Family-based analysis using a dense single-nucleotide polymorphism-based map defines genetic variation at PSORS1, the major psoriasis-susceptibility locus. Am J Hum Genet 2002; 71 (3):554-64.

Vencovsky J, Jarosova K, Ruzickova S et al. Higher frequency of allele 2 of the interleukin-1 receptor antagonist gene in patients with juvenile idiopathic arthritis. Arthritis Rheum 2001; 44 (10):2387-91.

Venturelli D, Travali S, Calabretta B. Inhibition of T-cell proliferation by a MYB antisense oligomer is accompanied by selective down-regulation of DNA polymerase alpha expression. Proc Natl Acad Sci U S A 1990; 87 (15):5963-7.

Verity DH, Marr JE, Ohno S et al. Behcet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. Tissue Antigens 1999a; 54 (3):213-20.

Verity DH, Wallace GR, Vaughan RW et al. HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behcet's disease. Tissue Antigens 1999b; 54 (3):264-72.

Verjans GM, van der Linden SM, van Eys GJ et al. Restriction fragment length polymorphism of the tumor necrosis factor region in patients with ankylosing spondylitis. Arthritis Rheum 1991; 34 (4):486-9.

Verma MJ, Lloyd A, Rager H et al. Chemokines in acute anterior uveitis. Curr Eye Res 1997; 16 (12):1202-8.

Vermeire S, Noman M, Van Assche G et al. Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. Gastroenterology 2003; 125 (1):32-9.

Vijgen L, Van Gysel M, Rector A et al. Interleukin-1 receptor antagonist VNTR-polymorphism in inflammatory bowel disease. Genes Immun 2002; 3 (7):400-6.

Waldron-Lynch F, Adams C, Amos C et al. Tumour necrosis factor 5' promoter single nucleotide polymorphisms influence susceptibility to rheumatoid arthritis (RA) in immunogenetically defined multiplex RA families. Genes Immun 2001; 2 (2):82-7.

Wang JT, Wang TH, Sheu JC et al. Effects of anticoagulants and storage of blood samples on efficacy of the polymerase chain reaction assay for hepatitis C virus. J Clin Microbiol 1992; 30 (3):750-3.

Ware CF, VanArsdale TL, Crowe PD et al. The ligands and receptors of the lymphotoxin system. Curr Top Microbiol Immunol 1995; 198:175-218.

Watanabe M, Iwano M, Akai Y et al. Association of interleukin-1 receptor antagonist gene polymorphism with IgA nephropathy. Nephron 2002; 91 (4):744-6.

Weaver CT, Harrington LE, Mangan PR et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006; 24 (6):677-88.

Wegscheider BJ, Weger M, Renner W et al. Role of the CCL2/MCP-1 -2518A>G gene polymorphism in HLA-B27 associated uveitis. Mol Vis 2005; 11:896-900.

Weinblatt ME, Kremer JM, Bankhurst AD et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340 (4):253-9.

Weiner A, BenEzra D. Clinical patterns and associated conditions in chronic uveitis. Am J Ophthalmol 1991; 112 (2):151-8.

Weinreb RN, Tessler H. Laboratory diagnosis of ophthalmic sarcoidosis. Surv Ophthalmol 1984; 28 (6):653-64.

Wildin RS, Ramsdell F, Peake J et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001; 27 (1):18-20.

Wilkinson RJ, Patel P, Llewelyn M et al. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. J Exp Med 1999; 189 (12):1863-74.

Willems M, Moshage H, Nevens F et al. Plasma collected from heparinized blood is not suitable for HCV-RNA detection by conventional RT-PCR assay. J Virol Methods 1993; 42 (1):127-30.

Wilson AG, di Giovine FS, Blakemore AI et al. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1992; 1 (5):353.

Wilson AG, Symons JA, McDowell TL et al. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997; 94 (7):3195-9.

Wolk K, Kunz S, Witte E et al. IL-22 Increases the Innate Immunity of Tissues. 2004; 21 (2):241-54.

Xu H, Dawson R, Crane IJ et al. Leukocyte diapedesis in vivo induces transient loss of tight junction protein at the blood-retina barrier. Invest Ophthalmol Vis Sci 2005; 46 (7):2487-94.

Yamaguchi E, Itoh A, Hizawa N et al. The gene polymorphism of tumor necrosis factor-beta, but not that of tumor necrosis factor-alpha, is associated with the prognosis of sarcoidosis. Chest 2001; 119 (3):753-61.

Yamashiro S, Kamohara H, Wang J-M et al. Phenotypic and functional change of cytokine-activated neutrophils: inflammatory neutrophils are heterogeneous and enhance adaptive immune responses. J Leukoc Biol 2001; 69 (5):698-704.

Yang Z-X, Liang Y, Zhu Y et al. Increased expression of Toll-like receptor 4 in peripheral blood leucocytes and serum levels of some cytokines in patients with ankylosing spondylitis. Clinical and Experimental Immunology 2007; 149 (1):48-55.

Yazici H, Yurdakul S, Hamuryudan V. Behcet's syndrome. Curr Opin Rheumatol 1999; 11 (1):53-7.

Yeh S, Li Z, Forooghian F et al. CD4+Foxp3+ T-regulatory cells in noninfectious uveitis. Arch Ophthalmol 2009; 127 (4):407-13.

Yen D, Cheung J, Scheerens H et al. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. J Clin Invest 2006; 116 (5):1310-6.

Yilmaz S, Aydemir E, Maden A et al. The prevalence of ocular involvement in patients with inflammatory bowel disease. International Journal of Colorectal Disease 2007; 22 (9):1027-30.

Yla-Herttuala S, Lipton BA, Rosenfeld ME et al. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci U S A 1991; 88 (12):5252-6.

Yoshida Y, Kumar A, Koyama Y et al. Interleukin 1 activates STAT3/nuclear factor-kappaB cross-talk via a unique TRAF6- and p65-dependent mechanism. J Biol Chem 2004; 279 (3):1768-76.

Zaidi AA, Ying GS, Daniel E et al. Hypopyon in patients with uveitis. Ophthalmology 2010; 117 (2):366-72.

Zheng YQ, Wei W, Dai M et al. Interleukin-1 receptor antagonist intervenes in signaling between different types of synoviocytes in rats with adjuvant arthritis. Acta Pharmacol Sin 2006; 27 (1):111-8.

Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007; 81 (3):584-92.

Zouboulis CC, Katsantonis J, Ketteler R et al. Adamantiades-Behcet's disease: interleukin-8 is increased in serum of patients with active oral and neurological manifestations and is secreted by small vessel endothelial cells. Arch Dermatol Res 2000; 292 (6):279-84.

Zouboulis CC, Kotter I, Djawari D et al. Epidemiological features of Adamantiades-Behcet's disease in Germany and in Europe. Yonsei Med J 1997; 38 (6):411-22.

MOORFIELDS EYE HOSPITAL NHS Trust

Patron: Her Majesty The Queen

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Patient Label

Centre Number::
Study Number: LlGS1013
Patient Identification Number for this trial:

CONSENT FORM
Title of Project: DNA polymorphism and gene expression analysis as predictors of disease severity and response to treatment in patients with anterior uveitis.

Name of Researcher: PROFESSOR SUSAN LIGHTMAN AND TEAM

Please initial

1. I confirm that I have read and understand the information sheet dated...
(version ... .) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time,without giving any reason, without my medical care or legal rights being affected

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from [company name] or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree to take part in the above study.

Name of Patient

Date

Signature

Name of Person taking consent (if different from researcher)

Date
Signature

Researcher

Date
1 for patient; 1 for researcher; 1 to be kept with hospital notes

Signature

MOORFIELDS EYE HOSPITAL

Patron: Her Majesty The Queen

Chairman
Sir Thomas Boyd-Carpenter
Chief Executive I. A. J. Balmer
City Road
London
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Patient Label

1. Study Title: DNA polymorphism and gene expression analysis as predictors of disease severity and response to treatment in patients with anterior and posterior uveitis

2. Invitation: You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or you would like more information.

Thank you for reading this.

3. What is the purpose of the study?: As you know, you suffer from inflammation inside your eye/s which in some patients results in loss of vision despite treatment. In other patients, the inflammation does not cause visual loss and we are trying to understand what actually causes damage to help patients with these type of diseases. In the inflamed eye, blood cells go into the eye and make substances which are called cytokines. Some of these are harmful to the eye while others are not. Ifwe can determine which are good ones and which are not, it would be possible to devise new treatments which would switch off the bad ones and hopefully less damage to the eye will occur. Although you have a chronic disorder we will only ask you for one sample and we will recruit patients for three years to the study

4. Why have I been chosen?: In order to really know the answer to this question, we are inviting all patients with active inflammation inside their eye to take part in this study.

5. Do I have to take part?: It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without

giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

6. What will happen to me if I take part?: If you agree to take part, you will be asked for a blood sample from your arm and then a sample of eye fluid if you feel you could do this. To take this we sit you at the slit lamp as for a usual eye examination and put local anaesthetic drops onto your eye to numb the eye. We then ask you to keep still and look straight ahead while a needle attached to a syringe takes the fluid from the side part of your eye. As the needle goes in, you may feel pressure but not pain. The sample takes a few seconds to take, the needle is removed and antibiotic cream put on. If you wish, the eye can be padded afterwards while you travel home.

7. What do I have to do?: If you would like to take part, we will ask you to sign the consent form and the samples will then be taken immediately if you are happy for this to be done now.

8. What is the drug or procedure that is being tested? This is not applicable to this study.

9. What are the alternatives for diagnosis or treatment? In this study, we are trying to identify
patients who are at risk of more severe disease and are not offering any other treatments at this stage.

10. What are the side effects of taking part?: when the local anaesthetic wears off from the eye, most patients do not feel any discomfort but if you do, you can take paracetamol tablets as you would if you had a headache. ? If you are concerned, please call us on 020 7566 226 during the working day.

11. What are the possible disadvantages of taking part?: In order to take part in this study, we need to take a blood sample and where possible a sample a fluid from your eye. Although taking eye fluid samples is something we do every day, some patients find the thought of it worrying. It is not a painful procedure but could cause mild discomfort once the local anaesthetic drops have worn off.

12. What are the possible benefits of taking part?: Your sample would contribute greatly to the knowledge about inflammation in the eye. It will therefore help us help people like you in the future who have inflammation and those that are damaged by it but the results will not help you at the present time.

13. What if new information becomes available?: Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue with the study. If you wish to withdraw, we will continue your normal care in the usual way as before the study started. If you continue, we may require you to sign an updated consent form.

14. What happens when the research study stops?: The results of the study will be very

carefully analysed to determine new treatment strategies based on this knowledge

15. What if something goes wrong? In many countries, taking eye fluids is a part of management of inflammation of the eye and we have done many hundreds here. No patient has suffered a problem because of the sample being taken.

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

16. Will my taking part in this study be kept confidential?: Yes. All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it except the letter to your OP which will let him know.

17. What will happen to the results of the research study?: All the information is presented to all the doctors involved in the management of patients with uveitis. This is done by presentation of the results at meetings and by publishing the information in the medical journals. No patient is individually identified in this but the results of the whole group are determined. Relevant patient groups are also informed of new advances in treatment. Some cells are stored for future study so that we can investigate new advances quickly when thy come along and help improve treatment prospects.

18. Who is organising and funding the research?: This study is carried out by Professor Lightman and her team and organised through the Institute of Ophthalmology and Moorfields Eye Hospital.

19. Who has reviewed this study?: This study has been approved by the Moorfields Eye Hospital Research Ethics Committee.

20. Contact for further information: The doctor involved in your eye care is part of the team that looks after patients with eye inflammation. The medical supervisor of the study is Professor Susan Lightman and she can be contacted via her secretary on 020 7566 2252.

You will be given a copy of this information sheet and a signed consent form for you to keep.

Thank you for considering taking part in this study.

.....

Susan Lightman FRCP FRCOphth PhD FMedSci Professor of Clinical Ophthalmology Consultant Ophthalmologist

APPENDIX 3

Clinical outcomes of patients with chronic anterior uveitis

AIMS:

The aims of this study were to:

1. Define the factors determining the ocular complications in a group of patients with chronic anterior uveitis.
2. Determine the visual outcome of these patients.

This research by the candidate in the clinical outcome of chronic anterior uveitis has contributed the following valuable information:

1. Posterior synaechia is not significantly associated with worse visual outcome in patients with CAU
2. Cystoid macular oedema, the most common cause of permanent visual loss in patients with uveitis is more likely to occur in patients in whom their uveitis is associated with a systemic autoimmune disease.
3. The overall long-term visual prognosis in patients with CAU is good regardless the subtype of CAU, despite patients with an associated systemic inflammatory disorder having an increased risk of developing cystoid macular oedema.

Published work:

1. Menezo V, Lightman SL. The development of complications in patients with chronic anterior uveitis. Am J Ophthalmol 2005;139:988-992.

Cytokine and chemokine polymorphisms in anterior uveitis

AIMS:

The aims of these studies were to:

1. Investigate the association between SNPs of key cytokine, chemokine and their receptor genes and AU.
2. Investigate the association between these SNPs and the development of chronicity and/or complications in AU.
3. Investigate their association with the presence of HLA-B27 antigen.

This study has contributed the following valuable information:

1. The identification of TNF-α -857T as markers of susceptibility to develop AAU.
2. The identification of potential SNPs, such as TNFRSF1A -201G/T and TNFRSF1A -1135C/T as potential contributors to the development of complications in patients with HLA-B27 associated AU.
3. The identification of the IL-1ra +2081T/C polymorphism as predictor of chronic disease in patients with AU.
4. The identification of MCP-1 polymorphism (MCP-1 -2076A/T) with a potential protective role against AU.

Published works:

1. Kuo NW, Lympany PA, Menezo V, Lagan AL, John S, Yeo TK, Liyanage S, du Bois RM, Welsh KI, Lightman SL. TNF-857T, a genetic risk marker for acute anterior uveitis. Invest Ophthalmol Vis Sci 2005;46:1565-1571.
2. Menezo V, Bond SK, Towler HMA, Kuo NW, Baharlo B, Wilson AG, Lightman SL. Cytokine gene polymorphisms involved in the chronicity and complications of anterior uveitis. Cytokine 2006;35:200-206.
3. Keo TK, Ahad MA, Kuo NW, Spagnolo P, Menezo V, Lympany P, Lightman SL. Chemokine gene polymorphisms in idiopathic anterior uveitis. Cytokine 2006;35:29-35