Current views on the diagnosis and aetiology of posterior uveitis

Ongkosuwito, J.V.

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Chapter 1

GENERAL INTRODUCTION

1.1 History of uveitis

Herophilos of Alexandria noted that the term uvea was coined because of its resemblance to a grape.[1] On dissection, the eye resembles a grape with the optic nerve as the stalk, hence the term uvea, which means grape in Latin. Uveitis is defined as inflammation of the uveal tract, consisting of iris, ciliary body, pars plana and choroid (Figure 1), but in practice it is also used to describe inflammation involving the vitreous, retina and optical nerve. This results in terminology such as iritis, pars planitis, vitritis, choroiditis, retinitis, and neuritis.

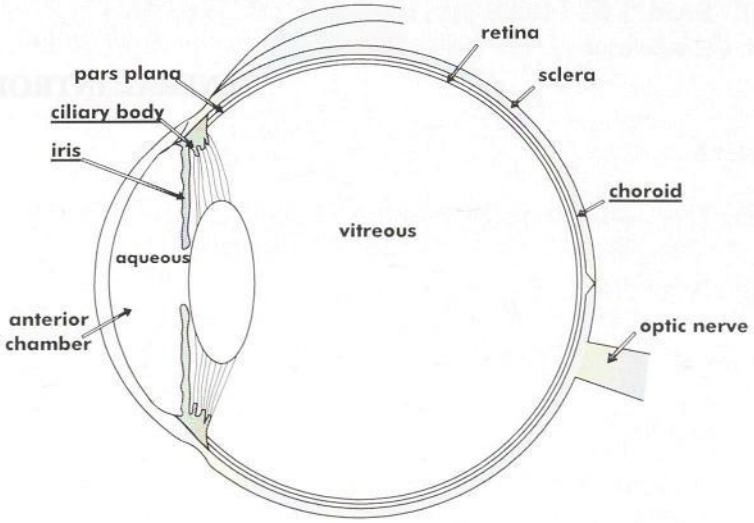

Figure 1. Localisation of uveitis
(the parts of the uveal tract are underlined)

A historical overview shows that there have been many changes in the concepts concerning the aetiology of uveitis. Before the development of the slitlamp, two forms of uveitis were distinguished: purulent, secondary to perforating trauma or exogenous infection, and nonpurulent uveitis, as a result of bacterial endotoxins or other substances giving a sort of hypersensitivity reaction.

Table 1 lists the dates of important insights into and discoveries of associations with uveitis. The development of the ophthalmoscope by Helmholtz[2] in 1851 was a significant breakthrough since this allowed visualisation of the fundus.

Table 1. Broad outlines of the discovery of associations with uveitis during this century.[2-10]

Time point	Aetiology
Untill –1910	Toxins from bowel or bacteria from dental or tonsillar infections: focal infection
1903	Autoimmune reactions to lens protein: phacoanaphylactic endophthalmitis
1907	Syphilis, rheumatic disease
1909	Tuberculosis
1931	Hypersensitivity to uveal proteins or bacterial toxins
1936	Sarcoidosis, brucellosis
1949	Weil's disease-associated uveitis (Leptospiral uveitis)
1952	Toxoplasmosis
1959	Cytomegalovirus (CMV) retinitis
1973	HLA-B27-associated acute anterior uveitis
1980	acquired immunodeficiency syndrome (AIDS)-> opportunistic infections
1982	Varicella zoster virus (VZV) in acute retinal necrosis (ARN), [1989-Herpes simplex virus type I (HSV-I)]
1987	Whipple's disease
1989	Lyme disease
1990-1997	Cat-scratch disease, drug-related uveitis (rifabutin, metipranolol, cidofovir)

Although Uhlenhut discovered in 1903 that animals react with inflammation to their own lens proteins, resulting in lens-induced uveitis, and others discovered that injection of uveal proteins could result in a form of uveitis resembling sympathetic ophthalmia, it was thought that the underlying factors in uveitis were either infectious organisms in the eye or focal infections such as dental infections. Until 1907 it was thought that the main cause of uveitis was syphilis. After the discovery of the tubercle bacillus, this organism was considered to be the most common cause of uveitis. Woods,[16] one of the leaders in the field of uveitis in the first half of this century, thought in 1944 that tuberculosis was a contributing factor in about 79%; this percentage had decreased to about 23% in 1954. During this period, organisms such as *Brucella* sp., and *Toxoplasma gondii* [6] were demonstrated in enucleated eyes and several serological and skin tests were developed. In 1936, Bruins Slot[4] pointed out that the histologic features of the eyes of patients with uveoparotid fever were identical to that of sarcoidosis, so that a definitive association between a systemic disease, such as sarcoidosis, was established. In 1941, Guyton and Woods described the aetiology of uveitis in 572 cases and concluded that the role of focal infections was rather small and that removal of the presumed origin (teeth, tonsils) was of little help.[17]

Woods[16] summarised the thoughts about uveitis in 1961 and utilised the most common classification until that time, based on the clinical or histological appearance: granulomatous or non-granulomatous uveitis. Granulomatous uveitis was used to describe the chronic tissue reaction, which follows infection, by various nonpyogenic agents. Histologically, it is recognised by 'clumps' (granulomas) of cells accompanied by necrosis. (The current definition of a granuloma is as follows: a focal area of granulomatous inflammation. It consists

of a microscopic aggregation of macrophages that are transformed into epithelium-like cells surrounded by a collar of mononuclear leukocytes, principally lymphocytes and occasionally plasma cells. In tuberculosis it is combined with central caseous necrosis[18]). Non-granulomatous uveitis was used to describe acute, nonpurulent inflammations, recognised histologically by oedema and capillary dilatation with little tissue destruction or overgrowth of connective tissue.[17] This classification was, however, inadequate since it seemed that some types of uveitis can present as both granulomatous and non-granulomatous, depending on the stage of the ocular disease, as for example in uveitis related to sarcoidosis. The acute stage presents as non-granulomatous and the chronic stage as granulomatous, despite the same aetiology.[19]

In 1973, Brewerton first reported the association between HLA-B27 and ankylosing spondylitis and thereafter the association between HLA-B27 and acute anterior uveitis (AAU)[8] In 1977, Wacker[20] characterised a soluble uveitopathogenic antigen from bovine retina (S-antigen) which can cause autoimmune uveitis (EAU) after injection into animals in the presence of appropriate adjuvants. Both of these findings contributed to the hypothesis that autoimmunity could play an important role in the aetiology of uveitis. In 1980, Rosenbaum et al.[21] developed another experimental uveitis model (endotoxin-induced uveitis (EIU)) based on the injection of lipopolysaccharide (LPS) into the footpads of Lewis rats. The EAU and EIU models have both proved to be of enormous importance in the understanding of the pathogenesis of uveitis and the development of new therapeutic regimens.

With the progression of other laboratory techniques and the advances in the field of immunology, the idea emerged that infections are of minor importance in the aetiology of uveitis. Other underlying factors or mechanisms can also play a role, such as autoimmune reactions, systemic diseases, and systemic or topical medication. The discovery of several antimicrobial agents also contributed to the decrease of infectious causes, as was seen for syphilis after the use of penicillin in this disease. In 1984[9] the human immunodeficiency virus (HIV) was identified as the cause of the acquired immunodeficiency syndrome (AIDS). After the manifestation of this syndrome, opportunistic infections such as CMV-retinitis were seen more frequently.[22] The discovery of new infectious organisms such as the spirochete *Borrelia burgdorferi* in 1982, and the improvement of PCR techniques also contributed to the increased diagnosis of infectious causes of uveitis in the last twenty years.

1.2 Classification of uveitis

Since 1987, the classification of uveitis is based on the localisation of the inflammation according to the recommendations of the International Uveitis Study Group[23]: anterior (iris, ciliary body), intermediate (pars plana, vitreous), posterior (retina, choroid) or pan- (the uveal tract as whole including the retina and the optic nerve) uveitis. In addition to the anatomical classification, the aetiology is used to make a subclassification.

Table 2. Classification of uveitis

Location	Aetiology	
	Infectious	**Presumed non-infectious**
Anterior	HSV, VZV *Mycobacterium* sp., *Leptospira, Treponema pallidum* (syphilis), *Propionibacterium acnes, Bartonella henselae*	HLA-B27-associated uveitis, Fuchs' heterochromic iridocyclitis, lens-associated uveitis, sarcoidosis, drug-induced uveitis (e.g. ß-blockers, rifabutin, IL-6 therapy)
Intermediate	*Borrelia burgdorferi, Toxocara* sp., *Tropheryma whippelii*, Human T-lymphotropic virus type I (HTLV-1)	Multiple sclerosis-associated uveitis, sarcoidosis, inflammatory bowel disease-associated uveitis (M. Crohn)
Posterior/ pan-	Parasites: *Toxoplasma gondii, Toxocara* sp., *Ascaris* sp., *Onchocerca volvulus* viruses: HSV, VZV, CMV, HIV bacteria: *T. whippelii, M. tuberculosis, T. pallidum* Fungi: *Candida* sp., *Aspergillus* sp., *H. capsulatum*	Sarcoidosis, Behçet's disease, white dot syndromes*: VKH, APMPPE, MEWDS, birdshot chorioretinopathy, sympathetic ophthalmia Presumed ocular histoplasmosis syndrome

* VKH= Vogt-Koyanagi-Harada disease, APMPPE= acute posterior multifocal placoid pigment epitheliopathy, MEWDS= multiple evanescent white dot syndrome

1.3 Incidence

The incidence of uveitis is currently estimated at 17/100.000.[24] The kind of uveitis seen varies per study, depending on whether the clinic was a referral centre or a community-based clinic (Table 4).[25] Anterior uveitis is more frequently seen in a general centre, while posterior uveitis is more frequently seen in a referral centre. In the study reported by McCannel et al.[25] CMV was considered to be the most frequently seen infectious cause of uveitis. The authors stated that this was probably due to the high prevalence rates for AIDS in the region where the study was done. This is in contrast to many other reports, in which *T. gondii* is listed as the most frequently seen infectious cause.[26,27]

The latest figure for the incidence of uveitis is from Leicester in the United Kingdom (1996),[28] where the total annual incidence was 7.4/100.000, with the highest incidence for idiopathic anterior uveitis, followed by HLA-B27-associated acute anterior uveitis and Fuchs' heterochromic iridocyclitis. HSV and toxoplasmosis had the highest incidence among the infectious causes. Overall, anterior uveitis (idiopathic or HLA-B27-related) had the highest incidence.

Infectious causes seem to have decreased in the past decades, probably because of the development of antimicrobial agents. Examples include the decline of ocular syphilis after the introduction of penicillin and the decrease of ocular tuberculosis after the development of tuberculostatics. Nowadays, infectious causes are mainly seen in posterior uveitis and account for approximately 15-52% of cases,[29,30] if HIV-infected patients are included, then the number of infectious causes is increasing. The number of infections causing uveitis is also largely dependent on the region under investigation. In certain regions of Africa, onchocerciasis used to be the most frequently seen infectious cause, while in the Western World this is toxoplasmosis.[26,31] Systemic (presumed non-infectious) diseases account for approximately 30% of all cases of uveitis.[32] The incidence of drugrelated uveitis is estimated at 0.5%.[15] In spite of the fact that several diagnostic tests are increasingly available and more diseases associated with uveitis are known, approximately 30-50% of uveitis cases are still classified under the term 'idiopathic' (unknown cause) (Table 3 and 4).

In most reports concerning the impact of blindness, uveitis is not considered as a distinct entity.[35] One study describing the causes of blindness in Germany[36] reports that the prevalence of blindness as a result of uveal inflammation is the highest in younger blind persons (3.6%) as compared to elderly people in whom the prevalence of uveal inflammatory disease accounted for 2.4% of the cases.[36] Uveitis causes about 10% of the visual handicaps in the world.[37] The main cause of blindness due to uveitis is cystoid macular oedema, followed by corneal opacities and macular inflammatory lesions.[38]

Table 3a. Anatomical localisation of uveitis as seen in a general versus a referral centre.[25]

	General centre		Referral centre	
	N=213	%	N=213	%
Anterior uveitis	193	90.6	129	60.6
Intermediate uveitis	3	1.4	26	12.2
Posterior/ panuveitis	13	6.1	51	23.9
Other (e.g. endophthalmitis)	4	1.9	7	3.3

Table 3b. Aetiology of uveitis as seen in a general versus a referral centre.[25]

	General centre		Referral centre	
	N=229	%	N=316	%
Infectious aetiology (total)	48	21.0	151	48
- CMV-retinitis	16	7.0	103	32.6
-HSV/ VZV	16	7.0	30	9.4
- *T. gondii*	9	4.0	11	3.4
- other	7	2.0	7	2.6
Associated with systemic disease (total)	43	18.8	35	11.1
-sarcoidosis	2	0.9	2	0.6
-HLA-B27	33	14.4	23	7.3
-other	8	3.5	10	3.2
Lens-associated uveitis	2	0.9	2	0.6
Idiopathic but categorised (total)	17	7.4	42	13.2
-Fuchs' heterochromic iridocyclitis	2	0.9	3	0.9
-Birdshot chorioretinopathy	0		2	0.6
-Intermediate uveitis	2	0.9	26	8.2
-other	13	5.6	11	3.5
Idiopathic but not categorised	119	51.9	86	27.2

Table 4. Aetiology of posterior uveitis in Europe in % of total uveitis cases

	1975 Finland[33]	1990 Portugal[34]	1993 The Netherlands[29]	1996 United Kingdom[28]
Anterior	84	60	52	56
Intermediate	n.s.*	4	9	8
Post/pan-	16	36	39	23
Infectious	15	23	15	16
Non-infectious	9	18	36	30
Lens-associated	n.s.	n.s.	n.s.	1
Idiopathic	76	49	40	27
Miscellaneous	n.s.	6	8	26

* n.s. = not specified

1.4 Immune response in the eye

As it is very important to preserve the visual function of the eye, this organ is protected against blinding damage by its localisation (inside a bony cavity) and an altered immune response (immune privilege). It was discovered more than a century ago that foreign tissues or antigens placed in the anterior compartment of the eye are not rejected and do not cause an inflammatory reaction, a phenomenon designated as immune privilege.[39] A status of immune privilege is also found in several other sites of the body, such as the subretinal space, brain, and testes.

In the eye, this immune privileged situation is maintained by intrinsic mechanisms to limit immune responses in the eye, such as the presence of blood ocular barriers (retinal pigment epithelium, epithelium of the ciliary body and iris vessels), the (disputable) absence of lymphatic drainage, the presence of a variety of immunosuppressive mediators (such as TGF-ß, α-MSH, VIP), and the constitutive expression of Fas-ligand on various ocular structures. The proliferation of T-cells, that are necessary to elicit an immune response is suppressed by factors present in the aqueous humour such as TGF-β.[40] The unique presence of Fas-ligand on corneal epithelial and endothelial cells, iris, and retina protects the eye against influx of activated, Fas-expressing T-cells because it triggers the apoptotic death of these cells.[41] Release of IL-10 by these apoptotic cells has been suggested as one of the key elements in the induction of ocular immune deviation.[42]

Besides these intrinsic mechanisms to protect the eye against severe inflammatory responses, immune privilege is also obtained through deviation of immune responses. The phenomenon that antigens placed in the anterior chamber of the eye elicit a deviant immune response is currently named 'anterior chamber associated immune deviation' (ACAID). The hallmarks of ACAID are antigen-specific deficits in delayed hypersensitivity (DH) and complement-fixing antibodies and a unique spectrum of regulatory T-cells: CD8+ cells that suppress DH expression and CD4+ cells that interfere with the induction of DH effector cells.[43] When these protective mechanisms fail as a result of, for example, an infectious agent that causes breakdown of the bloodocular barrier, the eye will be susceptible to more harmful inflammatory reactions. Due to breakdown of the bloodocular barrier, influx of factors that block activation of latent into active TGF-β may result in the abrogation of the immune privileged status of the eye.[44]

1.5 Aetiology: infectious versus 'non'- infectious

With the improvement of various laboratory techniques the understanding of the aetiology of several uveitis entities has increased. Not only the discovery of the infectious organisms but also the increasing knowledge of immunology gave a better insight into the aetiology of uveitis.

It is, however, still difficult to discriminate between infectious and non-infectious causes, because there are only a limited number of procedures that can be used in the diagnosis of uveitis. Choroidal or retinal biopsies are difficult to perform and may have serious complications. The discrimination between an infectious and a non-infectious cause is very important, because the therapeutic strategies are different. If an infectious cause is suspected, corticosteroid therapy may induce exacerbation or even worsen the visual outcome as seen in, for example, ocular toxoplasmosis.[45] On the contrary, corticosteroids seem very helpful in the treatment of non-infectious uveitis, as is seen in, for example, in ocular sarcoidosis and in HLA-B27-associated anterior uveitis.

There are a number of mechanisms that may be involved in the pathogenesis of infectious uveitis. The damage in the eye can be the result of

(-1-) the infectious organism itself, as was seen in patients with AIDS and CMV-retinitis in a period when highly active anti-retroviral therapy (HAART) was not yet available. In these patients, CMV-retinitis appears as necrotising retinopathy spreading rapidly over the retina. Anti-CMV therapy is needed to conquer the virus and prevent reactivation. The result of direct viral damage to the retina is also seen in patients with acute retinal necrosis (ARN). Histopathological examination of enucleated eyes with ARN showed large regions of necrosis with many viral particles.[10] Preliminary findings show that the visual prognosis is much better when antiviral therapy is administered directly into the vitreous than when it is applied systemically, thus indicating that rapid clearance of the virus is necessary to limit retinal damage [Van der Lelij, personal communication]. Patients with ocular toxoplasmosis treated with corticosteroids without anti-parasitic medication[45,46] and patients with AIDS and ocular toxoplasmosis may present with full-thickness retinal necrosis. Histopathological examination reveals little or no retinal inflammation, supporting the hypothesis that the damage in these cases is also due to proliferating organisms rather than the host immune response.[47]

Furthermore, (-2-) the host immune response against an infectious organism can result in damage to the retina. This mechanism is difficult to distinguish from the damage caused by the infectious organism, but a clear example is that of uveitis caused by *Toxocara* sp.. In 1974, Byers[48] reported that inflammatory signs due to the larva of *Toxocara* start after the death of the larva, and that these signs disappear after treatment with corticosteroids. Although small-scale studies were performed in which patients were treated with antihelmintics, most authors feel that these therapeutic agents do not have additional value.[49]

In case of recurrent ocular toxoplasmosis it seems that the inflammatory reaction seen at the border of an old scar is due to the immune response against the parasite, because ocular toxoplasmosis is usually a self-limiting disease. During the eighties it was thought that recurrence of ocular toxoplasmosis could be due to a hypersensitivity reaction against retinal antigens.[50] Others have suggested that retinal autoimmunity was not of major importance but could be an epiphenomenon following retinal inflammation.[51, 52] During active ocular toxoplasmosis, intraocular antibody production can be detected[53] and *T. gondii*-specific T-lymphocytes can be derived from ocular fluid samples.[54] These findings point to a host anti-parasitic immune response that leads to damage of ocular structures instead of a direct noxious effect caused by the parasite itself.

The latest example of inflammation due to the host immune response is the transient vitritis seen in patients with AIDS who have been treated with HAART and have a CMV-retinitis, this in contrast to CMV-retinitis in the pre-HAART period, when the vitreous was clear during CMV-retinitis. The authors proposed that the vitritis was the result of an increased host immune response against the virus.[55] The appearance of an unexplained vitritis was also reported in patients with non-active CMV-retinitis after HAART.[56] Others reported that in AIDS patients following immune restoration continuous anti-CMV therapy is no longer needed, as the host seemed to be able to control CMV replication.[57, 58]

Another mechanism that could result in ocular damage is (-3-) an 'autoimmune' reaction triggered by either infectious organisms or trauma. This is a rather speculative point, because there are only indications for possible mechanisms. One is molecular mimicry: cross-reactivity between the host and an infectious organism; the general assumption is that certain antigenic determinants (as small as 6 or 8 aminoacids) of an infectious organism have to be similar enough (compared to 'self') to induce a cross-reactive immune response, but with sufficient differences to elicit an immune response. This immune response is then also directed at self-protein and results in inflammation of those tissues expressing this antigen.[59] This mechanism has been described in an animal model for retinal S-antigen (S-Ag), whereby experimental autoimmune uveitis (EAU) could be induced with several viral proteins that had an aminoacid sequence homology with the uveopathogenic site of S-Ag.[60]

A recently reported example of molecular mimicry in ocular disease is that of uveitis in patients infected with the nematode *Onchocerca volvulus*. The cause of ocular damage in the posterior segment is not yet clear, but an association with the worm is equivocal. It was suggested that the damage is due to the presence of antibodies directed at retinal antigens such as anti-retinal S-antigen (S-Ag), but no cellular or humoral response was seen against either interphotoreceptor retinoid binding protein (IRBP) or S-Ag in patients with uveitis due to *Onchocerciasis*.[61, 62] McKechnie et al., however, discovered a retinal protein (hr44) that cross-reacts with the parasite antigen Ov39. This Ov39 antigen is able to induce class II expression on retinal microglia and

causes retinal disease in rats after immunisation with this antigen.[63] To what extent molecular mimicry plays a role in the aetiology of other 'presumed non-infectious' uveitis entities has to be investigated.

A second possibility might be an autoimmune mechanism following the breakdown of immunological tolerance that has been implicated as a cause of non-infectious uveitis. Examples are sympathetic ophthalmia (SO) and lens-associated uveitis (LAU).[64] SO appears after penetrating trauma in one eye involving the uvea, causing a granulomatous inflammation in both eyes (one eye 'sympathises' with the other). This was thought to be the result of an autoimmune response directed at uveal proteins.

LAU, formerly called phacoanaphylactic or phacogenic uveitis or endophthalmitis, may appear after extracapsular cataract extraction, when the lens cortex is not fully removed or especially when the lens capsule is ruptured, and the 'dropped' nucleus may provoke an inflammatory reaction in the vitreous. This is also thought to be an autoimmune reaction (against lens proteins). The general thought was that both uveal and lens proteins were sequestered and thus invisible for the immune system: i.e., that these proteins would not be recognised as 'self' when the immune system comes in contact with these proteins. If bloodocular barriers are damaged by (surgical) trauma, the immune system will elicit a response to these 'foreign' proteins. Despite the fact that these hypotheses have been around for a number of decades, no definite 'autoantigen' has been characterised for either SO or LAU.

To date, the only proven human autoimmune disease caused by an immunological reaction against retinal proteins is cancer-associated retinopathy (CAR). The CAR syndrome serves as a model to illustrate how retinal damage can manifest as an immune-mediated repercussion evoked by the patient's immune response to recoverin, a 23 kD autoantigen that is aberrantly expressed by the neoplasm (mostly small cell carcinoma of the lung).[65]

Some disorders can simulate chronic uveitis;[19] these disorders are classified under the term 'masquerade syndromes'. When the underlying cause has been treated, the 'uveitis' will also resolve. These disorders are mostly of malignant origin, as for example intraocular lymphoma, but sometimes non-malignant such as intraocular foreign bodies, retinal detachment, or drug reactions. As malignant disorders often require prompt therapy it is very important that a distinction can be made between, for example, intraocular lymphoma and an infectious cause.[66]

When patients are treated with corticosteroids, because a non-infectious cause is suspected, and the uveitis does not resolve, a diagnostic pars plana vitrectomy is indicated to exclude a 'masquerade syndrome'. The vitreous specimen that is obtained can be used for cellular analysis, but has to be processed rapidly to preserve the morphology and integrity of the cells.[67] Since it can be very difficult to evaluate a biopsy via a histopathological method, other diagnostic tests, such as the detection of cytokines and PCR to diagnose intraocular lymphoma, are currently under investigation.[68]

1.6.1 Laboratory procedures in uveitis: systemic factors

HLA

Since Brewerton reported the association of HLA-B27 with acute anterior uveitis, other HLA-associations have been reported for other uveitis entities (Table 5), indicating that genetic components play a role in their pathogenesis.

Table 5 HLA associations with uveitis.[8, 69-75]

HLA-molecule	Uveitis entity
HLA-B27	Acute anterior uveitis
HLA-A29	Birdshot chorioretinopathy
HLA-B51	Behçet's disease
HLA-DR15	Intermediate uveitis
HLA-DRw2/B7	Presumed ocular histoplasmosis syndrome
HLA-DR4/HLA-B53	Vogt-Koyanagi-Harada disease

In patients with acute anterior uveitis, HLA-typing is important and can result in early diagnosis of ankylosing spondylitis when patients are HLA-B27 positive.[76] An early diagnosis is very important in this disease as it may prevent disability in these patients when treated adequately at this stage. The other HLA- associations mentioned in Table 5 are not included in the diagnostic criteria, but are of great interest since they may lead to a better understanding of their pathogenesis.

Immunoglobulins

The presence of specific immunoglobulins in serum can be used to detect the presumed causative infectious agent of uveitis if this organism does not infect virtually all people in the population under investigation. Due to the availability of antimicrobial agents, the prevalence of syphilis has decreased, resulting in a very low seropositivity in the population. Therefore, positive serum titres may give an adequate indication of the cause of uveitis. In infections that affect a large part of the population seroconversion can be useful. The following changes are used to indicate acute infection: seroconversion from IgG negative to positive, the presence of IgM or IgA, or a four-fold increase in IgG level.[77] In Europe, most people have been infected with several viruses, such as VZV (chickenpox) and EBV (M. Pfeiffer), and some parasites such as *T. gondii;* thus, IgG against these organisms is readily detected in their serum. In Europe and Brazil, the percentage of patients seropositive for *T. gondii* increases with age, so that in the elderly population approximately 70% have been in contact with the parasite.[78, 79]

Several studies have reported that the presence of specific IgG in serum is of little help to investigate whether the uveitis is caused by the suspected organism or not, since no difference was found between infected patients with or without uveitis.[80, 81] Therefore, in these cases, the detection of intraocularly produced immunoglobulins is very important, and has been shown to be a reliable method to indicate active infection in the eye.

Cytokines

The absence of pro-inflammatory cytokines in serum does not reflect the situation in the eye, because IL-6, for example, could not be detected in the serum of patients with uveitis but could be detected in the ocular fluid. [82] There are, however, indications that increased levels of IL-8 and soluble intercellular adhesion molecule (ICAM) in serum from patients with intermediate uveitis disclose a predisposition for systemic involvement.[83, 84]

The presence of other cytokine-related substances, such as IL-1 receptor antagonist (IL-1ra)[85] and IL-2 receptor,[86] in serum has been investigated but these studies failed to demonstrate a clear diagnostic value of these tests. The presence of IL-1ra could be used to indicate the therapeutic effects of treatment with cyclosporin and corticosteroids in patients with Behçet's disease.[85]

Other factors

Most of these factors indicate the presence of 'immune'-mediated systemic diseases that can involve the eye. Angiotensin converting enzyme (ACE) and lysozyme are released by epitheloid cells and macrophages within sarcoid granulomas. Serum ACE is elevated in 60-90% of patients with active sarcoidosis,[87] and it seems to confirm uveitis in correlation with sarcoidosis in patients not accessible to biopsy.[87] The detection of increased ACE levels is highly specific, but can also be found in other uveitis-related disorders such as tuberculosis and leprosy.[89] Lysozyme levels usually parallel ACE activity, but an increased level is less specific than ACE. The combination of elevated ACE and lysozyme may carry a higher predictive value than either test alone.

The presence of anti-phospholipid antibody (anti-cardiolipin antibodies and lupus coagulant) and anti-double stranded DNA antibodies (ds-DNA) indicate systemic lupus erythematosus (SLE) in patients with retinal vasculitis.

Anti-neutrophil cytoplasmic antibody (ANCA) shows three reaction patterns: 1- cytoplasmic (c-ANCA), 2- perinuclear (p-ANCA) and 3-a diffuse pattern which is a non-specific reaction due to miscellaneous antibodies.[90] The presence of p-ANCA in patients with uveitis has recently been re-investigated. It appeared that patients who were p-ANCA positive and had uveitis were either HLA-B27 positive or had systemic evidence of immune-mediated diseases.[91] The presence of c-ANCA is an indication for Wegener's granulomatosis.[92] For general uveitis screening, however, the detection of these antibodies has a limited value, uveitis is present in only 16% of patients with Wegener's disease and this disease itself is very rare.

Anti nuclear antibodies (ANA) are present in patients with rheumatic diseases, in children with uveitis this should be measured as uveitis can precede rheumatic complaints.

1.6.2 Diagnostic procedures involving intraocular fluids

Wardrop (1782-1869) evacuated aqueous humour as a therapeutic measure to control the raised intraocular pressure and the acute anterior inflammation in uveitis patients. It was thought that after removing the aqueous, new aqueous would flow into the anterior chamber thereby increasing the amount of antibodies.[1]

In 1940, Irvine et al.[93] wanted to get better insight into uveitis by studying the aqueous humour. They measured 'sugar' levels and protein content and made smears to detect what cells were important, but the outcomes were not related to the aetiology. Bruckner did the first paracenteses for diagnostic reasons in 1919.[1] In 1943, Amsler and Verrey published a paper [94] on the practical use of anterior chamber paracentesis; in 1954 they published the results of some 4500 anterior chamber taps that were done to investigate the cytology and immunology of the aqueous. No relation could be detected between the cell types in the aqueous and the presumed aetiology of the uveitis. It was also very rare to find living organisms in the samples, so that the cultures of aqueous humour samples were often negative.[95] In 1954, Goldmann and Witmer[96] had shown that specific antibodies that were produced in the eye could be detected in horses with uveitis caused by leptospira and in a few patients with uveitis caused by tuberculosis. At the same time, Cavara detected viral particles of HSV in the aqueous humour during iritis.[97]

When, however, aqueous humour was used for diagnostic purposes, it was either cultured or inoculated into an animal to make the diagnosis of either viral or other infectious origin. This often resulted in false-negative tests and when the test was positive, contamination could not be excluded. Witmer[95] first used intraocular antibody production to determine the aetiology of uveitis in 693 patients with uveitis in 1978. He concluded that only 10% of the aqueous chamber punctures were of benefit to the patients, but this was the result of the large volume needed for the tests. Since many ophthalmologists were of the opinion that paracentesis had more disadvantages than benefit, most investigators only used antibody detection in serum. The presence of antibodies in serum, however, does not substantiate the cause of the ongoing inflammation in the eye as was shown for ocular toxoplasmosis.[80] Therefore, testing of the intraocular fluid for antibodies is useful, and the calculation of the Goldmann-Witmer (GWc) or Witmer-Desmonts coefficient is commonly used, although seldom outside Europe.

$$GWc = \frac{\text{antibody level ocular fluid}}{\text{antibody level serum}} : \frac{\text{total IgG ocular fluid}}{\text{total IgG serum}}$$

In 1973, Allansmith et al.[98] investigated the amount of immunoglobulins in several parts of the eye and concluded that the main immunoglobulin (Ig) present is IgG (MW 140,000). The mean level of 0.01 mg/ml in non-inflamed eyes was probably due to diffusion from the circulation.[98] Since the diffusion factor is taken into account when calculating the GW- coefficient, the result can become false-negative, as is the case during uveitis related to an acute systemic infection with high serum antibody titres. It might therefore be useful to use other immunoglobulin classes. In patients with ocular toxoplasmosis who are not suffering from an acute systemic infection, intraocular antibody production will aid in the diagnosis in approximately 75% of cases.[99]

After the development of the polymerase chain reaction (PCR) to detect DNA by Saiki et al. in 1988, many investigators thought that the use of PCR would contribute greatly to making a definite diagnosis because the organism can be detected directly and only a small sample size is needed. For culture or serology a larger volume was needed and both were often negative. Viruses and certain bacteria, such as anaerobic bacteria, are difficult to culture, so the result is often false-negative. The diagnosis can also be false-negative if the production of locally produced antibodies is not yet fully started. [100, 101] Although PCR is now very popular it also has its limitations. PCR as a diagnostic means in uveitis was first used by Fox et al.[101] in 1991 to detect viral DNA in vitreous or aqueous humour from patients with suspected cytomegalovirus retinitis and by Brezin et al.[102] to detect *Toxoplasma gondii* DNA. De Boer et al.[100] compared the use of the detection of locally produced antibodies and PCR and concluded that in immunocompetent patients, PCR is useful in the very acute stage of inflammation but that after about 4 weeks, calculating the GW-coefficient is more useful. In patients immunocompromised by infection with the human immunodeficiency virus or as a result of immunosuppressive therapy, PCR might be more useful since antibody production is disturbed, resulting in polyclonal antibody formation. In immunocompetent patients, PCR seems to contribute to the definite diagnosis in only 30%.[103]

Although many investigators have demonstrated the reliability of laboratory investigation of aqueous humour samples, aqueous chamber paracentesis is not yet routinely used for the diagnosis of uveitis, since most ophthalmologists do not have the possibility to investigate the samples because the volume is very small and access to laboratories experienced in the analysis of ocular fluids is limited. Before performing more interventional procedures, such as retinal or choroidal biopsies, it would be better to perform anterior chamber paracentesis, regarding which Van der Lelij and Rothova reported that it is a safe procedure with few complications, often providing an answer in cases presenting as a diagnostic dilemma.[104]

Cytokines

A great deal of research has been done to investigate the presence of inflammatory cytokines in ocular fluid samples from patients with uveitis in order to get a better understanding of the immunology of uveitis. Until now, only Chan et al. reported the useful application of cytokine detection as a diagnostic measure. In 1995, she reported the use of the ratio between IL-6 and IL-10 to discriminate between uveitis and an intraocular lymphoma.[105] In patients with uveitis, high levels of IL-6 and very low or undetectable levels of IL-10 were found in the ocular fluid sample. This was in contrast to patients with an intraocular lymphoma in whose ocular fluid very high levels of IL-10 and only low levels of IL-6 could be detected. The level of IL-10 corresponded with the number of malignant cells in the ocular fluid sample.

1.7 The aim of this study

When dealing with uveitis patients, it is of the utmost importance to discriminate between an infectious and a non-infectious aetiology, because the treatment of these two forms can be very different. Uveitis of infectious origin has to be treated with antimicrobial agents, while uveitis of non-infectious aetiology can be treated with anti-inflammatory or immunosuppressive therapy. When, however, uveitis of infectious origin is treated solely with immunosuppressive medication, it can result in an unnecessarily poorer visual outcome. To treat the patient or possibly come to other 'novel' therapeutic strategies, it is therefore important to be able to identify the cause of uveitis, and also to investigate whether other factors may be playing a role in the pathogenesis of uveitis to be able.

The role of the Epstein-Barr virus as a cause of uveitis is still a controversial issue. The first part of this study was therefore designed to investigate the role of the Epstein-Barr virus in the pathogenesis of uveitis and to analyse whether routine diagnostic screening for this virus is necessary (**Chapter 2**).

Ocular toxoplasmosis is still the most important cause of posterior infectious uveitis. Until now the diagnosis of ocular toxoplasmosis is confirmed either by PCR or by the detection of intraocular anti- *T. gondii* IgG antibody production; approximately 75% of cases can be confirmed using these methods. As the detection of IgA antibodies has been described to have a diagnostic value in encephalitis, the use of anti- *T. gondii* IgA detection in ocular fluids was evaluated in patients with ocular toxoplasmosis (**Chapter 3**). In the following, chapter the discrimination between primary ocular toxoplasmosis in the setting of either acute or chronic systemic toxoplasmosis is described. This discrimination is important and has implications for the therapy; primary ocular toxoplasmosis may mimic other focal uveitis entities (**Chapter 4**).

To investigate whether the presumed ocular histoplasmosis syndrome (POHS) in The Netherlands is associated with *Histoplasma capsulatum* or other infectious parasites, a search for antibodies and possible risk factors in Dutch patients with POHS was investigated and the results are presented in **Chapter 5**. No risk factors of either infectious or non-infectious origin could be identified in our patients. In the USA, an association with either HLA-DR2 or HLA-B7 was reported for patients with POHS. We performed HLA-typing in Dutch patients with POHS to investigate whether a same genetic predisposition can be found in our patients who have POHS. (**Chapter 6**).

To investigate whether a profile of immunoregulatory cytokines can point to a certain uveitis entity, we measured IL-2, IL-4, IL-6, IL-10 and IFN-γ in patients with acute retinal necrosis and ocular toxoplasmosis. The results were compared with the results of patients with 'autoimmune' uveitis and non-uveitic controls (**Chapter 7**).

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