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MYELOID CELLS IN AUTOIMMUNE DISEASES

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Myeloid Cells in Autoimmune Diseases

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To my beloved mother, wife and daughter

স্বপ্ন নয় - শান্তি নয় - ভালবাসা নয়,
হৃদয়ের মাঝে এক বোধ জন্ম লয়!
আমি তারে পারিনা এড়াতে
সে আমার হাত রাখে হাতে;
সব কাজ তুচ্ছ হয় , পন্ড মনে হয় ,
সব চিন্তা - প্রার্থনার সকল সময়
শূন্য মনে হয়,
শূন্য মনে হয়!

- জীবনানন্দ দাশ

ABSTRACT

Multiple Sclerosis (MS) and Type 1 Diabetes (T1D) are autoimmune diseases caused by dysregulation of the immune system. Monocytes/macrophages are myeloid cells that play a pivotal role in both induction and resolution of these diseases depending on the stage and microenvironment of disease course. Similar to monocytes/macrophages, microglia are CNS resident macrophages that during MS may also exhibit both pro-inflammatory and anti-inflammatory properties. The main purpose of my PhD project was to develop a method to induce a regulatory or suppressive phenotype of myeloid cells for use in adoptive transfer as a novel therapy in preclinical studies of autoimmunity.

In Paper I we tested the hypothesis whether there are any differences in activation states between mouse strains with different genetic backgrounds. We used congenic Nramp1 -susceptible and -resistant macrophages on BALB/c and C57BL/6 mouse backgrounds and determined fundamental differences in macrophage activation states between the two different strains as well as Nramp1-specific effects.

In Paper II we tested the therapeutic effect of M2 macrophages in T1D. Our results reveal that after a single adoptive transfer of IL-4/IL-10/TGF-β-stimulated macrophages > 80% of the treated mice were protected from disease development. In Paper III we explored the same treatment in a MOG-induced EAE model. We were able to demonstrate that intranasal administration of IL-4/IL-10/TGF-β-stimulated microglia can attenuate EAE development in DBA/1 mice. In Paper IV we translated the rodent M2 macrophage induction protocol to a human monocyte-macrophage setting. Our findings indicate a robust stimulation protocol for generation of an optimal, specific, stable, and immunosuppressive human monocyte-derived macrophage phenotype.

The results presented in this thesis collectively demonstrate induction of a regulatory phenotype IL-4/IL-10/TGF-β in various myeloid cells including rodent macrophages, microglia and human monocytes. The IL-4/IL-10/TGF-β-induced M2 cells had a potent deactivating effect on pro-inflammatory LPS/IFNγ-activated macrophages (M1), significantly suppressed T cell proliferation and induced Tregs. Several modes of action are thus indicated to explain the therapeutic clinical effects, which were particularly apparent during chronic disease states. Based on these results, further clinical development of this therapy is thus warranted.

LIST OF PUBLICATIONS

I. **Mia S,** Plantinga T, Andresen P., Holm, B. & Harris RA. Nramp1 and background gene effects determine distinct macrophage activation phenotypes.
Manuscript.

II. Parsa R, Andresen P, Gillett A, **Mia S**, Zhang XM, Mayans S, Holmberg D, Harris RA. Adoptive transfer of immunomodulatory M2 macrophages prevents Type 1 Diabetes in NOD mice. *Diabetes.* 2012 Nov;61(11):2881-92

III. Zhang XM, Lund H, **, Mia S**, Parsa R, Harris RA. Adoptive transfer of cytokine-induced immunomodulatory adult microglia attenuates experimental encephalomyelitis in DBA/1 mice.
GLIA. 2014 In Press

IV. **Mia S,** Warnecke A, Zhang XM, Malmström V, Harris RA. An optimized protocol for human M2 macrophages using M-CSF and IL-4/IL-10/TGF-β yields a dominant immunosuppressive phenotype.
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Sarman S, **Mia S**, Harris RA, Kvanta A, Aronsson M, van der Ploeg I. Reduced neovascularisation in interleukin-10-deficient mice in a model of ischemic retinopathy. (Manuscript)

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LIST OF ABBREVIATIONS

APC	Antigen Presenting Cells
Aβ	Amyloid Beta
AIRE	Autoimmune Regulator
BBB	Blood Brain Barrier
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CXCL13	Chemokine (C-X-C motif) Ligand 13
DA	Dark Agouti
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
EAE	Experimental Autoimmune Encephalomyelitis
EDSS	Expanded Disability Status Scale
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr Virus
ELISA	Enzyme-Linked Immunosorbent Assay
GWAS	Genome-Wide Association Study
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
HLA	Human Leukocyte Antigen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LPS	Lipopolysaccharide
MBP	Myelin Basic Protein
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
MS	Multiple Sclerosis
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Image
NK	Natural Killer
NFκB	Nuclear Factor kappa-light –chain-enhancer of activated B cells

PBMC	Peripheral Blood Mononuclear Cells
PLP	Proteolipid Protein
PVG	Piebald Virol Glaxo
qPCR	Quantitative Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RA	Rheumatoid Arthritis
rMOG	Recombinant MOG (amino acids 1-125)
RNA	Ribonucleic Acid
SNP	Single Nucleotide Polymorphism
T1D	Type 1 Diabetes
TCR	T Cell Receptor
TGF	Transforming Growth Factor
T_H	T Helper Cell
TLR	Toll-like Receptor
TNF	Tumor Necrosis Factor
T_{REG}	T Regulatory Cell
VitD	Vitamin D
VLA-4	Very Late Antigen 4

1 INTRODUCTION

1.1 AUTOIMMUNITY

Our immune system is designed to protect us from invading pathogens by detecting and eliminating them. Autoimmunity is the failure of the immune system to distinguish self from non-self, or in other words the failure to recognize the body's own proteins and to not react to them. More than 70 distinct autoimmune diseases affect 3-5% of the total population worldwide [1].

Autoimmune diseases can be organ-specific, like in Type 1 Diabetes (T1D) that only involves the pancreas, or Multiple Sclerosis (MS) that only affects the central nervous system (CNS; brain or spinal cord). Autoimmune diseases may instead be systemic, affecting multiple organs, such as in Systemic Lupus Erythematosus (SLE) where the skin, joints, kidney, brain, lung and other tissues are attacked. It is well accepted that many autoimmune diseases are more prevalent in females. Recent experimental studies have shown that this gender bias in autoimmunity is influenced by microbiota/commensal organisms. Under germ-free conditions the incidence of T1D in NOD mice was almost equal between males and females [2]. In another study it was reported that commensal microbes increased testosterone levels and protected NOD mice, and that following transfer of gut microbiota from males to females the incidence of disease was decreased in females [3].

Despite many years of study, the underlying mechanisms that lead to autoimmunity are still enigmatic, but it is believed that the combined influences of genetic and environmental factors play important roles in the development of autoimmune diseases. Most of the autoimmune diseases have association with MHC or HLA genes [4]. I will discuss genetic and environmental factors in detail in the context of specific diseases. From an immunological viewpoint, after escaping from central and peripheral tolerance mechanisms, autoreactive T and B cells may be activated in the secondary lymph organs through the processes of molecular mimicry or epitope spreading due to infection or injury. They then migrate to the assigned organ or tissue and destruction ensues through the actions of their cytokine secretion. Autoimmunity and tolerance are two sides of the same coin. To

understand autoimmunity we must understand tolerance. In the following section I will discuss how central and peripheral tolerance mechanisms normally maintain the disease-free state.

1.2 TOLERANCE

The thymus is the principle organ involved in development of T lymphocytes and plays a central role in immune tolerance. In the thymus a special type of antigen presenting cell (APC) called thymic epithelial cells (TEC) can express thousands of tissue-specific self-antigens (TSA) to developing thymocytes. TEC are regulated by a nuclear factor AIRE that controls ectopic expression of tissue-restricted antigens (e.g. insulin). The fate of the thymocytes depends on the avidity and affinity of TCR peptide-MHC interactions. According to the affinity hypothesis three possible scenarios can occur (Figure-1).

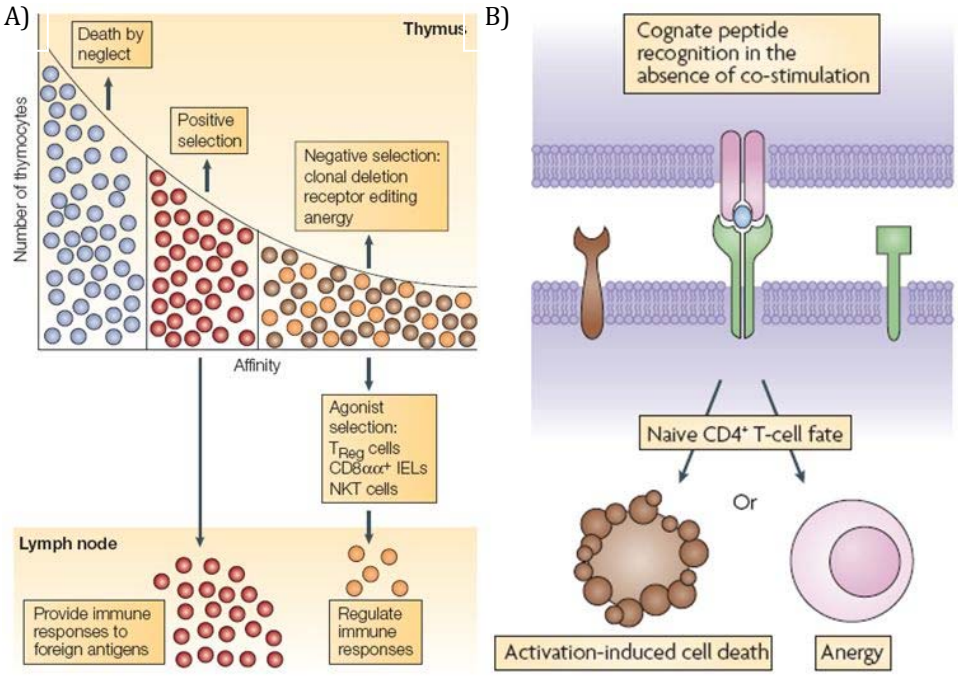

Figure 1: Mechanism of central tolerance [5] and peripheral tolerance[6] In central tolerance T cell progenitors undergo different selection process according to their the avidity and affinity of TCR peptide-MHC interactions. Cells might die by negligence, may survive or be deleted. (A) In peripheral tolerance, naïve T cells undergo anergy or activated induced cell death in the absence of co-stimulation (B).

Progenitors that have no affinity can die by neglect and this is thought to be the fate of most thymocytes. T cell progenitors that have low affinity to self-peptides can survive and differentiate, and this process is called 'positive selection'. If the thymocytes have strong affinity for their peptides then they are deleted through a process called 'negative selection'. In the periphery, a second round of affinity interactions will similarly determine T cell fate – activation-induced death, anergy or differentiation into natural Tregs [7].

B cell tolerance is also important for preventing autoimmune diseases and B cells also undergo the central and peripheral tolerance mechanisms. During central tolerance high affinity premature B cells specific for autoantigen are also deleted by the processes of anergy and receptor editing. Those with intermittent binding affinity towards self-antigen can survive and differentiate into mature B cells.

All self-reactive lymphocytes cannot be eliminated effectively by central tolerance. Some low affinity self-reactive lymphocytes can escape negative selection and enter into periphery. Once in the periphery mature T lymphocytes encounter self-antigen presented by Antigen Presenting Cells (APC). For full activation of $CD4^+$ T cells both T cell receptor (TCR) and co-stimulation are required. Peripheral tolerance is maintained by inhibition or expressing low levels of surface co-stimulation molecules in the absence of inflammation [6]. It has been demonstrated that CTLA-4, an inhibitory co-stimulatory molecule with a higher affinity for CD28 can inhibit T cell proliferation and induce anergy [8]. Similarly, the receptor PDL-1 can also induce a state of functional unresponsiveness to self-peptides and maintain tolerance [9].

1.3 MULTIPLE SCLEROSIS

The term Multiple Sclerosis was first coined by the French neurologist Jean-Martin Charcot in 1968. Despite intensive studies during the last 50 years, many aspects of the disease are still enigmatic [2, 10]. MS is a chronic inflammatory and demyelinating disease of the CNS and affects more than 2.5 million people worldwide, with a huge associated socio-economical burden. The prevalence in Sweden is 0.1-0.2% and women suffer more than men (with an approximate ratio of 2.5:1), as is common among autoimmune diseases [11]. Patients present with a

wide range of neurological defects depending upon the location of the demyelinated lesions in the CNS. Common neurological symptoms are coordination problems, balance disturbance, speech difficulties, cognitive impairment, severe fatigue, muscle weakness, visual problem and in severe cases, impaired mobility [12].

1.3.1 Clinical Course

According to the heterogeneity of the clinical courses, the neuroradiological appearances of lesions and the responses to treatment, MS is classified into four subtypes: relapsing-remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS) and progressive relapsing MS (PRMS) (Figure-2).

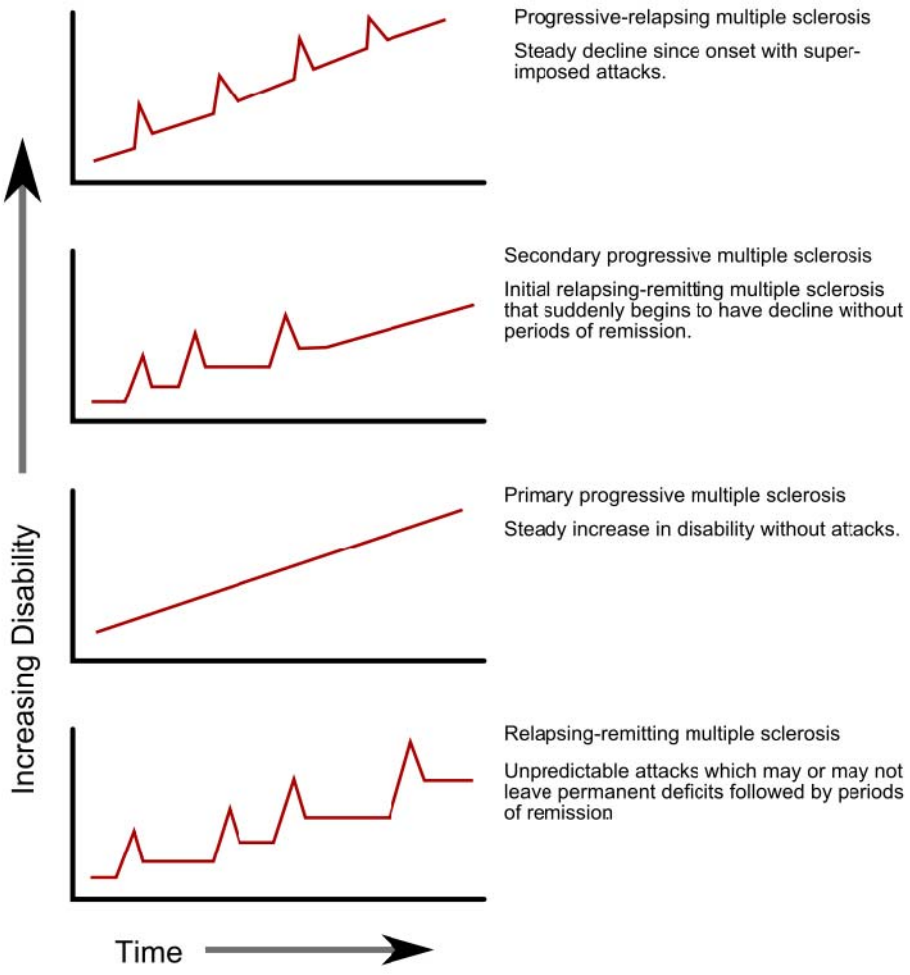

Figure 2: Different disease courses of MS. Patients commonly experience relapsing-remitting course and the majority of them end up with the secondary progressive variant. Some patients experience a progressive type from the beginning.

A majority of the patients start with a bout of inflammation then remain symptomless (remission) before experiencing another bout of inflammation (relapse). Patients with RRMS will eventually progress to SPMS. Approximately 10-15% of patients present with symptoms of PPMS from the very beginning. MS is diagnosed by a bout of neurological symptoms, the presence of oligoclonal bands in cerebrospinal fluid (CSF), and lesions evident using magnetic resonance imaging (MRI) scans [13-15]. The quality of life of MS patients is assessed using a 0-10 graded Extended Disability Scoring Scale (EDSS) [16].

1.3.2 MS is an autoimmune disease

In autoimmune diseases the regulatory mechanisms that check and balance self and non-self reactivity are impaired, and as a result the immune system attacks their own cells. Accumulating evidence suggests that MS is an organ-specific autoimmune disease mediated by autoreactive T cells with myelin specificity and appearance of autoantibodies. MS-like disease pathology can be induced in the Experimental Autoimmune Encephalomyelitis (EAE) animal models by active immunization of myelin-specific autoantigens or by passive transfer of myelin specific autoreactive T cells [17, 18].

During development, autoreactive T cells escape negative selection and if reactivated in the periphery they up regulate surface molecules, enabling more efficient adherence to the Blood Brain Barrier (BBB). Due to local chemokine gradients they invade the CNS and once reactivated secrete pro-inflammatory cytokines which subsequently attract further immune cells into the CNS, resulting in axonal damage that leads to the clinical symptoms [19].

1.3.3 Genetic Factors

Why some individuals develop MS and why there is selectivity towards the CNS is currently unknown. Many hypotheses have been generated but interactions among the environment, genetic predisposition, epigenetics and undefined risk factors play a pivotal role in MS susceptibility. The genetic component of MS and recurrent risk has been evaluated by familial clustering of cases. Recurrence risk in family members of those affected with MS is higher than in the general

population [20]. Twin studies have revealed that the recurrence rate in monozygotic twins is increased compared to in dizygotic twins [21, 22]. Conversely, for adopted individuals the risk is not increased with an affected non-biological relative [23].

Many linkage studies and genome-wide association studies have been conducted, but one region (locus) is unambiguously indicated, the Human Leukocyte Antigen (HLA) [24]. This remains the major genetic determinant for many other autoimmune diseases such as Type I Diabetes (T1D) and Rheumatoid Arthritis (RA). The strongest risk alleles for MS are MHC class II HLA-DRB1*15:01 and class I HLA-A*0301 alleles [25]. Conversely, HLA-A*0201 and HLA-C*05 contribute protective effects [26]. Lately, large studies have identified numerous candidate genes outside the HLA region. Among the non-HLA genes the interleukin-7 receptor (IL7R) and IL2 receptor alpha (IL2RA) genes were first identified and validated [27-29]. The latest MS GWAS identified more than 100 loci that contribute to MS susceptibility [30].

1.3.4 Environmental Factors

In addition to genetic susceptibility, environmental exposure is also required for the development of MS. The geographical distribution of MS reveals a distinct trend of prevalence, temperate regions having a higher susceptibility to MS. Immigrants from low-to-high prevalence regions before adolescence do not have an increased risk [31]. There are several potential explanations for this including lack of sun exposure, trauma, type of infection and education related to hygiene. There is geographical, biological and now immunological evidence that Vitamin D is an associated risk factor for MS [32]. Our lab and others have determined that Vitamin D seems to have a protective role against EAE [33].

Epidemiological studies indicate that, as for other diseases, smoking also gives a higher risk for MS [34]. Moreover, a number of viruses have been suggested to be associated with the pathogenesis of MS, particularly Human herpes virus 6 (HHV6) and Epstein Barr virus (EBV) [35, 36]. The role of viruses in MS pathology is still unclear, but it is likely that viruses can trigger inflammation

and through bystander activation via epitope spreading or molecular mimicry can cause specific myelin damage [37-39].

1.3.5 Immunopathogenesis of MS

It is known that healthy individuals possess autoreactive T cells along with their normal T cell repertoire [40]. However, the question is why MS develops selectively in persons. Despite extensive research of the underlying pathogenesis the answer to this question is still not clear. The prevailing thought is that individuals with genetic susceptibility are influenced by environmental factors like viral infection, and that this can trigger an autoimmune disease development, including MS.

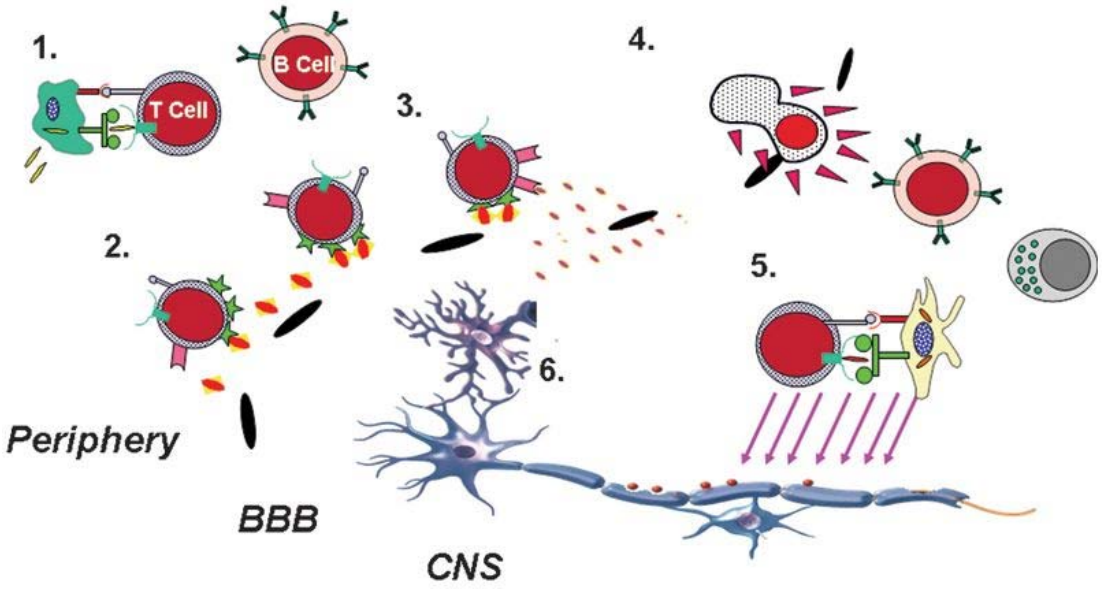

Figure 3: Pathogenesis of MS [41]: Activation of immune cells including T cells , myeloid cells and B cells (stage 1). Upregulation of adhesion molecules (stage 2). Active secretion of cytokines and chemokines (stage 3). Matrix proteases (stage 4). Reactivation of autoreactive T cells (stage 5). Demyelination and axonal loss (stage 6). BBB-Blood Brain Barrier; CNS-Central Nervous System.

It has been historically considered that the brain is an immune-privileged area. However, recent studies demonstrate that naïve lymphocytes can traffic into the inflamed CNS [42]. It is clear that the primary events that manifest the disease are activation of autoreactive T cells but where these T cells are activated is not very clear-cut. Inflammation in the periphery or CNS can activate these autoreactive T cells. Myelin-specific T cells can cross-react with foreign antigens

such as viral peptides through the process called molecular mimicry, and can be activated [43]. It was presumed that $CD4^+$ T cells are crucial for developing MS but it is now well accepted that $CD8^+$ T cells play a vital role in the immunopathogenesis of MS.

Activated T cells upregulate the adhesion molecules ICAM-1 and VCAM-1 and their ligands LFA-1 and VLA-4, respectively, that regulate the migration of leukocytes across the BBB [44] (Figure - 3 stage 2). Antigen presenting cells such as monocyte-macrophages and DCs secrete pro-inflammatory cytokines such as TNF-α, IL-6, IL-12, IL-1β, and chemokines including CCL2, CCL5, CXCR3 that cause tissue injury and promote further infiltration of monocytes and lymphocytes into the CNS. Moreover, monocytes and macrophages produce different tissue lytic enzymes, particularly MMP-9 [45], that disrupt the BBB and facilitate leukocyte infiltration (Figure - 3 stage 3). When autoreactive T cells enter into the CNS and encounter their specific antigen they become reactivated and this leads to secretion of pro-inflammatory cytokines including IFNγ, IL-17, IL-21, and activate other immune cells such as microglia (Figure - 3 stage 5). In the CNS microglia play a vital role as antigen-presenting cells and orchestrate a pro-inflammatory environment. Myelin-specific T cells, particularly $CD8^+$ T cells, together with microglia can cause damage to oligodendrocytes (which produce myelin) and subsequent injury can lead to demyelination and predispose axons for irreversible damage. Myelin damage can also expose CNS components for subsequent attack through epitope spreading as different myelin antigens become liberated

1.3.6 Immune cells in MS

$CD4^+$ T cells are thought to be the most dominant cellular population in MS pathology. However, most researchers agree that $CD8^+$ T cells are also equally important. It has been found that $CD8^+$ T cells are more numerous than $CD4^+$ T cells in MS lesions at different stages of disease [46]. Brain biopsies from two MS patients revealed $CD8^+$ T cells are clonally expanded around lesion tissue [47].
$CD4^+$ T cells or helper T cells are subdivided into Th1 and Th2, and more recently discovered Th17 cells according to their signature cytokine production and expression of transcriptional factors. Th17 cells are considered to be the initiators of the disease, but Th1 play a more important role in disease chronicity [48].

Recently, the observations of autoantibody and complement deposition around MS lesions renewed interest in B cells. It has been reported that peripheral depletion of B cells using the drug Rituximab can reduce MS disease activity [49].
Dendritic cells are professional antigen presenting cells critically involved in naïve T cell activation and differentiation. DCs from MS patients display an activated phenotype with increased expression of activation markers [50]. Moreover, DCs from MS patients can skew differentiation of Th1 and Th17 cells during disease.
NK cells can play both effector and regulatory functions through their different subpopulations. It has been shown that $CD56^{dim}$ NK cells are primarily cytotoxic while $CD56^{bright}$ NK cells exert regulatory function [51]. A recent study observed that the NK2 NK cell subpopulation is increased during the remission stage of MS compared to during relapse [52].

N.B. Macrophages and microglia are discussed elaborately in later parts of the thesis.

1.3.7 Current therapy for MS

There are few therapeutic options available today for MS patients (Table-1). Most of the drugs are not very potent and effective, only slowing down the progression of the disease. To date, no drug has been shown to be effective against PPMS or SPMS forms of the disease. The standard treatment regime for MS is parenteral immunomodulatory IFNβ and polypeptide Glatiramer acetate. These drugs are only capable of reducing the frequency of relapses and delay disability, but fail to prevent progressive MS. Adverse effects also limit these drugs to use over time. The pharmacokinetics and pharmacodynamics of these drugs are not fully understood, but there are several mechanisms proposed. IFNβ may enhance production of regulatory cytokines [53], reduce T_H1 pathology [54, 55], and inhibit T_H17 differentiation [54, 56, 57]. Polypeptide Glatiramer acetate promotes T_H2 polarization and restores deficient $CD4^+CD25^+Foxp3^+$ regulatory T cells through modulating monocytes [58, 59].

After a successful trial in Crohn's disease, Natalizumab, a humanized recombinant monoclonal antibody raised against human α4 integrin of the adhesion molecule very late antigen 4(VLA-4) on leukocytes, emerged as a new

potent therapy for MS [60]. Natalizumab inhibits the extravasation of immune cells over the BBB into the CNS [61]. However, severe adverse effects in the form of progressive multifocal leukoencephalopathy (PML) have been observed [62, 63].

Combination therapy along with corticosteroids is thought to be a good option to obtain the maximum effect, due to heterogeneous pathological nature of MS. But unexpected adverse effects, additive or synergistic, can be a potential threat [64, 65]. Recently, several oral medications have been shown to be beneficial and well tolerated with moderate safety profiles (Table-1). Fingolimod [66-68] and Cladribine [68] are two oral drugs that have been recently approved by the US and European communities. Despite continuous evolving efforts to develop potent and safer drugs it is also very important to develop biomarkers for prognosis and early diagnosis. These will allow us to take accurate and timely decisions to choose the right drugs for the right patient.

Table-1: MS therapy, their modes of action and adverse effects

Therapeutics	Brand name	Mode of Action	Adverse effect
IFNβ	Rebif, Avonex	Reduce T_H1 pathology and inhibits T_H17 differentiation	Injection side reaction, flu-like symptoms
Glatiramer acetate	Capaxone	Promotes T_H2 polarization and activates M2 monocytes	Injection side reaction, flashing, chest pain
αVLA4	Tysabri/ Natalizumab	Inhibits the extravasation of immune cells over the BBB into the CNS	Multifocal leuko-encephalopathy (PML)
Corticosteroids	Medrol	Suppress immune response	Systemic side-effects
FTY720	Fingolimod	Spingosine1–phospate receptor modulator that inhibits lymphocyte egress from lymph nodes to CNS	Well tolerated with some non-specific adverse effects
Leustatin	Cladribine	Purine nucleotide analog causes leukocytes apoptosis	Lymphocytopenia and herpes zoster infection are common

1.4 EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

Obtaining human MS patient materials entails many ethical and practical issues. Animal models of human disease circumvent some of the practical issues and were developed in order to generate greater levels of understanding of basic disease pathology. Nowadays they are always employed in order to test the pharmacokinetics and pharmacodynamics of newly developed therapeutic agents to control disease.

In 1933, Experimental Autoimmune Encephalomyelitis (EAE) was introduced by Thomas Rivers in monkeys through multiple intramuscular injections of rabbit brain emulsion [69]. EAE is the most widely used animal model for human MS, mimicking the different histological and immunological features of MS. EAE can be actively induced in mice, rats, guinea pigs, marmosets, rabbits and primates, but its clinical and pathological outcome varies depending on the genetic background, antigen used for immunization or even the immunization protocol. EAE can also be induced passively by transfer of myelin–specific autoreactive T cells. Although EAE does not occur spontaneously, there are some TCR congenic models in which EAE can be developed spontaneously. EAE can otherwise be induced by immunization of the animal with different CNS antigens. These include Myelin Basic Protein (MBP), Myelin Oligodendrocyte glycoprotein (MOG) Phospholipid Protein (PLP), Spinal cord homogenate or peptides of these proteins along with adjuvant.

Although EAE is widely regarded as a useful animal model for MS there are some serious shortcomings regarding translating effective therapeutics from bench-to-bedside. IFNγ, anti-TNF-α and more recently a monoclonal antibody against CD28 were all tested in animal models and provided outstanding results, but the latter caused a disastrous side-effect (cytokine storm) in a small trial and was immediately postponed [70]. One reason for this discrepancy between mice and Man could be use of microbiologically clean and a genetically limited number of homogenous rodent strains. Another reason might be that while EAE and MS are both neuroimmunological syndromes, the underlying immunopathological mechanisms differ, EAE primarily supporting $CD4^+$ T cell-driven autoimmunity

while $CD8^+$ T cells are prevalent in MS lesions, for example.

1.5 T1D

Type 1 Diabetes is an autoimmune-mediated disease resulting from destruction of insulin-producing β-cells of the pancreas. As a result, insulin deficiency occurs in the body and needs to be supplied exogenously thereafter. This deficiency can lead to acute complications such as hyperglycemia and or ketoacidosis and chronic complications such as neuropathy, retinopathy and nephropathy [71-73]. T1D develops gradually in genetically predisposed individuals, taking months-to- years to develop full-blown disease. At the time of clinical debut the β-cell mass is nearly completely destroyed and insufficient to maintain metabolic function.

T1D typically develops in childhood or early puberty but can develop at any age. The common symptoms of untreated T1D include high blood sugar level, hyperglycemia and high sugar level in urine, excessive thirst (polydipsia), an increased frequency of urination (polyuria), increased appetite (polyphagia) despite weight loss and fatigue.

After the successful isolation of insulin from animals in 1921 by Fredrick Banting and Charles Best [74] led to insulin therapy, T1D is no longer considered a life-threatening or lethal disease although patients still develop complications. A significant percentage of patients develop serious chronic complications including microvascular diseases such as diabetic retinopathy (damaged retina), diabetic nephropathy, kidney diseases (e.g. glomerular nephritis), neuropathy and macrovascular diseases such as atherosclerosis and lipid disorders. Diabetic complications are the leading cause of morbidity and mortality worldwide and life expectancy can be reduced up to 10-15 years from T1D complications. Glycosylated haemoglobin HBA_{1C} is an important prognostic marker to assess the risk of developing secondary end organ complications. The abundance of HBA_{1C} in pediatric T1D patients is 8% higher than in other T1D patients [75].

1.5.1 Incidence and epidemiology

The incidence of T1D has steadily increased all over the world during the last 2 decades, the average increase being 2.5-3% worldwide [76]. The disease is more

prevalent in children aged below 5 and younger. Despite continuous effort to manage the disease current trends predict the number of T1D cases will have doubled by 2020 [77]. The incidence rate of T1D varies among different parts of the world, ranging from the lowest (0.1/100 000 per year in children ≤ 14 years of age) in China and Venezuela [78] and highest (64.2/100 000 per year in children ≤ 14 years of age) in Finland and Sardinia [79]. The incidence in Sweden is also very high at around 30/100 000 per year. This striking heterogeneity or disparity of incidence among the different countries is suggestive of not only genetic influences, but there must be environmental and epigenetic changes playing a vital role in susceptibility to T1D.

1.5.2 Genetic risk factors

T1D is a polygenic disease. Twin studies and family cluster studies revealed that genetic factors are an important determinant for susceptibility to T1D. The risk of having disease in an affected sibling is approximately 6% compared to an average risk of 0.4% for unaffected persons. The concordance rate for monozygotic twins is estimated at 21-53% and is much higher when one twin is diagnosed at an earlier age. Conversely, in dizygotic twins the concordance rate is around 16%. Human Leukocyte Antigen (HLA) on chromosome 6p21 is the main and strongest region associated with T1D. This region encrypts important functions of the immune system including T cell selection, antigen presentation and immune responses [80]. Two extended risk alleles DRB1*04-DQA1*03:01-B1*03:02 and DRB1*03:01-DQA1*05:01-B1*02:1 present around 90% cases of T1D either alone or in combination before age 18 [81]. Conversely, the DR15-DQA1*01:02-DQB1*06:02 allele is associated with strong protection which is carried by less than 1% in T1D cases. Genome-wide association studies and linkage analyses have identified more than 40 genetic variants that associate with T1D susceptibility [81]. Other than HLA, there are some important genes associated with T1D, including INS (insulin gene), which has a odds ratio of 1.25, interleukin-2 RA (*IL-2RA)* with an odds ratio of approximately 1.6, Protein Tyrosine Phosphatase Non- receptor 22 *(PTPN22)* odds ratio 2, and Cytotoxic T lymphocyte protein 4 (CTLA4) with an odds ratio of around 1.25.

1.5.3 Environmental Factors

The steady increase in the incidence of T1D across geographical areas and its undetermined inheritability both suggest a role of environmental factors in developing T1D. The environment might trigger or accelerate the disease process in genetically predisposed individuals. These shared environmental influences might be prenatal or related to diet, exposure to infection and other factors.

Many studies in animals or humans indicate that infectious agents such as viruses can be pathogenic or protective. Congenital rubella syndrome and enteroviruses, particularly rotaviruses, are linked with a higher incidence of early onset T1D. While some researchers have shown that Coxasackie B4 virus is detrimental for T1D, others show it can prevent T1D[82]. It is been suggested that virus infection can break immunological tolerance: i) by secreting pro-inflammatory cytokines such as IL-6, TNF-α, IL-8 that are harmful for β cells; ii) by molecular mimicry, e.g. the autoantigen GAD65 and enterovirus share conformational structures; and iii) increased presentation of autoantigens through bystander activation. There is growing evidence that commensal microorganisms play a role in developing T1D, especially *Bacteroides spp.* [83]. In regard to this infection hypothesis it has been observed that T1D only develops in NOD mice housed under sterile conditions [84]. Another interesting epidemiological observation is that the incidence of T1D is higher in developed countries in which hygiene is optimal and enterovirus infection is less, in contrast to underdeveloped countries in which the converse is true [85].

Dietary components such as cow's milk have been suggested as environmental triggers. A positive correlation has been determined between the consumption of milk and incidence of T1D in Northern European countries. It was also determined that early introduction of cow's milk increases the risk for T1D [86, 87].

Finally, epidemiological studies have demonstrated a strong relationship between Vitamin D and the incidence of T1D [88]. Vitamin D is an important immunomodulatory agent that is activated by sun exposure. Individuals in northern European countries such as Finland and Sweden in which winter sun exposures are limited produce less Vitamin D and have a higher incidence of T1D

than individuals in Southern Europe. Recent studies reveal that dietary supplements of Vitamin D reduce the incidence of T1D [86].

1.5.4 Pathogenesis of T1D

The immunopathogenesis of T1D is difficult to explore due to the relative inaccessibility of the human pancreas, making the animal model of T1D quite a good model. The initiation and progression of T1D has been defined in three main stages: i) insulitis or stage of recruitment of islet-specific T cells; ii) progressive stage or activation of APCs; and iii) accumulation of immune cells or full-blown disease stage [82]. After an insult in a genetically predisposed and environmentally susceptible individual, macrophages, dendritic cells, NK cells, $CD4^+$ and $CD8^+$ T cells infiltrate in the periphery of islets of Langerhans [89, 90] (Figure - 4). These cells secrete pro-inflammatory chemokines and cytokines, especially IL-1β, TNF-α, IL-12 and IFNs that destroy β-cells directly and activate macrophages.

These activated macrophages and DCs prime pathogenic islet-specific T cells in the pancreatic lymph nodes [91]. Chemokines, particularly CXCL10, CCL2 and CCL3, play a vital role in recruiting further monocytes and lymphocytes into the pancreatic islets. β-cells can themselves produce chemokines and their secretion is controlled by NF-κβ and STAT-1 transcriptions factors [92]. In NOD mice, depletion of CCL3 improves symptoms of insulitis and prevents development of T1D [93].

There are substantial indications that T cells are pivotal cells for initiation and progression of the disease. It was believed that only autoreactive $CD8^+$ T cells induced the disease but studies of NOD mice have demonstrated that both $CD8^+$ and $CD4^+$ T cells are essential for the pathology of the disease. It is assumed that $CD8^+$ T cells are more important at the beginning of the disease and that $CD4^+$ T cells are significant throughout the disease. It is unclear exactly how the T cells kill or damage β-cells, but it is assumed that killing by autoreactive $CD8^+$ T cells is perforin-dependent. The incidence of T1D in NOD mice lacking perforin is reduced and onset of the disease is delayed. Conversely, perforin alone is insufficient to cause the disease unless there are high amounts of IFN-γ and TNF-α produced [82]. The FAS pathway is another key regulatory mechanism for immune

mediated β-cell death and inhibition of autoreactive T cells [90].

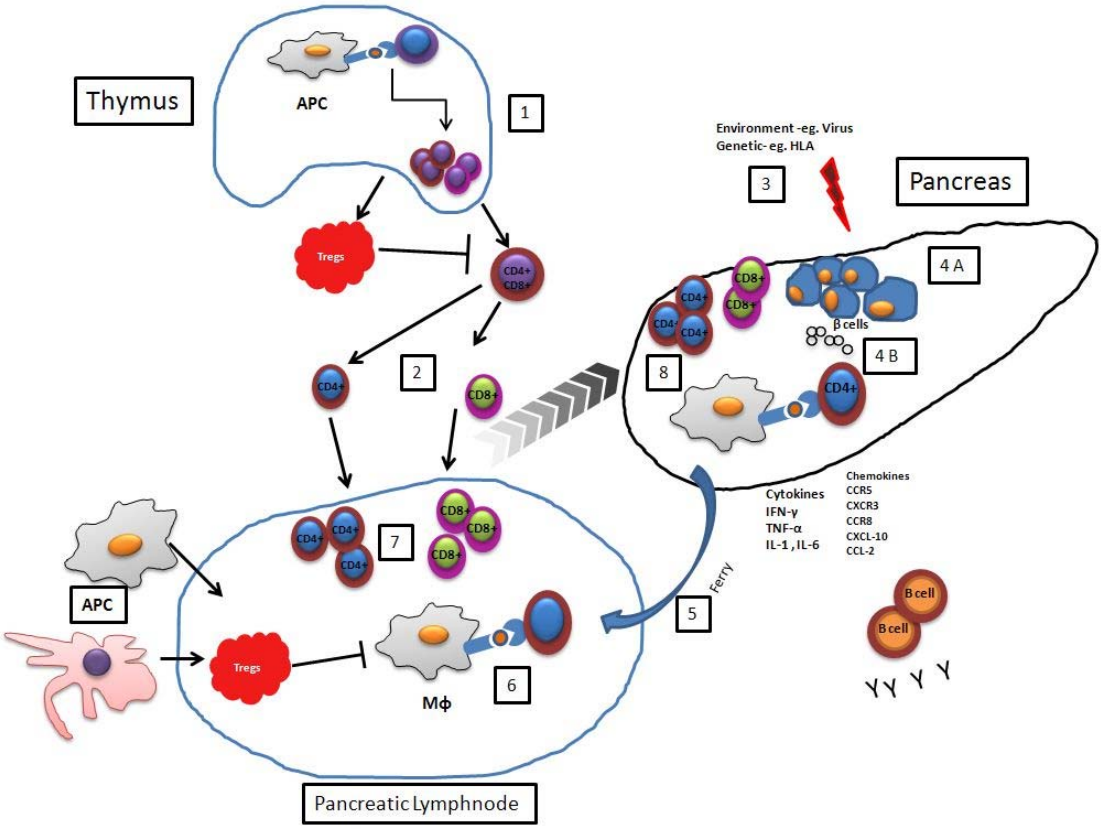

Figure 4: Pathogenesis of T1D: 1) escape negative selection; 2) autoreactive lymphocytes in circulation; 3) environmental trigger; 4A) activation of APCs and insulitis; 4B) β-cell apoptosis; 5) ferry autoantigen to pancreatic lymph nodes; 6) activation and 7) clonal expansion; 8) homing to pancreas and further damage to Islets of Langerhans

β-cell apoptosis might be the important form of death in T1D and the products of dying cells provides a potential danger signal to the immune cells during inflammation [92] (Figure - 4). Conversely, apoptotic cells in low numbers in a non-inflammatory environment might lead to induction of tolerance against β-cells.

It has been shown that B cells and NK cells are also involved in the development of T1D, depletion or inactivation of these cells preventing development of T1D in the mouse model [94]. NKG2D expressed by NK cells is associated with β-cell damage [95]. There is large consensus that T1D develops due to imbalance between pathogenic T cells and regulatory T cells.

At full-blown disease autoantibodies are present in 85-90% of T1D cases.

There are four main autoantibody specificities implicated in T1D, including antibodies against insulin, Glutamic acid decarboxylase (GAD65), tyrosine phosphatase-like protein (IA-2) and zinc T8 transporter (ZnT8). These autoantibodies can be detected for a long time (7-10 years) before disease diagnosis [73]. Measurement of islet autoantibodies is one of the screening tools for diagnosis, risk assessment and prognosis of T1D. Detection of at least two autoantibodies elevates the risk of T1D as high as 71% and with four autoantibodies the risk is even higher at around 90% [80].

1.5.5 Therapy for T1D

A life-long exogenous supply of insulin is the conventional treatment for T1D. The associated metabolic abnormalities lead to a significant proportion of patients developing chronic microvascular and macrovascular diseases and the risk of hypoglycemia and weight gain all add to a reduced life expectancy of 10-15 years [96]. A definitive cure for T1D thus needs to address induction of immunological tolerance and prevention of further destruction of β-cells. Numerous efforts to this end have been made and are ongoing to prevent both the emergence of T1D (primary prevention), to reverse it (secondary prevention) or to preserve the function of residual β-cells following diagnosis (tertiary prevention) [97].

Antigen-specific therapy has been proven safe in new onset T1D. The rationale of using insulin was to induce tolerance and to prevent development of autoantibodies [81]. Treatment with insulin was tested using different routes of administration, Parentral (DPT-1 trial) [98], oral (DPT-1, TrialNet) [99, 100] or intranasal (INIT, DIPP trial) [101]. While no clinical outcome was significant [81]. Oral administration was found to be effective in spontaneous and induced animal models [97]. The failure of insulin treatment in humans is thought to be related to the state of the disease and the dosages of antigen used. It might be possible that after the emergence of multiple antibodies, insulin cannot exert its effects to reverse autoimmunity [102].

GAD65 autoantibody reactivity seems to be great tool for diagnosis of T1D in modern clinical practice [97]. In mouse models, administration of GAD65 via mucosal and subcutaneous routes prevented development of T1D [103, 104].

Vaccination with GAD65 with aluminium hydroxide (alum; a conventional adjuvant) demonstrated positive results in patients with recent onset of T1D in Phase I and II clinical trials. The results of a Phase III were disappointing, there being no significant effects on preserving residual C-peptide (endogenous insulin production is measured by the levels of C-peptide levels), glycated hemoglobin level (HBA1c) or hypoglycemia rate [105, 106].

A number of immunomodulatory agents has been tried and is growing exponentially. In the past decade two humanized anti-CD3 monoclonal antibodies trials have been carried out with Otelixizumab and Teplizumab, respectively [107, 108]. These agents were designed to block the proliferation of effector T cells and subsequently stimulate the function of regulatory T cells to induce immunological tolerance. Unfortunately, in Phase II clinical trials both failed to preserve C-peptide, to lower HBA1c levels and to reduce insulin usage [109]. Both drugs are associated with self-limiting transient lymphopaenia fever, arthralgia and impaired liver functions [110]. Similarly, with anti-CD20 Rituximab [111] and CTLA4-Ig (Abatacept) [112], after initial transient maintenance of insulin secretory functions the effects were lost in Phase II clinical trials. Anti-cytokine therapy such as anti–interleukin 1 (Anakinra) anti-TNF-α has been used in a number of autoimmune diseases. The aim of using anti-cytokine therapies is to readdress the T_H1 vs. T_H2 pattern [113]. In murine models anti-TNF and IL-1 therapy were found to be protective but translation of these finding to humans is a major challenge.

Experimental models and recent clinical trials indicate that islet transplantation (using the 'Edmonton protocol') could be a viable alternative to current treatment options despite some serious issues. Shortage of donor pancreata, islets and MHC-mismatched allografts make transplantation inefficient in a long-term perspective [114, 115]. Stem cell therapy in the generation of new β-cells might be a promising reality, but again, ethical issues regarding generation of *in vivo* functional β-cells and the prolonged usage of immunosuppressive drugs to overcome the immune response and rejection of allogeneic tissue will be major challenges to resolve [116]. Alternative approaches to cell therapies may thus be warranted.

1.6 MACROPHAGES

Macrophages have been considered as indispensable effector cells of the immune systems. Eli Metchnikoff discovered 'phagocytes' 100 years ago and received the Nobel prize. It was believed that the human monocyte is the only precursor of tissue macrophages, but growing evidence has revealed that tissue resident macrophages actually self-maintain in a stochastic manner with minimum contribution from circulating monocytes in steady state [117]. By using fate-mapping, parabiosis studies and adoptive transfer of total bone marrow (BM) cells, it was demonstrated that monocytes are not the progenitor cells for lung, splenic red pulp, peritoneal or bone marrow (BM) tissue macrophages in the steady state [118, 119]. Congenital monocytopenia or reticular dysgenesis is a rare human disease in which the monocyte population decreases but the tissue macrophage number remains normal [120]. All this evidence suggests that tissue resident macrophage development is not monocyte-dependent. It is immensely important to know the role of tissue macrophages and BM-derived macrophages in homeostasis, inflammation and repair.

In mice, macrophages are derived from at least three different lineages, (Figure - 5) starting from the primitive ectoderm of the yolk sac that populates $F4/80^{hi}$ tissue resident macrophages such as brain, splenic, liver, pancreatic and lung macrophages [119]. This lineage is controlled by CSFR1 and its ligands IL-34 and CSF1 [121]. After that the fetal liver takes over the haematopoiesis and gives rise to Langerhans cells. The most important lineage, BM gives rise to circulatory monocytes ($Ly6C^{+}$) and subsequent progeny such as $F4/80^{low}$ tissue macrophages, DCs and patrolling monocytes ($Ly6C^{-}$) [122].

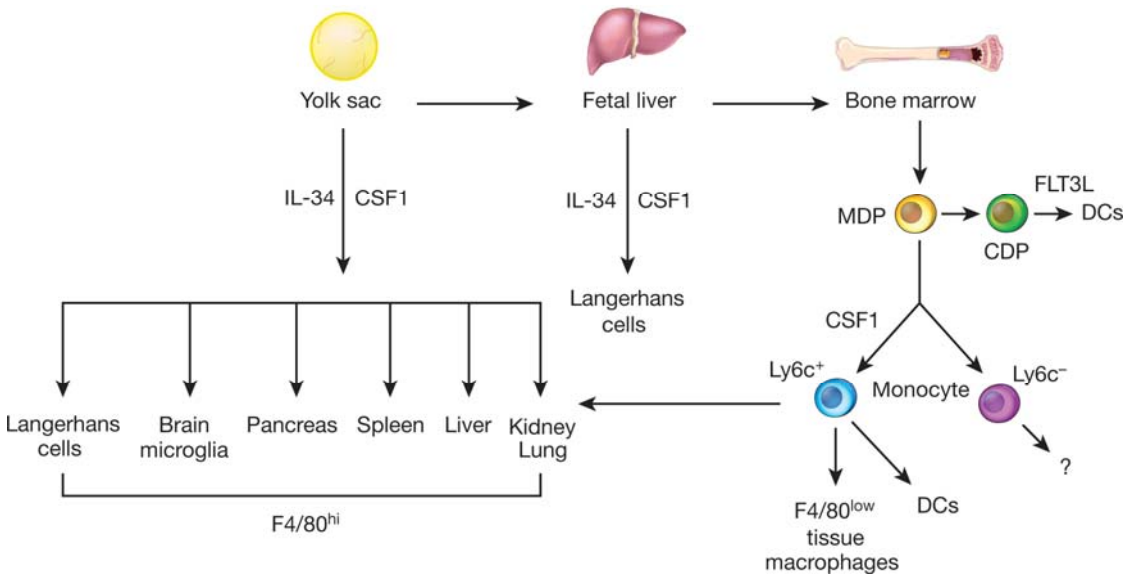

Figure 5: Macrophage lineages in mice [122] In steady state, murine macrophages develop from at least three sources. Yolk sac gives rise to microglia pancreatic, red pulp splenic macrophages, liver, kidney and lung macrophages. Langerhans cell develop mainly from fetal liver. Circulating monocytes and DCs developed from bone marrow.

Plasticity and diversity are the hallmarks of the monocyte–macrophage lineage. Functional classification of macrophages depends on the state of inflammation. Both in human and rodents, macrophages are defined into Classically activated or pro-inflammatory (M1), and Alternatively activated or anti-inflammatory (M2) macrophages, although it is clear that in reality the classification is not black-and- white and there is an overlapping spectrum of activation phenotype [123].

M1 macrophages are induced by the Th1 cytokine IFNγ and microbial products such as LPS. The M1 phenotypes are potent effector cells capable of killing intracellular microorganisms including viruses and tumor cells [124]. They can secrete copious amounts of pro-inflammatory cytokines including IL-12, IL-1β, IL-15, IL-18, TNF-α [125] and chemokines such as CCL15, CCL20 and CXCL8-13 [126]. In addition, M1 cells can produce nitric oxide (NO) through inducible NO synthase (iNOS) and express high levels of MHC II and co-stimulatory molecules CD80/CD86, thereby enhancing antigen presentation and adaptive immune response to microorganisms and tumors [124, 127].

Conversely, M2 macrophages were named because they could be induced 'alternatively' by IL-4 instead of IFNγ [128]. These alternatively activated macrophages produce anti-inflammatory cytokines, scavenge cellular debris and apoptotic cells, and promote the processes of angiogenesis, tissue remodelling and repair [124, 129].

Currently, M2 macrophages can be generated in culture in at least 3 different ways, yielding M2a, M2b and M2c subsets. These different subtypes of M2 macrophages exhibit different functions but in general they have the common phenotype of producing low to absent pro-inflammatory cytokines and poor production of NO.

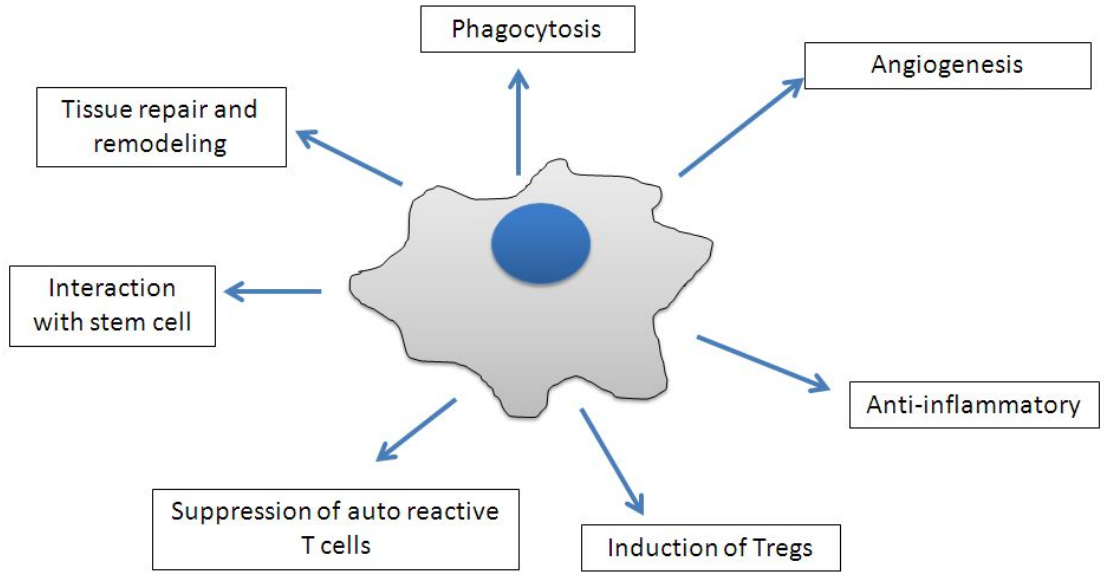

Figure 6 General functions of macrophages

M2a macrophages are activated by IL-4 or and IL-13 and secrete anti-inflammatory IL-10 and chemokines (CCL17/18/22/24), express a high level of mannose receptor (MR; CD206) and exhibit arginine 1 (Arg-1) activity. They are involved in killing extracellular pathogens including parasites [124], efferocytosis, angiogenesis, tissue remodelling and wound healing (Figure - 6). M2b macrophages are induced by immune complexes together with toll-like receptor ligands, secrete large amounts of IL-10, low levels of IL-12 and TNF-α and IL-6, and express higher levels of CD80/CD86. M2c macrophages are activated in the

presence of anti-inflammatory stimuli such as IL-10, glucocorticoid hormone or transforming growth factor (TGF-β) and they are also known as *deactivating macrophages*. M2c macrophages produce suppressive cytokines such as IL-10 and TGF-β and can suppress T cell proliferation and promote Tregs [130]. This phenotypic classification of macrophages is very unlikely to represent the complex *in vivo* state of macrophage activation in different diseases. In mouse studies a more flexible and broad classification has been suggested which encompasses a range of overlapping functions encompassing classically activated macrophages, wound healing macrophages and regulatory macrophages [123].

1.7 MONOCYTES

Monocytes are dynamic and versatile mononuclear phagocytes and are involved in steady state homeostasis, innate immune surveillance, establishment and resolution of inflammation [131, 132]. Monocytes are large circulating cells that comprise 5-10% of total circulating lymphocytes in humans and 4% in mice. Monocytes originate from the BM and can circulate into the blood or reside in the spleen as a reservoir before mobilization during an inflammatory process [133]. A recent study reported the possibility of monocyte development outside the bone marrow as an extramedullary hematopoiesis during atherosclerosis [134]. In 1939 Elbert and Florey reported that monocytes emerge from the blood circulation and give rise to tissue macrophages [135]. Accumulating evidence now indicates that tissue macrophages in mice in steady state do not derive from circulating monocyte but are rather derived from an extra-hematopoietic origin in the yolk sac or fetal liver [118, 119].

In 1989 Ziegler-Heitbrock and colleagues reported [136] that human monocytes can be distinguished into two main subpopulations on the basis of expression of cell surface antigens CD16 (Fcγ receptor III) and CD14 (a receptor for lipopolysaccharide): classical ($CD14^{++}CD16^{-}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes. Classical monocytes comprise 85-90% of the total circulating monocyte pool and the remaining 10-15% are non-classical or patrolling monocytes [137].

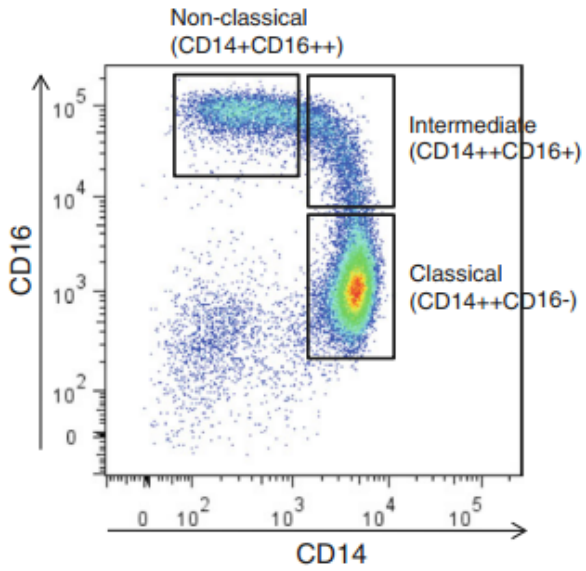

Figure 7: Sub-populations of human monocytes. Human monocytes are subtyped according to CD14 and CD16 expression into 3 major sub-populations:

1. Classical ($CD14^{++}$ $CD16^{-}$)

2. Intermediate ($CD14^{++}$ $CD16^{+}$) and 3. Non-classical ($CD14^{+}$ $CD16^{++}$)

However, Grage-Griebenow and colleagues reported that $CD16^{+}$ monocytes consist of at least two subpopulations with discrete functions [138]. Thus the intermediate population ($CD14^{++}CD16^{+}$) that express both CD14 and CD16 was first defined. Phenotypic and functional differences between the three subsets of human monocytes are depicted in Table-2. Numerous terms and assigned functions have been used by different research groups, making the monocyte subpopulation definitions confusing. This confusion was amplified when the heterogeneity of murine monocyte subpopulations was compared and contrasted with human monocyte subpopulations. Some researchers determined classical monocytes to be the most proficient producers of TNF-α, IL-1β, IL-6, CCL2 and CCL3 in response to LPS [139]. Conversely, Rossel *et a*l [140] reported that the intermediate subpopulation produces more TNF-α, IL-1β and IL-6.

However, Wong *et al* [141] found that non-classical monocytes are the highest producers of TNF-α and IL-1β. Similarly, there is inconsistency regarding IL-10 secretion. Skrzecynska-Moncznik *et al* [142] determined intermediate monocytes as the highest producer of IL-10 in response to LPS and zymosan, but recent studies report that classical monocytes produce the highest amounts of IL-10 [141, 143].

Table-2: Phenotypic and functional differences between the three subsets of monocytes

	Classical CD14^{++}CD16-	**Intermediate CD14^{++}CD16^{+}**	**Non-classical CD14^{+}CD16^{++}**
Approximate proportion of total monocytes	85%	5%	10%
Cytokine secretion in response to LPS	IL-10, TNF-α, IL-1β higher acc. to Cros *et al* [139]	IL-6, IL-18 TNF-α, IL-1β higher acc. to Rossel *et al* [140]	TNF-α, IL-1β higher acc. to Wong [141]
Surface markers	Higher CCR1, CCR2 CD62L Lower CX_3CR1 MHC II	Higher CX_3CR1, MHC II Lower CCR1, CCR2, CD62L	Higher CX_3CR1 MHC II Lower CCR1, CCR2, CD62L
In relation to disease	Higher CD14 co-related with disease severity and poor outcome	Increased in acute and chronic inflammation and infectious disease.	Increased in acute and chronic inflammation and infectious disease.
Functional heterogeneity	Superior Phagocytosis, anti-apoptotic	Pro-angiogenic, T cell proliferative and stimulatory	Patrolling pro-apoptotic, T cell proliferative and stimulatory

One possible explanation for these varying results from different research groups might be the different isolation procedures and different gating strategies employed. In order to resolve the confusion in 2010 the International Union of Immunological Societies (IUIS) proposed a consensus nomenclature for different subsets of monocytes [144].

As in humans, mouse monocytes are also phenotypically and functionally heterogeneous and can be differentiated on the basis of variable Ly-6C

expression. Ly-6C^{high} comprise 50-60% of mouse monocytes in the steady state and express higher CCR2, adhesion molecule L-selectin (CD62L) and lower chemokine CX_3CR1 levels, being phenotypically more equivalent to human classical ($CD14^{++}CD16^{-}$) monocytes. During inflammation the number of Ly-6C^{high} CX_3CR1^{low} $CD62L^{+}$ increases and they produce a higher amount of TNF-α, IL-1β. [145]. Conversely, the Ly-6C^{low} CX_3CR1^{high} $CD62L^{-}$ subset patrols the vasculature in steady state and more phenotypically resembles human $CD16^{+}$ cells [146].

Questions might arise whether monocytes are effector cells or just developmental intermediate precursor cells between bone marrow and tissue macrophages. It is now well accepted at least in the mouse that tissue macrophages can be replenished other than from bone marrow-derived monocytes in steady state. Conversely, during inflammation bone marrow-derived monocytes can respond to pro-inflammatory cues and are postulated to infiltrate inflamed tissue and differentiate into macrophages and DCs [147, 148]. During infection with *Listeria monocytogenes* Ly-6C^{high} CX_3CR1^{low} $CD62L^{+}$ monocytes differentiate into DCs that produce high amount of TNF-α, NO, ROS (known as Tip DCs). Interestingly, the Ly-6C^{low} CX_3CR1^{high} $CD62L^{-}$ subset has been reported to differentiate into M2-like (alternatively activated) macrophages in the presence of the same pathogen (*Listeria monocytogenes*)[147-149]. Similar findings have been observed in a spinal cord injury model [150] and in myocardium healing [151].
In conclusion, different subsets of monocytes can differentiate into either DC or macrophages that can be activating or inhibiting depending upon the nature of an infection or the local environment.

1.8 MICROGLIA

Microglia was first distinguished as resident immune cells in the CNS by Pio Del Rio Hortega in 1919. He named microglia from the Greek work 'Glia' that means 'glue' to provide support to other neural cells [152]. Their principal functions are to respond quickly against microbial invaders in the brain. They are distributed throughout all parts of the brain and they constitute 5-20% of the total glial cell population within CNS [153].

Microglia have been somewhat mysterious cells of the immune system. Despite intensive research the precise origin of the cells has long been debated. Two major hypotheses were equally convincing. One school of researchers considered microglia derived from myeloid progenitors that populated the brain pre-natally or post-natally [154]. In contrast, another school demonstrated that microglia actually have a mesodermal origin [155]. It is easy to speculate that microglia should be derived from the monocyte lineage due to their high similarity in marker expression, phagocytosis behaviour and activation pattern. But recent accumulating evidence suggests more and more that microglia originate from the primitive yolk sac (Figure-5)[156, 157].

Ramified microglia are known to be resting microglia. Almost any manipulation in the form of inflammation or infection to the brain quickly activates microglia and converts them into an amoeboid form. Along with morphological transformation, microglia express a diverse array of cell surface and intracellular receptors in response to invaders. A body of evidence indicates that like monocytes and macrophages, microglia also adopt different M1 and M2 activation states, the former being associated with destructive neuroinflammation and the latter with its resolution.

During neuroinflammatory or neurodegenerative conditions different activation states of microglia can co-exist. For example, a higher expression of pro-inflammatory and anti-inflammatory gene profiles in microglia in the vicinity of Alzheimer's amyloid deposits has been reported.

Therefore, some degree of classical activation is required to limit the Aβ deposit despite the collateral damage [158], and alternatively activated microglia can restore the normal functions of the surrounding with greater risk of Aβ deposition. Along with coexistence, another important determinant of microglial phenotype is the timing of their activation during various phases of diseases. In the EAE model, inhibition of microglial activation through tissue plasminogen knockout leads to delayed onset of the disease but increased severity and delayed recovery from neurological symptoms [159, 160].

Table- 3: Microglia function in healthy and disease state

Source: Modified from Luo ad Chen, 2012 [161]

		Conditions	Microglia function
In steady state		Healthy resting state	Surveillance, homeostasis, fixed cell and motile processes, minimal expression of cell surface markers and release of cytokines and chemokines, not involved in phagocytosis
In disease state	Neuro-protective	Axotomy of the optic nerve	Efficient clearance of myelin debris
		Traumatic injury	Clear glutamate without evoking inflammatory mediators
		Ischemia	Synthesis of tumor necrosis factor, engulfment of harmful invading neutrophil granulocytes
		Alzheimer's Disease	Internalize and degrade amyloid beta
		Multiple sclerosis	Secrete soluble mediators that trigger neural repair and usually contribute to the creation of an environment conductive for regeneration
	Neuro toxic	Parkinson's disease	Releasing various kinds of noxious cytokines, reactive oxygen species
		Multiple Sclerosis	Express iNOS and generate toxic ROS which might injure neurons
		Alzheimer's Disease	Produce of chemokines, neurotoxic cytokines and reactive oxygen and nitrogen species that are deleterious to the CNS

Heterogeneity of microglial function, whether it be neuroprotective or neurotoxic, depends upon a number of determinants such as type of injury or

inflammation, different phases of disease, crosstalk with other regulatory components including brain cells and infiltrating T cells, and last-but-not-least age–related dysfunctions of microglia. A number of studies have shown that neurons send 'on' and 'off' signals that influence microglial activation. Moreover, released factors from neurons can facilitate the survival of microglia [162-164]. The normal ramified morphology of microglia is replaced by 'dystrophic' microglia in the ageing brain. A number of studies have shown that senescent microglia not only have a significantly reduced size of telomere length, a marker of ageing, but also an altered inflammatory profile in that they convert from a neuroprotective phenotype to a neurotoxic phenotype [165, 166].

During CNS insults microglia become activated and monocyte-macrophages infiltrate the CNS and accumulate at the site of injury and contribute to both damage and repair [167]. Due to a lack of a microglia-specific marker, within CNS lesions it is difficult to distinguish between activated microglia and macrophages because both exhibit similar morphologies and express many similar markers. Despite this super similarity, microglia and macrophages have recently been demonstrated to have some differences, which are listed in Table-4.

CCR2 expression was determined to be low in microglia from both healthy and diseased animals compared to in macrophages [168]. Moreover, microglia can be discriminated from monocytes on the basis of surface marker expression of Ly6C [169]. Microglia can been isolated by their relatively low expression of CD45 compared to peripheral blood monocytes or macrophages [170], but using this marker it is difficult to distinguish infiltrating macrophages from local microglia in a tumour setting [171]. Microglia can present antigen to T cells and can express MHC II like other APCs, although it has been shown that during antigen presentation microglia express low or absent levels of co-stimulatory molecules.

Table- 4: Difference between microglia and monocytes-macrophages

Microglia	Monocyte-macrophage
Microglia require different growth factors. Use IL-34 for their maintenance [172]	Monocyte–macrophages use CSF-1 for their growth and maintenance
Microglia are $CD11b^{+}$ and $CD45^{low}$ [170]	Monocyte-Macrophages are $CD11b^{+}$ and $CD45^{high.}$
Microglia express higher Galectin-3, TGF-β receptor 1, and TREM2 [173-175]	Comparatively lower expression

1.9 IMMUNOTHERAPY

In 1796 Edward Jenner discovered vaccination against smallpox and started one of the greatest revolutions in the history of immunotherapy. This journey has continued and immunotherapeutic strategies for treating different diseases, including autoimmune diseases, have emerged as novel therapeutic options. Immunotherapy is a special type of immunological approach that uses immunological tools to modulate the disease outcome. Due to broadening of our understanding regarding the cellular and molecular processes involved in diseases, the specific targeting of immune molecules has been increased.

Targets that have received the most attention include i) cytokines that shape the nature of the inflammatory process, ii) various cell types that mediate the damage at the site of insult, iii) enzymes that are critical for breaking the extracellular matrix or helping penetration of blood vessels, iv) surface receptors and immunoglobulins that initiate or inhibit cell signalling and other toxic mediators including complement components and NO [176].

1.9.1 Cytokines

Isaacs and Lindenmann discovered IFN cytokines that protect cells from viral infection in 1957. After that ground-breaking discovery cytokine research has had great momentum and since then more than 90 cytokines and cytokine receptors have been identified [177]. Cytokines are low molecular weight proteins with a wide range of actions including T cell growth (IL-2, IL-4, IL-17, IL-15 and

IL-21), inflammation (TNF-α, IL-6, IFN-γ, IL-1, IL-17, GM-CSF) and inhibition of inflammation (IL-10, TGF-β, IL-4), tissue repair or wound healing (IL-4, IL-13, TGF-β) [176]. In autoimmune diseases treatment with cytokines is generally limited, in contrast to blocking of cytokines. A number of cytokines blocked by monoclonal antibodies have been approved and available for therapy.

1.9.1.1 TNF-α

TNF-α is one of the most extensively studied and clinically used cytokines. More than a million patients have been treated with anti-TNF [176], particularly those with Rheumatoid Arthritis. The pathogenic role of TNF-α has been observed in many diseases and confirmed in animal models of Rheumatoid arthritis, MS sepsis, Inflammatory bowel diseases (IBS) and Psoriasis [177]. There are different TNF-α blocking agents that are effective in different forms of RA. Infliximab, a mouse human chimeric antibody, was first introduced and subsequently Adalimumab (Humira) a fully humanized antibody (to reduce immunogenicity) was developed. It has been shown that TNF-α blocking antibody has increased efficacy if administered along with the immunosuppressive drug methotrexate [178].

Although TNF-α blocking antibody is effective for many patients with arthritis, unfortunately not all patients respond equally [179, 180], and paradoxically TNF-α blocking agents increase frequency [181] demyelination and disease activity in progressive MS [182] or other autoimmune diseases [183]. The possible explanation is that TNF receptor construct fail to penetrate the BBB during disease process.[184]

Along with benefits there are some adverse effects that outweigh the risk benefit ratio. The most common side-effect of TNF-α blocking antibody is an increased risk of infection. But it is hard to measure whether disease itself increases the susceptibility or it is due to treatment, for example reactivation of tuberculosis [185] and pneumonia [186]. Moreover, a meta-analysis of TNF-α blocking agent studies revealed that it might enhance cancer risk, although this is not proven [187].

1.9.1.2 IL-6

IL-6 is another important cytokine that plays a vital role in many human autoimmune diseases and their animal models. IL-6 has a detrimental role in development of EAE [188, 189], plays a key role in Collagen-Induced Arthritis (CIA)[190] and it has been shown that IL-6 knockout mice were resistant to developing autoimmune myocarditis [191]. IL-6 has a differential role for induction of pathogenic T_H17 cells so blocking of IL-6 also has synergistic effects on IL-17-induced autoimmune diseases. In several models of IBD the severity of these models is markedly reduced in IL-6 deficient mice [192]. Tocilizumab is a humanized antibody against membrane bound IL-6Rα that is effective when combined with methotrexate in patients unresponsive to TNF-α blockers [193-195]. A recent study has reported that tocilizumab monotherapy is better than methotrexate in RA treatment [195] and juvenile idiopathic arthritis [196].

1.9.1.3 IFN

Although IFNs are known to be capable of blocking viral replications, growing evidence suggests that these cytokines are involved in immune pathogenesis of different autoimmune diseases. Type 1 IFN levels are higher in sera of patients with systemic autoimmunity [197]. INF-γ (Type II) producing T_H1 cells have been associated with many autoimmune diseases including MS, RA, SLE and psoriasis. INF-β is the first line of treatment for RRMS, although half of the patients are non-responsive. In experimental models IFNβ blocks T_H1-induced EAE but exacerbated T_H17-induced EAE [198]. Similarly INF-γ was protective in EAE [199] but its blockade worsened the diseases [200], suggesting that T_H1 cells might not be the driving force for organ-specific autoimmune diseases. Nevertheless, Steinman in his most recent review argued against that view [201].

1.9.1.4 IL-1

The IL-1 family consists of a group of cytokines that play vital roles in inflammation by acting hand-in-hand with IL-6 and TNF-α. Anakinra, a recombinant human IL-1 receptor antagonist, has been demonstrated to be

modestly effective against RA. Inhibition of IL-1 improves β-cell function in the pancreas and therefore IL-1 blockers are attractive agents in T1D [202].

1.9.1.5 IL-17

IL-17 is pro-inflammatory cytokine that has been in the core of discussion for contributing to the pathogenesis of different inflammatory diseases. T_H17 cells are a major source of IL-17 and have been linked to many autoimmune diseases such as RA, MS, Myocarditis, IBD and Psoriasis. AIN457 and LY2439821 are two humanized monoclonal antibodies against IL-17 that were reported to be safe and efficacious against RA [203]. However, the response rate was lower compared to TNF-α and IL-6 inhibitors. IL-17 and T_H17 cells have a prominent implication in the induction of EAE, IL-17 targeted vaccines preventing EAE [204].

1.9.1.6 Other cytokines

IL-22 is a member of the IL-10 family and mainly produced by T_H17 cells. Contemporary studies have revealed that IL-22 plays a critical role in many autoimmune diseases including RA [205], MS [206] and SLE [207]. Antibody against IL-22 might have an ameliorating action in autoimmune diseases.

It has been shown that GM-CSF has been associated with MS, RA and IBD and their animal models. Blocking antibodies against GM-CSF protect from RA and EAE. Recent studies have reported that GM-CSF can ameliorate dextran sodium sulphate-induced colitis [208].

IL-23 is a heterodimeric cytokine composed of p19 and p40 subunits. The p40 subunit is shared with the IL-12p35 subunit of IL-12. Mice lacking IL-23 are resistant to developing EAE [209] and CIA [210]. The Ustekinumab monoclonal antibody against the p40 subunit of both IL-23 and IL-12 is effective against psoriasis [211], psoriatic arthritis [212] and Crohn's disease [213].

1.9.2 Monoclonal Antibodies

In 1975, Köhler and Milstein reported the hybridoma technology for production of mouse monoclonal antibody [214]. That brought a revolution in the

field of immunology, serology and cell biology. Since then, due to advancement of molecular engineering technologies the transition from first mouse to chimeric, and then to fully humanized monoclonal antibody, has taken place to avoid immunogenic mouse components [215, 216]. Monoclonal antibodies are highly specific and they possess long half-lives which enables precise action and allows infrequent dosing [217].

However, a wide range of adverse effects is associated with monoclonal antibody therapy. In Table-5 some of the important monoclonal antibodies, their indications and possible side-effects have been outlined.

1.9.3 Cell Therapy

Cell therapy has been pioneered for the treatment of malignancies and is now being tested in autoimmune diseases, especially the transfer of autologous hematopoietic stem cells. Different cells are involved in the pathogenesis of autoimmune diseases but autoreactive T and B cells play a particularly vital role. Conversely, preliminary studies indicate that Tregs, regulatory macrophages and DCs and mesenchymal stem cells can all be a feasible and potential therapeutic option to induce tolerance for many diseases. For the sake of interest, in the following section some of the potential cells for immunotherapy in autoimmunity will be discussed.

Table-5: Monoclonal therapy in autoimmune diseases

Target	mAb	Type	Indication	Side effects
α4 integrin	Natalizumab (Tysabri)	Humanized	RRMS [218] IBD	Hypersensitivity reactions, PML,[218] Hepatotoxicity
CD20 on B cells	Rituximab (Rituxan)	Chimeric	MS, [219]RA Non Hodgikn's Lympoma	Acute infusion reaction, Immunogenecity, PML
CD3 antigen on T cells	Muromonab	Mouse	Acute resistant allograft rejection, T1D[107]	Cytokine storm [220] Severe acute infusion reaction, Immune-suppression and infection, immunogenicity
CTLA-4	Ipilimumab		IBD	Hypersensitivity, enterocolitis, dermatitis
CD25	Daclizumab	Humanized	Transplant allograft rejection Autoimmune uveitis[221], MS [222]	Hypersensitivity, colitis
CD52 On B, T, and NK cells	Alemzutumab	Humanized	MS [222] B cell lymphocytic leukaemia, Graft-versus-host diseases	Infusion reaction, hypersensitivity and immunogenicity, cytokine storm
CD28	TGN1412	Humanized	B Cells Lymphocytic Leukemia	Cytokine storm

Table is modified from [223]

1.9.4 T cell-based Immunotherapy

Both $CD4^+$ and $CD8^+$ cells are involved in MS, T1D and RA. $CD4^+$ T cells can be effector cells or regulatory cells depending upon the role they play in the immune response. Effector $CD4^+$ T cells is subdivided into Th1, Th2 and more recently discovered Th17 cells according to the expression of signature cytokines and their transcriptional factors. Th1 cells are induced by IL-12 and they produce INF-γ. It has been shown in the EAE animal model of MS that blocking of INF-γ prevented disease, but that antibody against INF-γ in human exacerbated disease [224]. Similarly, inhibition of IL-12 (IL-12p35) does not infer resistance to development of EAE [225] because it shares the p40 subunit with IL-23. Gene deletion of both subunits of IL-23 prevents development of disease [209].

IL-23 is a potent inducer of T_H17 and recently it has been shown that T_H17/Th17 is more important in pathogenesis of MS. Th17 cells can be blocked by polarizing cytokines such as IL-6, IL-1β and IL-23 or effector cytokines like IL-17, IL-22, IL-23 [226, 227] and transcriptional factor ROR-γt. IL-2 acts as a growth factor for T lymphocytes that stimulates their clonal expansion and maturation. Blocking of IL-2 receptor deactivated T cell proliferation and improved clinical manifestations in EAE [228].

T-cell anergy is another option to deactivate autoreactive T cells. Anti-CD3 monoclonal antibody Muromonab suppresses T cell activation and proliferation and consequently ameliorates disease [229-231]. Antigen-specific therapy may restore self- tolerance and inactivate autoreactive T cell by promoting $CD4^+$ T cell apoptosis, anergy or deletion through TCR crosslinking. Although it is a highly specific therapeutic approach, due to the heterogeneous nature and variability of self-reactive antigens implicated in individual disease processes then this approach has yet to be fully efficacious in any setting of autoimmunity. For example, oral dosing of myelin basic protein (MBP) efficiently induced dose-dependent tolerance in EAE [232] but in clinical trials did not show any significant result [233-235]. Likewise, depletion of $CD4^+$ T cells using anti-CD4 antibody ameliorated EAE but was ineffective in MS [182].

Regulatory T cells (Tregs) are immensely important for induction of tolerance. Functional abnormality as well as decreased frequency of Tregs is well documented in different autoimmune diseases both mice and humans, including T1D, SLE RA and MS [236-238]. Tregs might therefore be a major therapeutic target. Ideally, Tregs therapy can be achieved either by increasing the endogenous Tregs population by *in vivo* administration of TGF-β, retinoic acid low doses of IL-2, Rapamycin and Cyclosporin [198, 239], or alternatively by isolating and expanding *in vitro* antigen-specific Tregs and then adoptively transferring them into patients [236, 240].

There are number of hurdles needed to be overcome before Tregs cell therapy can be adopted routinely. Firstly, the major flaw of Tregs therapy is to establish a protocol that enables a stable and sufficient amount of pure Tregs for adoptive transfer. Secondly, it has been reported that Tregs are highly antigen-specific and that they can switch their functional phenotype to pathogenic T cells [241, 242].

1.9.5 Targeting other cells

Although T cells have received extended attention regarding the development of many autoimmune diseases for decades, B cells have recently received serious attention, especially after the outcome of B cell depleting antibody Rituximab treatment in RA [243]. Apart from RA, B cells are critically involved in the pathogenesis of SLE, Sjögren's syndrome and MS [244].

In more recent years, stem cell therapies and particularly mesenchymal stem cells (MSC) have been emerging in autoimmune diseases as promising tools for immunotherapy. Initially, *in situ* differentiation of these cells was thought to be the basis of treatment, but now their immunomodulatory properties strongly support their therapeutic application. Emerging evidence indicates that MSC therapy in several animal models is beneficial. For example, in different models of EAE the administration of MSC delayed onset of disease and reduced demyelination [245-248]. In a small pilot study of advanced MS patients, autologous bone marrow-derived MSCs were given intrathecally, 50% of the patients having benefit [249]. Beneficial effects of MSC therapy have also been reported in animal models of SLE

[250], CIA [251] and T1D [252]. Although current experimental studies and pilot clinical data indicate that MSC therapy is feasible and promising, a long-term follow-up will be necessary to draw a conclusion regarding potential safety and efficacy [253].

1.10 NRAMP1/SLC11A1

A clear difference in disease susceptibility has been observed in endemic areas of disease and epidemics including mycobacterium tuberculosis and leprosy. The same susceptibility differences are viewed in several rodent strains after immunization to induce EAE. Nearly 40 years ago mouse susceptibility to *Salmonella typhimurium, Leishmania donavani, and Mycobactarium bovis* was determined by a host genetic factor [254]. This genetic factor Nramp 1 (Natural resistance-associated macrophage protein 1) or Slc11a1 (solute carrier family 11 member 1) was mapped by genetic linkage analysis and reverse genetic strategies as a candidate gene that has been shown to influence early phase of bacterial replication in reticuloendothelial cells. Later, positional cloning of Nramp1 identified *Bcg, Ity* and *Lsh* are being identical to Nramp1. A single nucleotide polymorphism (SNP) gives rise to two allelic forms that are associated with resistance (^{r}) or susceptibility (^{s}) to infection with intracellular bacteria. Monocytes, macrophages and polymorphonuclear cells express Nramp1 on their late endosomal and lysosomal membranes [255].

Nramp1 functions as a divalent cation transporter and transports iron into phagosomes. Susceptibility of many intracellular parasites depends on intra-phagosomal iron availability. Nramp1 contributes to the antimicrobial functions of macrophages by pumping the iron from phagolysosomes of infected individual towards the cytoplasm and thereby depriving microorganism of essential growth factors that prevents their replication. Alternatively, it has also been shown that Nramp1 concentrates iron within phagosomes, where, together with low pH generates ROS via the Fenton reaction to kill invading microorganism [256, 257]. Nramp1 also plays a role in macrophage activation. It regulates the secretion of IL-1β, TNF-α CXC chemokines, iNOS and NO [256]. In humans, SLC11A1 is associated with various autoimmune diseases including MS [258], RA, Sarcoidosis and Crohn's diseases [257].

2 AIMS OF THE THESIS

This thesis aimed to establish and characterise a stable suppressive and immunomodulatory phenotype of myeloid cells, especially monocyte-macrophages and microglia with a view to adoptive transfer in autoimmune diseases including MS and T1D.

Specific scientific goals were:

Study 1: To compare macrophage activation states between two mouse strains with different genetic backgrounds.

Study 2: To discern optimal suppressive macrophage activation phenotype and to investigate their role in a T1D model.

Study 3: To determine similar phenotypes of microglia and to investigate their role in a MOG-induced EAE model.

Study 4: To characterize and determine the optimal human macrophage adoptive transfer protocol for immunotherapy.

3 METHODOLOGICAL CONSIDERATION

Materials and methods used in this thesis are extensively described in each respective article. In the following section different aspects of some of the methods will be reviewed.

3.1 CHOICE OF ANIMAL MODELS

Three main animal models are used to study the pathological features of MS: i) Toxic model; ii) viral models; iii) EAE models. MOG-induced EAE models in mice were used in my thesis. To study MS and T1D we employed animal models that are widely used. For MS we used a MOG-DBA/1 mouse model (Paper III) established in our lab that nicely corresponds to the clinical manifestation with CNS lesions. Several models of EAE have been developed to mimic the different disease courses (relapsing remitting and progressive form) of MS (Figure - 9). EAE is induced by administration of rMOG subcutaneously without co-administration of pertussis toxin (PT). It is known that PT enhances vascular permeability and can influence the susceptibility of EAE induction. In that case it is an advantage to exclude the confounding effects of PT in rMOG-induced EAE. The model has a chronic disease course but without classic relapses and remissions, yet is still very acute and severe. Disease severity is measured according to a standard severity scale (Figure - 8).

There are certain advantages of mouse models over other rodent models. Numerous inbred and congenic strains are available, and at the same time more mouse-specific reagents and tools are available.

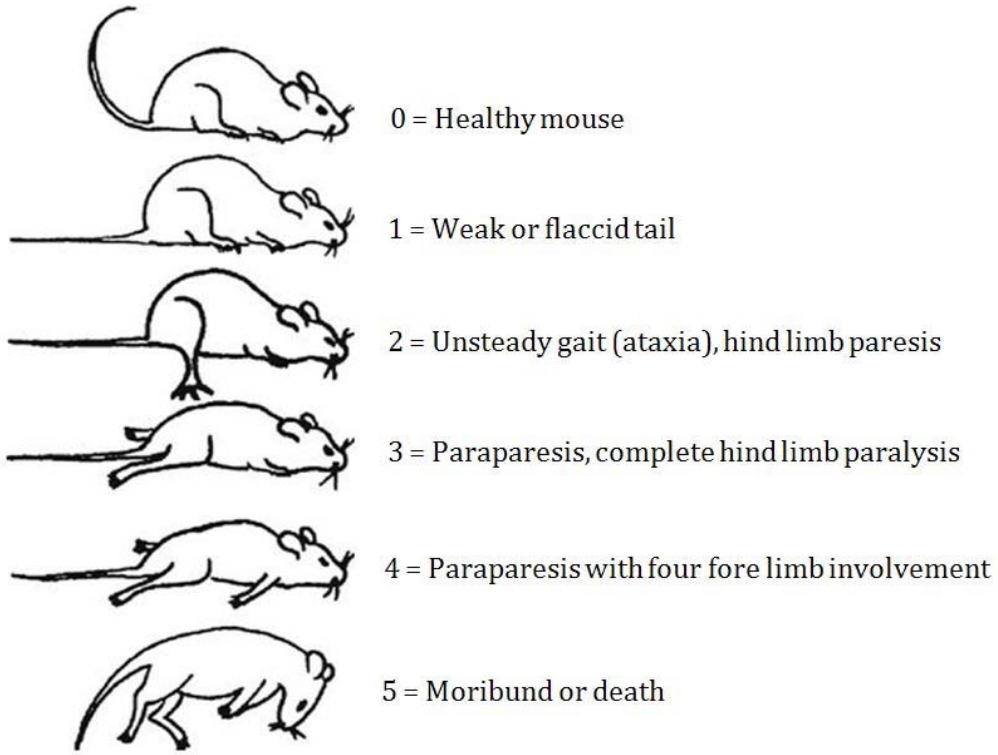

Figure 8: Clinical symptoms or severity of EAE model. Severity scales graded as follow 0= no clinical symptoms or healthy animal; 1=tail weakness or flaccid tail; 2= hind limb paraparesis or hemiparesis; 3= Hind limb paralysis or hemiparalysis; 4=tetraplegia; 5= moribund or death.

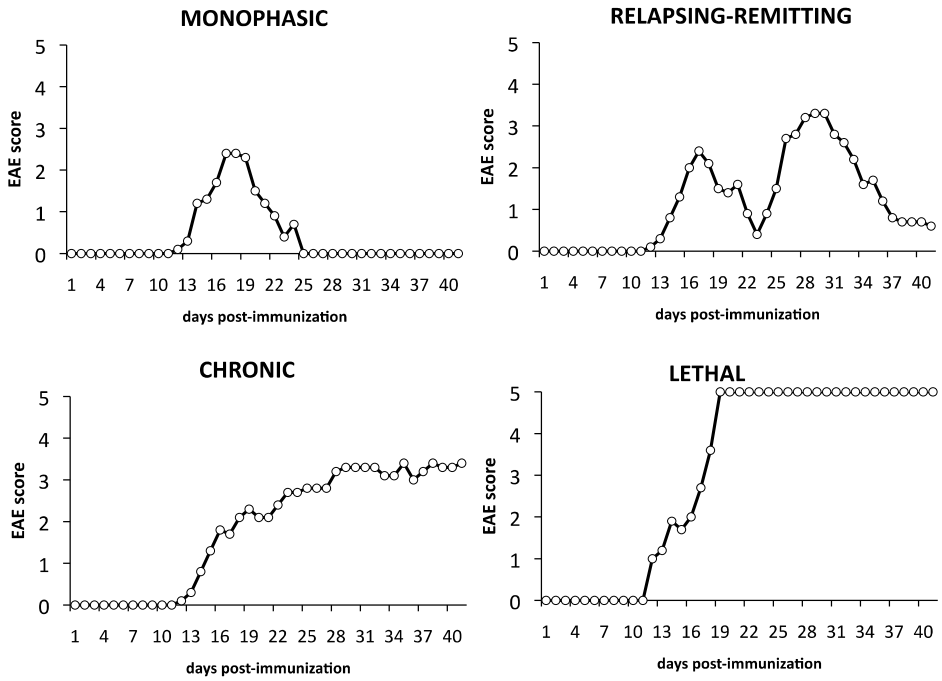

Figure 9: Representative graph of various clinical disease courses observed in EAE model. The x-axis represents the post-immunization days and the y-axis shows severity of the disease.

While it might be considered appropriate to test new therapeutic agents in a more chronic model, there is the ethical consideration of continuation of severe disease for extended times.

3.1.1 T1D Animal Models

The NOD mouse and BB rats are two of the most favoured spontaneous models for studying the etiopathogenesis of T1D. Pathological similarities with human disease have made these widely used animal models of T1D. The NOD mouse model was established in 1980 by a Japanese researcher Makino *et al* from a closed colony of JCI:ICR strain that developed cataracts [259].

At around 3-4 weeks of age the onset of disease in NOD mouse occurs spontaneously in germ-free conditions with initial insulitis. Immune cells, particularly dendritic cells and macrophages, initiate infiltration surrounding the islets and then $CD4^+$, $CD8^+$ T cells and B cells infiltrate. Pancreatic antigens are presented to the antigen presenting cells in the lymph node and autoreactive T cells are primed. Over the following few weeks the infiltration continues aggressively and autoreactive T cells start to destroy functional β-cells in islets of Langerhans [260-262]. Although much emphasis has been given on the role of T cell, macrophage and dendritic cell maturation and functional defects, lower NK cell activity and deficiency in $CD4^+CD25^+$ regulatory T cells play pivotal roles in breakdown of tolerance [263]. Female NOD mice develop full-blown diabetes by 30 weeks and incidence in males is lower [264]. After discovery of the NOD mouse model our understanding of how T1D develops in humans has greatly improved. However, disappointing results in translating many therapies effective in NOD mice to humans has raised concerns of using the model to mimic the human condition.

BB (Bio-breeding) rat models utilise two strains, BB-DP that is diabetes-prone and BB-DR that is diabetes-resistant. The incidence of disease in BB rats both in males and females is equal, as in humans. The only disadvantage of this model is development of T cell lymphopenia which is not evident in human and NOD mouse model [265]. Despite limitations in both models they play a pivotal role in further exploring the plausible anti-T1D therapy and their evaluation.

There are number of chemical substances that are used to induce symptoms of diabetes in both rats and mice. Alloxen was used initially as a β-cell cytotoxic agent. However, renal toxicity was so prominent an adverse effect it had to be discontinued. Another significant disadvantage with alloxen-treated mice was that they recovered spontaneously, and so this model was difficult to interpret therapeutic efficacy with [265].

Steptozotocin (STZ) is also β-cell cytotoxic agent and it induces DNA breakage which in turn inhibits insulin biosynthesis and its secretion, leading to β-cell death due to energy deprivation [266]. Interestingly, it has been shown that administration of low doses STZ in pre-diabetic (7-8 weeks) NOD mice delayed the onset of disease [267] and a single dose of STZ in young NOD mice protected from spontaneous diabetes [268]. It is believed that STZ causes dose-dependent β-cell apoptosis which induces Tregs and thereby self-tolerance [265].

NOD, NOD-BDC2.5 and NOD-FoxP3-GFP mouse models were used to study T1D in paper II. Most of the experiments were performed using female NOD mice. The incidence of disease in NOD was 60-80% in females and 20-30% in males. The NODBDC2.5 mouse was used for assessment of *in vivo* T cell proliferation and the NOD-FoxP3-GFP mouse was used to assess the *in vivo* induction of Tregs.

3.2 ISOLATION OF MONOCYTES:

Buffy coats of healthy donors were obtained from the Department of Transfusion medicine of Huddinge and patient blood was acquired from the Neurology and Rheumatology clinics. Peripheral blood mononuclear cells (PBMC) are a major source of monocytes. There are number of possible way of isolating monocytes after obtaining PBMC. Each isolation kit has some pros and cons regarding enrichment and number of cells. I compared the efficacies of the $CD14^+$ kit and negative selection kit from Miltinye and another kit from Stemcell Technology (Figure-10). There was an obvious heterogeneity in terms of purity and number of cells using these different methods, as shown in Figure-10. We finally decided to isolate monocytes using the $CD14^+$ kit as this yielded the most homogenous cell population. Monocytes were cultured and incubated overnight before stimulation.

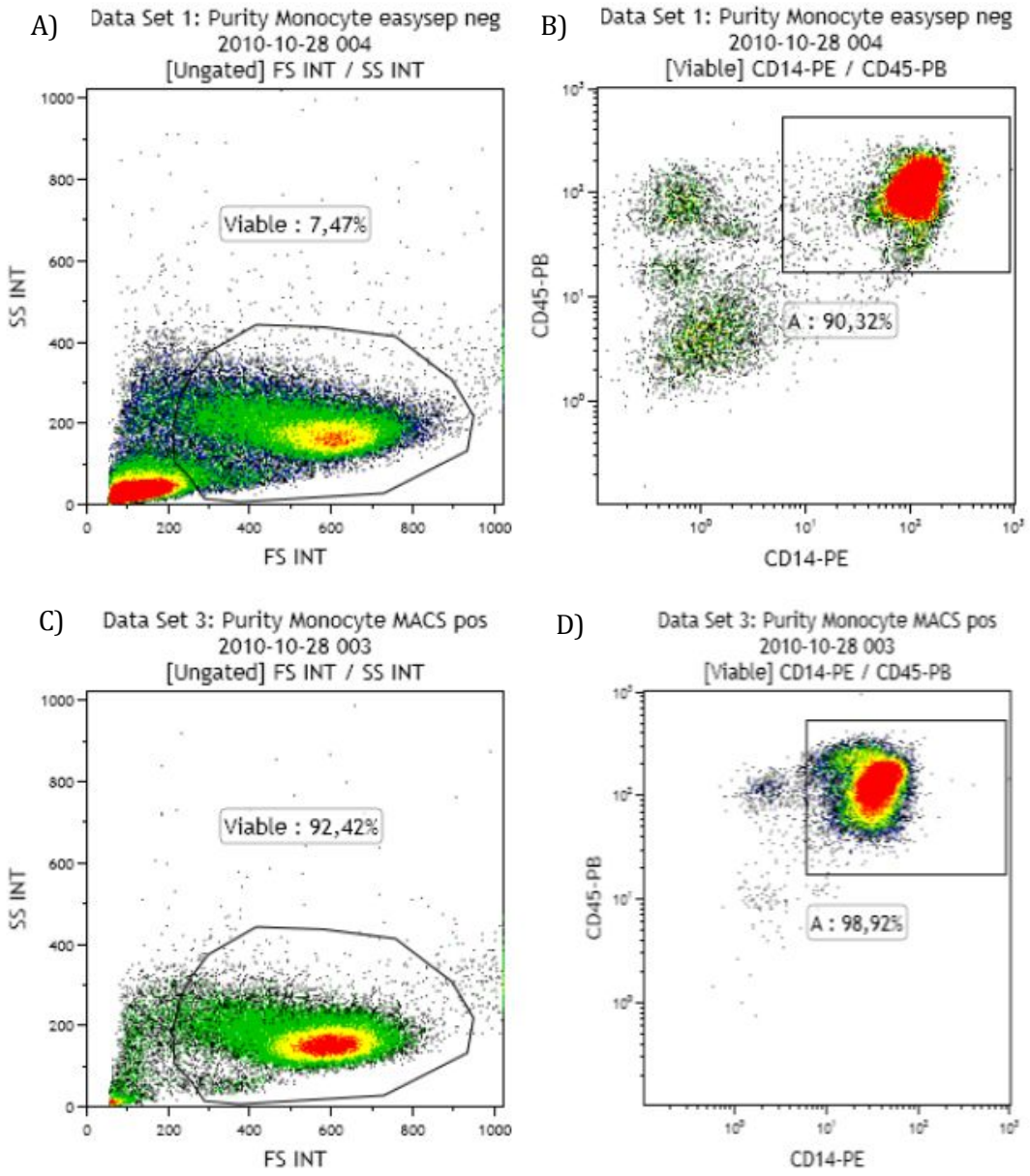

Figure 10: Representative figures of human monocyte isolation with kits from two different companies. The upper two figures (A & B) showed a less viable and pure homogeneous population; on the other hand the lower two figures (C & D) showed more viability and more homogeneous population.

3.3 RT-PCR

Real time Polymerase Chain Reaction is a powerful method for expression of genes. mRNA was extracted from cells using SUPERSCRIPT and reversely transcribed to complimentary DNA (cDNA) and amplified and quantified simultaneously using SYBR green as a fluorescent dye.

During mRNA extraction different protocols were followed to obtain optimal cDNA, but it was evident that even after increasing the number of cells mRNA levels did not improve accordingly. To avoid RNA degradation we transcribed the cDNA immediately. mRNA purity was measured using the Nanodrop method and integrity were assessed by capillary electrophoresis. Primers and reference genes/housekeeping genes were selected and normalization was performed by comparing the expression values with those of housekeeping genes. The results were expressed as relative fold expression by comparing with standard curves obtained by serial dilution from a pool of undiluted samples.

RT-PCR is very reliable, sensitive, reproducible and cost effective method for measuring a number of genes regulating disease. However, one limitation is that not all mRNAs are equivalently transcribed into proteins.

3.4 T CELL SUPPRESSION ASSAY

Suppression of T cell proliferation has been reported as a key feature of M2 monocytes in both tumour and inflammatory biology [269, 270]. To address the suppression capacity of regulatory monocytes we stimulated the monocyte/macrophages (Papers I and II) with different M2 induction (alternative activation) protocols for different time points and co-cultured with anti-CD3 activated PBMC/splenocytes. To optimize the assay we had to consider the density of cell populations, duration of co-stimulations, concentration of anti-CD3, to compare the effect of autologous versus allogeneic cells, and the use of additional anti-CD28 stimulation of PBMC or splenocytes. We optimized the number of monocyte/macrophages concentration by culturing fixed number of PBMC/splenocytes. In the end a 24 hour induction with 72 hours of co-stimulation using 1:4- 1:8 monocytes: PBMC density and 1:16 macrophages: splenocytes density of the cells (Figure-11) were determined to be the most effective protocols.

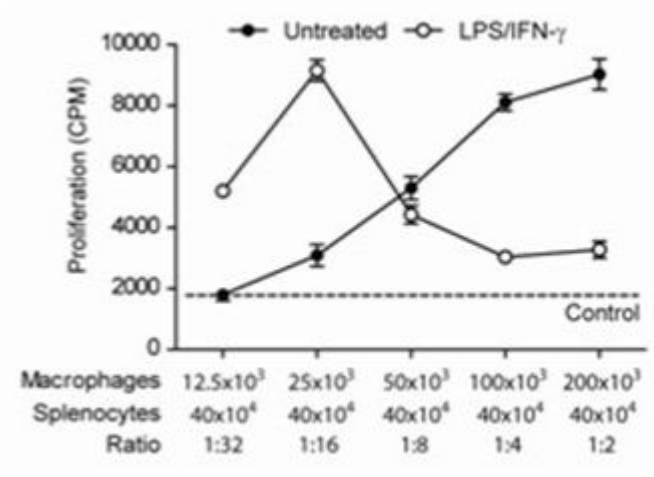

Figure 11: Optimisation of T cell suppression assay. The figure shows the 1:16 ratio of murine macrophages and PBMC gave rise to an optimal suppression assay.

3.5 FLOW CYTOMETRY

FACS analysis is a rapid, relatively simple and robust method for quantitative measurement of cells and assessment of expression of surface markers. This method is particularly useful for identification and distinction of sub-populations from a heterogeneous cell mixture based on their fluorescence scattering properties, although efficient setting of compensation is a major challenge. To address compensation pitfalls careful selection of fluorophores is needed so that spillover effects can be minimized. Isotype controls were used and dead cells were gated away before analysis.

3.6 INTRA-NASAL ADMINISTRATION

Intra-nasal administration was first developed by Frey in 1989 [271]. It is a non-invasive, practical method that bypasses the Blood Brain Barrier (BBB) to deliver therapeutic agents into the CNS. The BBB is made of tight junctions that not only prevent diffusion of a variety of pathogens and toxic substances into the brain and spinal cord, but that also hinders the access of therapeutic agents.

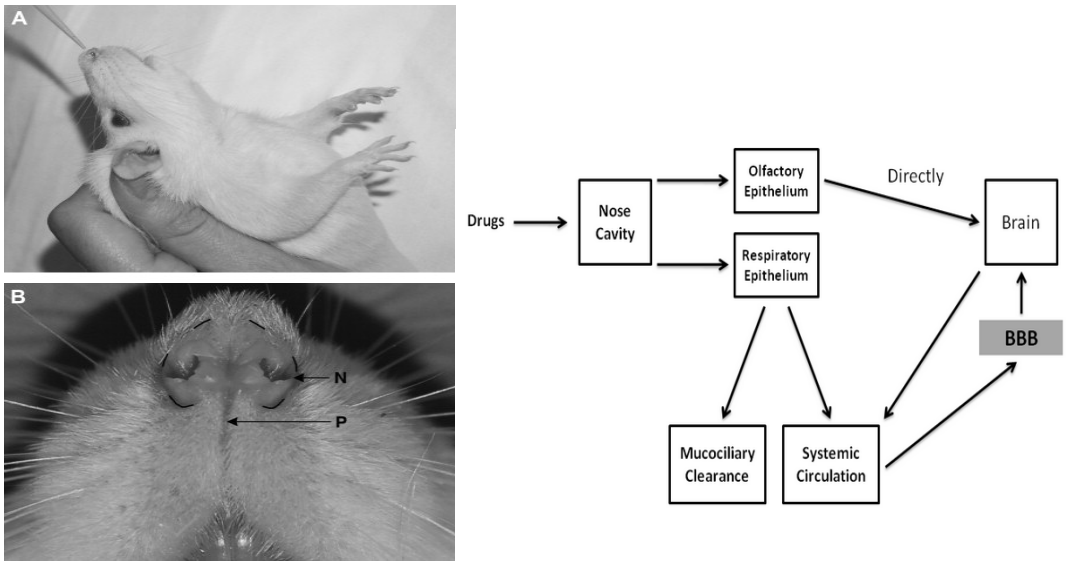

Figure 12: Intranasal delivery of cells and mechanism of entry of cells into brain.

Intra-nasal delivery methods are preferable in targeting the CNS while keeping the systemic exposure to a minimum (Figure-12). As a result it eliminates systemic loss and limits potential side-effects. A wide range of therapeutics including insulin like growth factor, nerve growth factor (NGF), estrogen, dopamine and cytokine have been reported to effectively reach the CNS following intra-nasal administration [272].

3.7 IMMUNOHISTOCHEMISTRY (IHC)

Despite all the modern imaging developments, IHC still remains an important tool for identifying proteins in tissues of interest. We used IHC in Papers II and III for staining pancreata and mouse brains, respectively. IHC is an easy, simple and inexpensive method but there are many technical considerations. For pancreatic islets we fixed the tissue with pre-warmed 4% paraformaldehyde and acetone and for mouse brains we fixed with 4% paraformaldehyde. It should be noted that the choice of fixative is a vital technical issue that can modify the tissue architecture and cell morphology. A fixative should be chosen based on tissue type. Formalin-fixed paraffin embedded tissue can be stored for longer times, but then tissues must be treated with antigen retrieval techniques to unmask the epitopes. Several non-specific binding sites can interfere with IHC results, so it is recommended to block non-specific binding to avoid false positive staining. After blocking, antigen-antibodies complex could be visualized either by directly conjugated antibody or individually by an unlabelled primary antibody that amplifies the signal from the tissue of interest, and then a secondary antibody labelled with a fluorophore

which reacts to the primary antibody. Biotinylated and streptavidin IHC is a convenient method but it has been criticized as being more subjective rather than uniform (accepted) threshold level of positivity. Sensitivity and specificity is also an issue for numerous antibodies available in the market.

4 RESULTS AND DISCUSSION

The main purpose of the PhD project was to develop a means of inducing a reproducible suppressive phenotype of macrophages for adoptive transfer as a novel therapy in preclinical studies of autoimmunity, which ultimately following proof-of-concept could be tested in patients (such as MS, T1D and RA). The four papers included in the thesis represent the combined efforts to characterize and compare the different morphological and functional activation states of macrophages, microglia and monocytes, and to test how effective these myeloid cells were in modulating experimental disease.

4.1 PAPER I: POLARIZATION OF MACROPHAGE CELL LINES

Background: Pro-inflammatory M1 and anti-inflammatory M2 macrophage activation states have been described and are generally considered extremes of a spectrum of activation phenotypes. There is a known genetic variability of susceptibility to pro-inflammatory autoimmune diseases in different mouse strains. Nramp1 has been described as a master regulator of macrophage phenotype, with Nramp1^{s} and Nramp1^{r} genotypes also being associated with disease-relevant macrophage phenotypes.

Hypothesis: Background gene effects will be more significant than Nramp1 gene effects in determining macrophage activation states.

Methods: Congenic Nramp1^{s} and Nramp1^{r} macrophages on C56BL/6 and BALB/c backgrounds were stimulated with an extensive panel of M1 and M2 protocols and the resultant respective pro-inflammatory and anti-inflammatory immunophenotypes were determined.

Results: Fundamental differences in activation states were apparent based on strain-specific background genes. Nramp1 phenotype was clearly associated with IL-10 production.

REFLECTIONS

This study gave me the opportunity to learn the principles of cell culture and to screen a number of different macrophage activation protocols. To be able to maintain the four distinct lines while preserving their integrity was a good challenge and means of assessing my new lab skills.

4.2 PAPER II: MYELOID THERAPY IN T1D

Background: T1D is a chronic autoimmune disease characterized by progressive destruction of insulin-producing pancreatic β-cells that leads to subsequent loss of glucose control and acute clinical complications.

Hypothesis: Considering that pancreatic destruction is a pro-inflammatory process, adoptive transfer of anti-inflammatory macrophages would reduce loss of β-cells and stabilize insulin production.

Methods: Bone marrow-derived macrophages were polarized using IL-4+IL-10+TGF-β into an anti-inflammatory state and adoptively transferred i.p into NOD mice in which insulitis was well established.

Results: The study result revealed that after a single transfer of M2 macrophages, >80% of treated NOD mice were protected from T1D development for at least three months. The few β-cells remaining at start of treatment seemed to be protected from further pro-inflammatory-mediated destruction.

REFLECTIONS

This study introduced me to the field of T1D, arguably the most economically draining disease on the planet. The experimental model in NOD mice is not difficult to learn as it is spontaneous, but as such it requires large groups of animals as the exact time for clinical debut cannot be predicted. Optimising the immunohistochemical staining was also rather straightforward when the tissue structure (islets) is so distinct. The possibility to use OPT through collaboration introduced me to this most modern and novel immunohistochemical staining technique.

4.3 STUDY III: MYELOID THERAPY IN MOG-EAE

Background: Microglia are the resident immune competent cells in the CNS, performing similar 'housekeeping' functions as monocytes/macrophages. Despite the harmful effects of microglia during CNS inflammation, many studies also report beneficial effects, especially through crosstalk with other CNS cells such as neurons. EAE is the experimental model of MS that is characterized by neuroinflammation and progressive demyelination, leading to clinical loss of motor functions.

Hypothesis: As we had previously demonstrated in Paper II that transfer of IL-4+IL-10+TGF-β-stimulated macrophages could prevent T1D in NOD mice, in this study our working hypothesis was that microglia could be similarly activated and adoptively transferred in order to modulate MOG-EAE. The rationale was that microglia should feel 'more at home' in the CNS, and that intranasal administration would be the fastest and easiest route for transfer.

Methods: Adult microglia were stimulated *in vitro* with IL-4+IL-10+TGF-β prior to i.n adoptive transfer at different time points during MOG-EAE in DBA/1 mice.

Results: A single transfer reduced the clinical symptoms of ongoing EAE at most of the time-points except day 0, but was most effective when administered during established disease (day 15). M2 microglia could suppress T cell activation and IL-17 production *in vitro*.

REFLECTIONS

This study introduced me to the field of Multiple Sclerosis and its experimental models. I learned how to induce and evaluate EAE development in three different models in DBA/1, C57BL/6 and NOD mouse strains. There were numbers of technical challenges for this project: i) Intranasal administration of microglia in a mouse involve very small volumes. Administration of a sufficient number of cells to give a clinical effect while using the lowest buffer volume but still preventing cell clumping required careful experimentation; ii) proving the migration of cells into the affected area when lesion site is unpredictable and the numbers of cells are small meant sectioning of a lot of CNS tissue; iii) the protocol for culture of adult microglia was established.

4.4 PAPER IV: POLARIZATION OF HUMAN MONOCYTES

Background: Monocytes circulate in the blood and first become mature macrophages upon infiltration into tissues. In the blood they are exposed to M-CSF, and first in tissues are they exposed to GM-CSF.

Hypothesis: Having established in the previous studies that an optimal anti-inflammatory M2 phenotype could be induced through specific activation with IL-4+IL-10+TFG-β, we hypothesized that a similar phenotype could be induced in human monocytes.

Methods: Human blood donor monocytes were stimulated *in vitro* and phenotyped for surface receptor expression, secreted mediators and functional activities. The effect of pre-differentiation with M-CSF or GM-CSF was additionally addressed. Finally, monocytes from patients were tested for their ability to be specifically polarized.

Results: An efficient anti-inflammatory M2 phenotype could be induced by IL-4+IL-10+TFG-β, and this was exacerbated by pre-differentiation with M-CSF. A similarly immunosuppressive M2 phenotype could be induced in monocytes from patients with pro-inflammatory autoimmune diseases.

REFLECTIONS

For this particular project I had expected a straightforward time applying the technical knowledge attained in the previous studies. It became obvious at an early stage that human cells do not behave in a similar way to murine cells, and variability between donors was much more extreme than between individual animals. While the methods of culture of bone marrow-derived macrophages and microglia were relatively standardized, a number of different methods are used in purification of human monocytes. I thus learnt a lot through comparing and contrasting the purity, yield and effect on purified cells of different isolation procedures originating from different companies. I tested both positive selection and negative selection kits. The negative selection protocol yielded a more pure population but the yield was not good, while a higher yield of lower quality was the result of positive selection. Considering that I wanted to work with patient samples, I preferred to select quantity over purity. Otherwise, I think negative selection is better option as it gives rise to an untouched population.

4.5 MYELOID CELLS

Immortal cell lines are very useful in many biological experiments simply because they are readily available and can be expanded easily without limitation. As macrophage cell lines are derived through immortalization with tumour cells then their ability to prolifically proliferate is a feature that is not shared with *de bono* macrophages. It is now understood that while tissue resident macrophages have some ability to expand, monocyte-derived macrophages do not. Another unwanted feature of cell lines is that on prolonged cell culture they may be prone to alteration of genotype and phenotype. In has also been shown that cell lines may be functionally different from primary cell lines [273].

Cell lines can thus not be expected to perfectly reflect their *in vivo* counterparts. However, acceptance of the use of cell lines differs in different biological fields. Sometimes primary cells are difficult to isolate and undergo senescence after only a few passages. Even primary cells, once removed from their *in vivo* location, will lack all the local environmental cues that govern their ultimate *in vivo* phenotype, and so even primary cell cultures are only an approximation of their true *in vivo* counterparts.

In Paper I, I utilized a panel of 4 macrophage cell lines in order to assess the effect of specific genetic background on activation states. The cell lines were chosen because they are congenic for Nramp1 (Slc11a1), a so-called master regulator of macrophage function. While the Nramp1^{r} 'resistant' and Nramp1^{s} 'susceptible' genotypes have been previously studied independently for each separate strain, BALB/c and C57BL/6, our intention was to compare all four cell lines together so that both Nramp1 genotype and background strain gene effects would be apparent. Activation phenotypes could thus be coupled to either Nramp1- or strain-dependencies.

Through comparison of wild type C57BL/6 (Nramp1^{r}) and wild type BALB/c (Nramp1^{s}) macrophages, it was evident that their cytokine production in response to pro-inflammatory stimuli differed, with lower TNF-α and significant IL-10 levels being characteristic of BALB/c but not C57BL/6 cells. This result clearly indicated a background strain dependence of activation states. Our group has

previously reported a similar finding in rat macrophages, with pro-inflammatory stimulations leading to different macrophage activation phenotypes [274]. Thus macrophages from the EAE-susceptible Dark Agouti (DA) rat produced IL-23 in response to LPS, while the EAE-resistant Piebald Virol Glaxol (PVG) strain produced IL-12. Similarly, there is an inherent difference in EAE susceptibility between BALB/C and C57BL/6 strains, BALB/C being highly resistant while C57BL/6 can suffer a severe disease course [275-277]. Thus background gene effects on macrophage activation may in part explain the relative degree of pro-inflammatory autoimmune disease-induced pathology in target organs.

The novelty of my study was to then investigate Nramp1 effects. We determined that TIMP-2 was significantly more highly expressed in both cell lines carrying the Nramp1^{r} allele, irrespective of strain background. Similarly, IL-10 was more highly expressed in cell lines with the Nramp1^{s} allele. This result indicates that Nramp1 also has a basal influence in determining the macrophage activation phenotype. There are four Nramp1 alleles in humans, and one is associated with autoimmune disease development while one is associated with susceptibility to infectious diseases [278]. That these two alleleic forms of the same intracellular transporter molecule can determine such different functionalities of the cells in which they are expressed is a good demonstration of how potent specific gene effects can be on determining immune reactions.

As well as comparing cell lines and primary cells, I have also compared and contrasted different populations of myeloid cells in my studies. The activation protocols have been applied to mouse bone-marrow macrophages and microglia, and to human monocytes. One can question why I did not ever use mouse monocytes? The simple explanation is that the yield of monocytes from mouse blood would have needed large numbers of mice. As bone marrow cells are the progenitors of blood monocytes, as opposed to tissue resident macrophages, the use of bone marrow is both acceptable and yields a rather homogenous population.

The rationale of using microglia in the EAE therapy study (Paper III) was that as CNS resident cells, microglia would be more effective than macrophages in

regulating CNS pathology. Up until recently researchers wishing to study microglia utilized embryonic or neonatal brains as sources. However, a novel methodology for culture of adult microglia was recently described [279], demonstrating a great functional difference between neonatal and adult variants. We thus consider that adult microglia would be more representative of the cells potentially involved in pathology during MOG-EAE, and thus used adult microglia in our therapy study.

In order to study human monocytes I tested a variety of purification protocols. The yields, degree of purity, and effect on the resultant monocytes varied immensely. In the end I selected a positive selection following density gradient centrifugation as the best all-round purification method.

4.6 CHARACTERIZATION OF DIFFERENT ACTIVATION STATES:

We tested the different panels of stimulations both in cell lines and primary cells and determined comparable phenotypes in both. As well as stimulatory cytokines, we included other agents such as the adjuvant aluminium hydroxide, vitamin D, flagellin and dexamethasone, all of which have been independently reported to yield M2 phenotypes. In Paper I, I tested a variety of combinations of these stimuli in an attempt to assess if there were synergistic effects. In accordance with the literature, an M2a wound healing phenotype is induced by the action of IL-4+IL-13, and an M2c deactivating phenotype by IL-10. We were particularly interested in the effect of a combination of IL-4+IL-10, as this was presumed to induce a combined M2a/M2c phenotype. These dual properties would be advantageous in the future studies assessing their effect post-adoptive transfer in models of autoimmunity.

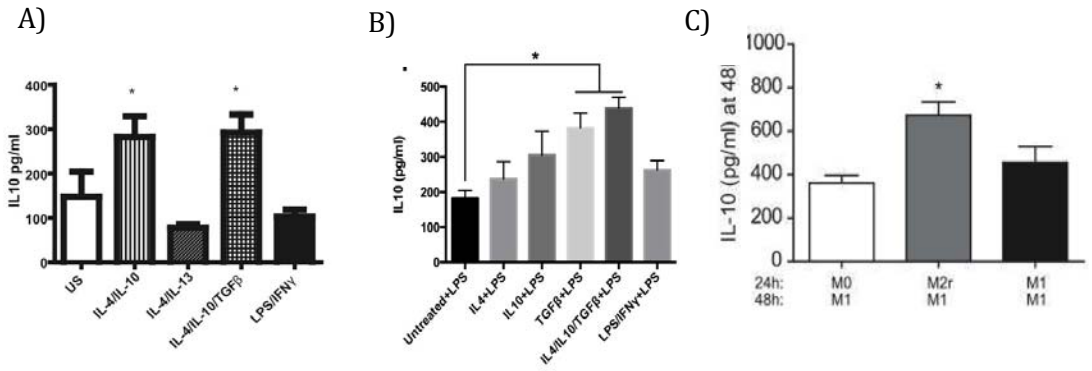

Figure: 13 IL-10 secretion of M2 (IL-4+IL-10+TFG-β) stimulated human monocytes (A) rodent microglia (B) and macrophages (C). Cells are initially stimulated with M2 protocol for 24 hours, washed and re-stimulated with LPS/IFNγ for another 24 hours and IL-10 was measured after 48 hours.

In Paper II we continued to refine the stimulation protocols, and added TFG-β to the IL-4+IL-10 stimulation protocol as this cytokine has additional, advantageous, immunosuppressive properties. Interestingly, while TFG-β appeared to synergise with IL-10, it also seemed to somewhat antagonize the actions of IL-4. Nonetheless, the triple combination of IL-4+IL-10+TFG-β was finally concluded to give the best 'all-round' phenotype.

In Paper IV, I demonstrated that the same triple cytokine combination could be used to polarize human monocytes. Thus I can conclude that irrespective of myeloid cell type (macrophage, microglia, monocyte) a comparable immunosuppressive M2 phenotype can be induced using the same protocol (Figure - 13). In this study I also investigated the effect of pre-differentiation with growth factors M-CSF and GM-CSF, which are often reported in the literature alone to induce M2 and M1 states, respectively. I demonstrated that M1 and M2 protocols could be applied to monocytes differentiated with either M-CSF or GM-CSF, but that the action of M-CSF was to enhance an M2 phenotype, and that of GM-CSF was to enhance an M1 phenotype. This is a significant aspect if the therapy will be tested in human disease states.

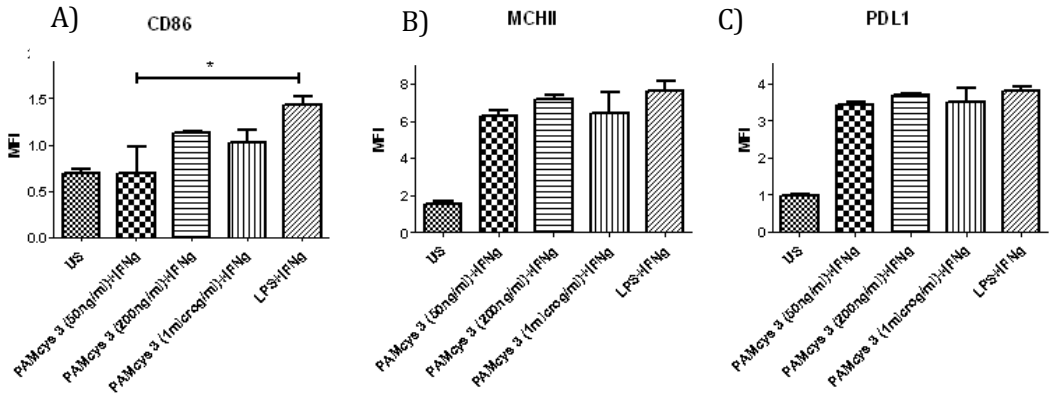

Figure: 14 Surface receptor expressions of human monocytes. Human monocytes were stimulated with LPS/IFNγ or different concentration of PAMCys3 for 24 hours. Surface marker A) CD86 B) MHC II and C) PDL-1 (CD274) were measured.

In my presented studies I did not address the variability of M1-inducing protocols. As it will be doubtful to obtain formal regulatory approval for using LPS to activate human cells prior to transfer into humans, alternative stimulation protocols were investigated. A panel of TLR-activating substances was applied and pro-inflammatory macrophage phenotypes were determined. I could conclude that the combination of Pam3Cys+IFNγ could induce a similar phenotype to that induced by LPS+IFNγ (Figure -14).

4.7 PERIPHERAL BLOOD RATHER THAN AFFECTED TISSUE

MS and T1D are organ-specific autoimmune diseases, with MS pathology occurring in the CNS during MS, and in T1D the β-cells of the pancreas are affected. In many instances the only readily available source of tissue for study is peripheral blood. Naturally, a question might arise as to whether it is relevant to study peripheral blood rather than the affected tissue. In animal models it is easier to collect tissues from the brain or pancreas, but in humans this is problematic for obvious ethical and practical reasons. The dilemma is thus whether peripheral blood is capable of reflecting the pathological reality in affected tissues. Although peripheral blood is used as an accessory tool to diagnose disease states it is not usually a confirmatory one. This is true for the presence of oligoclonal bands in peripheral blood plasma, for example, in MS. Conversely, in T1D blood is tested for autoantibodies to pancreatic antigens, and these associate with lesional

autoreactivity [280]. Autoantibodies even emerge before symptoms arise, but some individuals positive for autoantibodies do not progress to full-blown T1D. This demonstrates that screening of peripheral blood may even provide useful information before the onset of disease. Due to inaccessibility of the affected tissues it is also often difficult to follow-up the progression of the disease and response to therapy. In this case, peripheral blood or whole blood can be used as a surrogate marker to assess the immune response to a particular therapy.

4.8 CAN AN ANIMAL MODEL REFLECT THE REAL PATHOGENESIS IN HUMANS?

Animal models are crucial for studying disease pathogenesis as well as in developing new therapies, especially for inaccessible organ-specific autoimmune disease such as MS and T1D. However, questions can be raised as to how a small animal model can faithfully represent within such a short span of time the complex human disease pathology that develops in patients.

The relevance of using an animal model depends how well the animal model reproduces the specific immunological characteristics of the human disease. Although it is clear that there are major differences between the rodent and human immune systems they share many common principles. Nonetheless, translation of experimental treatment outcomes into humans gives mixed results, and some with tragic consequences [70]. Successful examples include Copaxone, Natalizumab and Fingolimod, three drugs developed for treatment of MS following promising pre-clinical studies.

EAE is a classic model for MS but it is criticized for not fully representing the human disease, which is heterogeneous and can affect the whole CNS. Moreover, while there are four different pathological patterns described in human MS [281], there is no single animal model that can represent all four of these patterns. In addition, use of inbred animal strains limits the individual variation, as a single strain can only mimic a single human individual. Another disadvantage of EAE models is that they are not spontaneous, being induced by active immunization with an autoantigen emulsified in an aggressive immune adjuvant. There are at least four major autoantigens described for MS: MOG, MBP, PLP and αβ-crystallin,

but no patient exhibits autoreactive T and B cell responses to all four of these autoantigens. An experimental model based on immunization with just one of these proteins can thus only be used to study that specific protein in pathogenesis. Another aspect of mouse EAE is that different models are used in different strains of mice using different immunization protocols, with different disease courses. The model that our lab developed, in DBA/1 mice following MOG+CFA immunization, has a particularly severe pathology.

In contrast, human T1D is replicated by the spontaneous NOD mouse model and BB rat model. The NOD model in particular is considered quite a good model of human disease, as it shares the features that autoreactivity to many pancreatic autoantigens occurs and that this is detectable prior to clinical disease onset, and because disease develops both spontaneously and progressively. However, the NOD model has been criticized for studies of therapy, as there are many different ways of modulating disease course if animals are treated early during the insulitis stage [262].

Both of these T1D models have been extensively used in preclinical studies aimed at testing newly developed drug preparations. The vast majority of the studies that report significant treatment effects involve therapeutic intervention prior to, or simultaneously with disease induction (prophylactic). In human diseases the patients have already passed these phases as they have been diagnosed as patients, and so therapeutic interventions during more chronic disease phases would be more applicable to study. Indeed, two-thirds of the autoimmune diabetes therapeutic interventions are conducted during the very early stage of the disease and terminate too early [280], only 7% of NOD mice experiments being followed up to 32 weeks [282]. When therapeutic interventions are tested in EAE models, the MOG-C57BL/6 model has often been used, primarily because clinical disease is mild in this model and therefore easy to modulate. In contrast, the MOG-EAE model that was developed in our lab [283] develops the most severe of all mouse pathologies, with lesions throughout the CNS rather than only being confined to the spinal cord (as in the C57BL/6 model). That we were able to modulate the disease course in this model is therefore even more impressive.

It is clear from this discussion that animal models are indispensible for ethical or practical reasons, but that we need improve them so that they can more closely reflect the immunopathological aspects of human disease.

4.9 ROUTES FOR M2 ADOPTIVE TRANSFER

Route of administration for any therapy is vital for its efficiency. Intra-nasal administration is a non-invasive route of administration that can bypass the BBB and easily deliver therapeutics to the CNS through olfactory or vascular pathways [284, 285]. Since the pathophysiology of MS occurs in the CNS we reasoned that i.n administration of M2 myeloid cells might be successful in this disease setting (Paper III). Indeed, comparison of i.v and i.n administration of IFNβ in the EAE model revealed that i.n administration yielded a higher concentration in the CNS and cervical lymph nodes, with associated improved therapeutic efficiency [286]. We observed a therapeutic effect following M2 administration in MOG-EAE in DBA/1 mice whether the route of administration was i.n or i.v (Figure-15). Interestingly, in an experimental model of Alzheimer's disease an M2 macrophage transfer leads to accumulation of transferred cells into the lungs, and no access to the CNS (our unpublished results). In the T1D NOD model we injected M2 macrophages i.p, yet they homed to the inflamed pancreas.

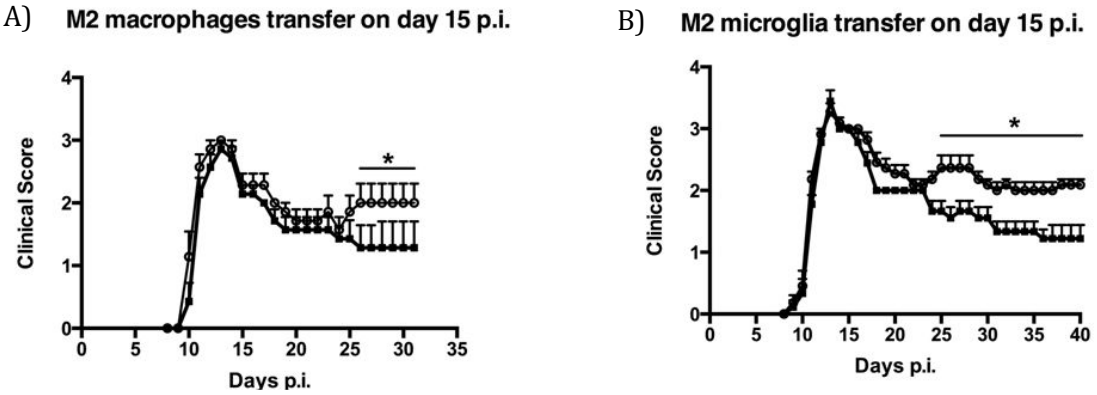

Figure15: Adoptive transfer of M2 (IL-4+IL-10+TFG-β) macrophages and microglia attenuated severity of EAE. Transfer of macrophages on day 15 p.i by intravenous (A) and microglia intranasal (B) route in MOG induced DBA/1 mice reduced the symptoms of EAE.

Taking this data together, it seems that in order for M2 cells to reach the target organ the inflammatory process must be in full swing, with presumably sufficient chemokine signals to attract the immunomodulatory macrophages. This is the case for the inflammatory processes during MOG-EAE and T1D, but obviously not for the milder inflammatory process characterizing Alzheimer's. When we tested earlier time-points in both MOG-EAE and T1D experimental models there was less or no protective effect, further demonstrating that this therapeutic approach is most suitable for chronic disease states. This is an important aspect if M2 therapy will be translated into the clinic, as chronic autoimmune disease states (e.g. SPMS) are usually untreated.

4.10 THERAPEUTIC EFFICACY OF MYELOID THERAPY

In our T1D model we observed a positive therapeutic effect if we adoptively transferred immunosuppressive M2 macrophages at a late phase of the disease, just prior to clinical debut, and we followed the mice for 3 months thereafter. If we conducted transfer at earlier time-points (e.g. week 10 when there is some insulitis but little physical loss of beta cells) there was no treatment effect. Likewise, we conducted an extended study of the kinetics of M2 microglia transfer in the MOG-EAE model, and again observed the best treatment effect during late disease as opposed to during early disease phases. In particular, if M2 cells were transferred at the same time as MOG/CFA immunization then there was no protective effect whatsoever.

What can one can conclude from this apparent time-dependency in therapeutic efficacy of myeloid cell transfer? Firstly, it would appear to be most effective when target organ tissues are damaged, so an established local pro-inflammatory responses needs to be ongoing. This is presumably necessary in order to provide the chemokine signals used by the transferred M2 cells to migrate to the site of damage, and the use of specific chemokine inhibitors or chemokine-deficient mice together with cell tracking analyses would be one way of further exploring this concept. Secondly, the transferred M2 cells did not seem to inhibit the infiltration of effector immune cells (Th17, Th1), so the pathogenic process during these advanced disease stages was apparently ongoing.

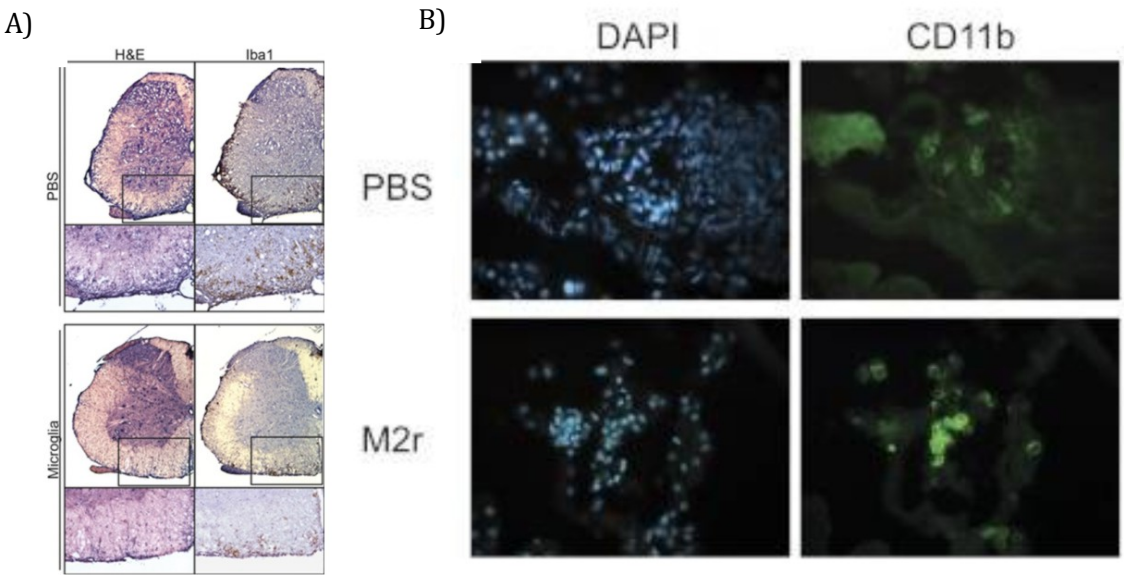

Figure 16: Mice treated with M2 (IL-4+IL-10+TFG-β) microglia and macrophages reduces inflammation. Histopathology of lumbar spinal cord revealed a reduced degree of inflammation in mice treated with IL-4+IL-10+TFG-β (M2) microglia in MOG-induced EAE model (A) and pancreata of NOD mice showed less infiltrating cells in M2 treated mice and more macrophages in close proximity to remaining islets (B).

This was particularly clear in the OPT imaging studies in the NOD mouse model, in which infiltrating T cells (green fluorescence) was equally apparent in 23 week old pancreata in untreated and treated animals.

Taken together, these studies of two chronic autoimmune disease states demonstrate a significant therapeutic effect of adoptive myeloid cell therapy using cells stimulated with IL-4+IL-10+TGF-β (Figure-16). While the effect in the MOG-EAE model might not initially seem as impressive as the effect in the T1D model, it is important to remember that the MOG-EAE model is particularly aggressive, and so the apparent alleviation in clinical symptoms is indeed significant. That effective therapy could be initiated so late in the T1D model, just prior to clinical debut, also indicates the significant efficacy of the myeloid therapy approach at modulating a raging pathological process.

4.11 MECHANISM OF ACTION OF MYELOID THERAPY

There are many potential effects of the M2 macrophages and microglia that were transferred in the NOD and MOG-EAE models, respectively. While infiltration of effector T cells appeared to be unchanged, we provided evidence in both models that their proliferative capacities and effector functions (cytokine production) could be inhibited in the presence of M2 cells *in vitro*. Macrophage regulation of T cell proliferation has been reported both in cancer and inflammation [269, 270] but the mechanisms underlying this are not very obvious. In our studies (Paper II and Paper III) we concluded that TGF-β has a prominent role in the suppression of T cell proliferation. Importantly, M1 functions could also be inhibited by M2 cells. So the pro-inflammatory actions of both T cells and macrophages could be modulated by an excess of immunosuppressive M2 myeloid cells. Whether M2 cells have comparable immunosuppressive capacities towards $CD8^+$, $CD4^+$ Th17, Th2 or Th1 T cells, NK cells or even neutrophils will require further analyses in specific tests of both cell phenotype and function.

An additional aspect is the ability of M2 cells to induce Tregs, something we demonstrated in BM macrophages from both NOD and DBA/1 strains of mice in our studies. There have been few previous studies addressing this action, but the most convincing of them proposed a bi-directional signalling between Tregs and M2 cells in a mutually inducing and propagating loop [287, 288]. In this context I assume that iTregs were induced in our *in vitro* assays (Figure -17), but a crosstalk of M2 cells with nTregs *in vivo* cannot be ruled out. It would make immunological sense that at a site of pro-inflammation, an M2-Tregs crosstalk would strengthen the immunosuppressive effect and this may even be important for induction of healing processes.

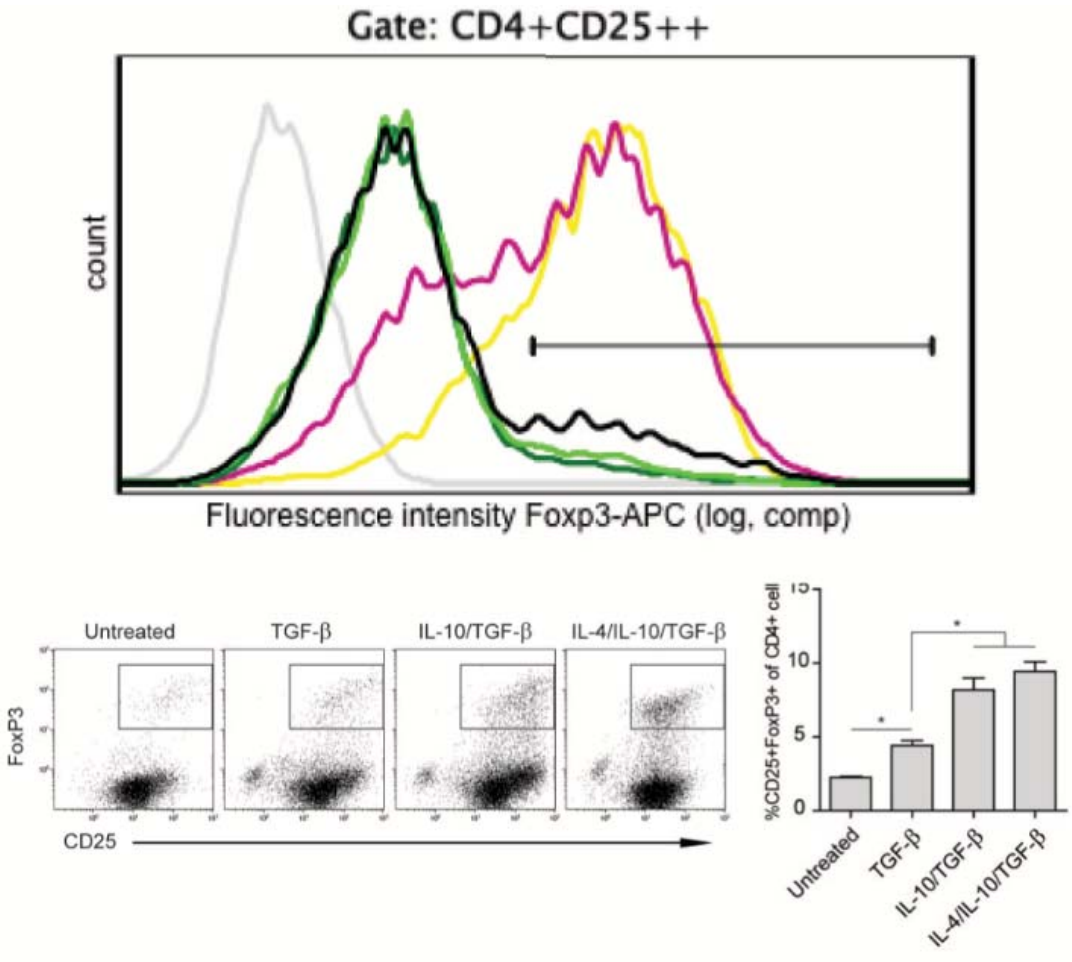

Figure: 17 M2 (IL-4+IL-10+TFG-β) stimulation can induce Tregs *in vitro*. IL-4+IL-10+TFG-β stimulated human monocyte-derived macrophages can equally express FoxP3 expression as natural Tregs (upper figure). Rodent Macrophages (lower figure) and microglia (not shown here) are also capable of inducing Tregs *in vitro* (lower figure).

One cannot rule out a potential effect on metabolism at the inflammatory site if there is a vast infiltration of highly metabolic M2 cells, thereby competing with effector cells locally, although I consider this less important. Additional modes of action may be in potentiating the mechanisms involved in wound healing (and tissue repair), and this certainly deserves further study. Finally, a role for M2 cells in attracting, stimulating or aiding the actions of stem cells is also a possibility, and this would presumably rely on a local action at the site of inflammation. Several of these lines of research are currently being investigated in the lab, and it will be interesting to follow these developments.

4.12 MYELOID CELL THERAPY IN HUMANS

There is increasing interest in applying myeloid cell therapy in clinical settings, and to date there have been reports of its use in spinal cord injury. Unfortunately the results have not been impressive [289], in contrast to the animal models in which clinical benefit has been demonstrated [290-292].

If I start to address the question as to why the outcomes in the clinic have not met the expectations from preclinical experience, then there are at least two aspects to consider. Firstly, the activation phenotype must be stable. While functional plasticity in myeloid cells is implied by their ability to adopt specific phenotypes following specific stimulations, whether macrophages are capable of exhibiting these different phenotypes successively i.e. 'switching' is still debated. A few studies have reported that macrophages are capable of switching their phenotype *in vitro* [293, 294], while others conversely conclude that the initial stimulus determines the final activation state of the cells [295]. In all my studies it was apparent that the IL-4+IL-10+TGF-β stimulation protocol could induce in either rodent macrophages/microglia or human monocytes a stable phenotype that resisted simultaneous or subsequent *in vitro* challenge with LPS/IFNγ. In Paper II we also demonstrate that the M2 phenotype was still partly intact (based only on surface marker staining) on recovery from the *in vivo* inflammatory site post-transfer. We need to be cautious to claim phenotypic stability *in vivo*, but we think that if a more stable immunostimulatory macrophage phenotype would be used in clinical studies then the trial outcome might be more favourable.

Secondly, the patients selected for clinical treatment have been the most severely injured (i.e. complete spinal cord injury grade A according to AIS). We know that around 90% of the functional pancreatic β-cell mass has already been destroyed when T1D debuts. There might be an upper threshold, after which it will be impossible to modify tissue-destructive pathology, and so irrespective if the adoptively transferred cells are competent in their role, then they might not be able to improve the pathological situation. So even if in our studies of transfer of M2 macrophages the best effect seemed to be apparent during more established disease states, just prior to clinical debut in T1D (Paper II), at day 15 in MOG-EAE (Paper III), then it still might be critical that there is an active disease process

ongoing. In the CNS during diseases such as MS, it is still unclear if there is a transition between neuroinflammation and neurodegeneration, or whether chronic disease is more the latter than the former process, in which case myeloid therapy might not be effective. These are issues that should be considered if and when new clinical trials are designed.

Use of autologous or allogenic monocytes is an important issue for any potential cell therapy. Autologous cells would be expected to be more advantageous as there will be no graft-versus-host immune reaction issues. However, for the very young, or the very old, in which either numbers of available cells or their functional capacities, respectively, might be significant issues. Conversely, use of donor cells might be additionally affected by their individual variation and immune capabilities. I noted considerable individual variation of human monocytes among the donors, and such variation in normal human monocytes has been previously reported. One possible advantage of using the IL-4+IL-10+TGF-β stimulation protocol following M-CSF differentiation for donor monocytes might be that due to their demonstrated effective immunostimulatory properties, they will also be able to somewhat withstand graft-versus-host immune reactions.

A final consideration is to put the potential of myeloid therapy into context. Do I believe that it will be able to cure patients with chronic diseases? I think that the conclusion that can be drawn from my presented studies is that there is great potential for the specifically differentiated and stimulated M2 macrophages to elicit immunosuppression, and hints that healing processes are also stimulated. So maybe myeloid therapy is part of a solution, but combination with transplantation of organs, stem cells or other immune cells may be a more realistic vision of the future.

5 FUTURE PERSPECTIVES

The aim of the thesis was to establish a regulatory myeloid cell phenotype both in rodents and humans as a novel therapy for autoimmune diseases including MS and T1D.

We have been able to define an immunomodulatory phenotype in rodent macrophages and microglia that is capable of suppressing autoimmune diseases. We have shown that human monocytes can generate the same phenotype both in healthy and chronically diseased patients. In the future we need to solve some other related questions before we can proceed to a pilot study using GLP cell facilities. We need to investigate how many cells will be needed and what will be the route of administration, and also address the potential risk of malignant transformation.

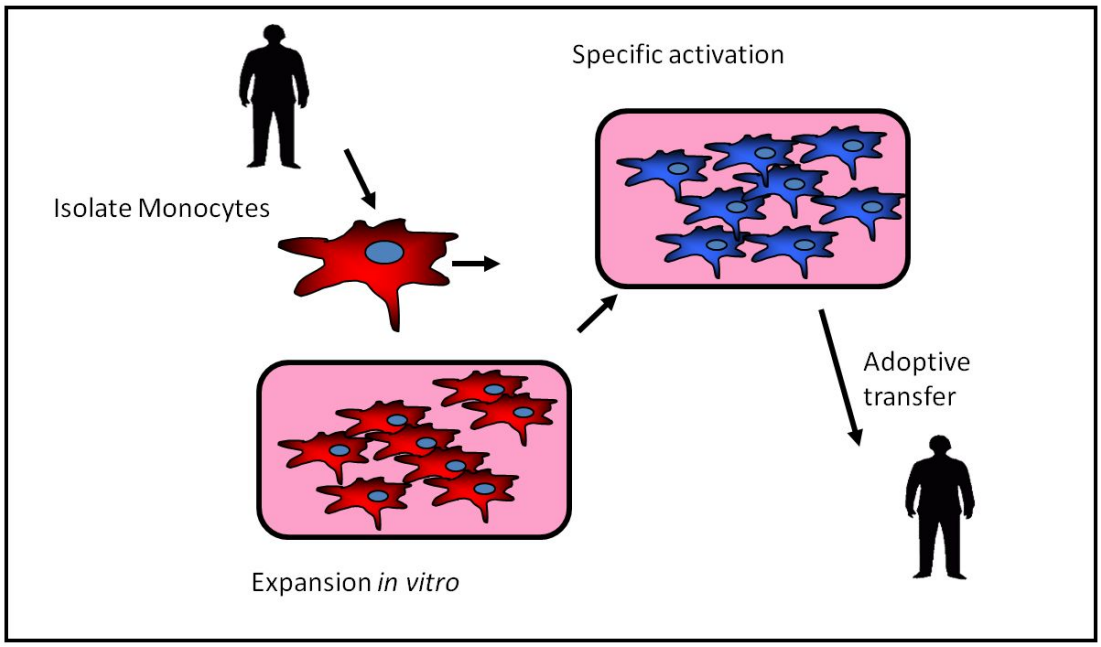

Figure 17: Schematic illustration of monocytes-macrophage therapy in autoimmune diseases. Monocytes will be isolated from patients or donors, and after *ex-vivo* stimulation adoptively transferred back into patients**.**

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Petrik, Thank you so much for all the encouragements, motivations and good wishes. I know you will do great in career.
Cecilia, I wish your future career would be great.
Emelie, **Maria** good luck to you both.

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7 REFERENCES

1. Marrack, P., J. Kappler, and B.L. Kotzin, *Autoimmune disease: why and where it occurs.* Nat Med, 2001. **7**(8): p. 899-905.
2. Yurkovetskiy, L., et al., *Gender bias in autoimmunity is influenced by microbiota.* Immunity, 2013. **39**(2): p. 400-12.
3. Markle, J.G., et al., *Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity.* Science, 2013. **339**(6123): p. 1084-8.
4. Klein, J. and A. Sato, *The HLA system. Second of two parts.* N Engl J Med, 2000. **343**(11): p. 782-6.
5. Hogquist, K.A., T.A. Baldwin, and S.C. Jameson, *Central tolerance: learning self-control in the thymus.* Nat Rev Immunol, 2005. **5**(10): p. 772-82.
6. Miller, S.D., D.M. Turley, and J.R. Podojil, *Antigen-specific tolerance strategies for the prevention and treatment of autoimmune disease.* Nat Rev Immunol, 2007. **7**(9): p. 665-77.
7. Sakaguchi, S., *Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self.* Nat Immunol, 2005. **6**(4): p. 345-52.
8. Eggena, M.P., et al., *Cooperative roles of CTLA-4 and regulatory T cells in tolerance to an islet cell antigen.* J Exp Med, 2004. **199**(12): p. 1725-30.
9. Fife, B.T., et al., *Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal.* Nat Immunol, 2009. **10**(11): p. 1185-92.
10. Shah, P.P., et al., *Induction of neuroplasticity and recovery in post-stroke aphasia by non-invasive brain stimulation.* Front Hum Neurosci, 2013. **7**: p. 888.
11. Landtblom, A.M., et al., *Distribution of multiple sclerosis in Sweden based on mortality and disability compensation statistics.* Neuroepidemiology, 2002. **21**(4): p. 167-79.
12. Compston, A. and A. Coles, *Multiple sclerosis.* The Lancet, 2002. **359**(9313): p. 1221-1231.
13. Filippi, M., et al., *Association between pathological and MRI findings in multiple sclerosis.* The Lancet Neurology, 2012. **11**(4): p. 349-360.
14. Fangerau, T., et al., *Diagnosis of multiple sclerosis: comparison of the Poser criteria and the new McDonald criteria.* Acta Neurologica Scandinavica, 2003. **0**(0): p. 061120065753028.
15. Hawkes, C.H. and G. Giovannoni, *The McDonald Criteria for Multiple Sclerosis: time for clarification.* Mult Scler, 2010. **16**(5): p. 566-75.
16. Ziemssen, T., *Multiple sclerosis beyond EDSS: depression and fatigue.* J Neurol Sci, 2009. **277 Suppl 1**: p. S37-41.
17. Goverman, J., *Autoimmune T cell responses in the central nervous system.* Nat Rev Immunol, 2009. **9**(6): p. 393-407.
18. Lassmann, H., *Models of multiple sclerosis: new insights into pathophysiology and repair.* Curr Opin Neurol, 2008. **21**(3): p. 242-7.
19. Zamvil, S.S. and L. Steinman, *Diverse targets for intervention during inflammatory and neurodegenerative phases of multiple sclerosis.* Neuron, 2003. **38**(5): p. 685-8.
20. Robertson, N.P., et al., *Age-adjusted recurrence risks for relatives of patients with multiple sclerosis.* Brain, 1996. **119 (Pt 2)**: p. 449-55.
21. Ebers, G.C., et al., *A population-based study of multiple sclerosis in twins.* N Engl J Med, 1986. **315**(26): p. 1638-42.
22. Westerlind, H., et al., *Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden.* Brain, 2014. **137**(Pt 3): p. 770-8.
23. Sawcer, S., *The complex genetics of multiple sclerosis: pitfalls and prospects.* Brain, 2008. **131**(Pt 12): p. 3118-31.

24. Olerup, O. and J. Hillert, *HLA class II-associated genetic susceptibility in multiple sclerosis: a critical evaluation.* Tissue Antigens, 1991. **38**(1): p. 1-15.
25. Lincoln, M.R., et al., *A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis.* Nat Genet, 2005. **37**(10): p. 1108-12.
26. Brynedal, B., et al., *HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis.* PLoS One, 2007. **2**(7): p. e664.
27. Gregory, S.G., et al., *Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis.* Nat Genet, 2007. **39**(9): p. 1083-91.
28. Lundmark, F., et al., *Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis.* Nat Genet, 2007. **39**(9): p. 1108-13.
29. Weber, F., et al., *IL2RA and IL7RA genes confer susceptibility for multiple sclerosis in two independent European populations.* Genes Immun, 2008. **9**(3): p. 259-63.
30. International Multiple Sclerosis Genetics, C., et al., *Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis.* Nat Genet, 2013. **45**(11): p. 1353-60.
31. Hammond, S.R., D.R. English, and J.G. McLeod, *The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia.* Brain, 2000. **123 (Pt 5)**: p. 968-74.
32. Correale, J., M.C. Ysrraelit, and M.I. Gaitan, *Immunomodulatory effects of Vitamin D in multiple sclerosis.* Brain, 2009. **132**(Pt 5): p. 1146-60.
33. Adzemovic, M.Z., et al., *Efficacy of vitamin D in treating multiple sclerosis-like neuroinflammation depends on developmental stage.* Exp Neurol, 2013. **249**: p. 39-48.
34. Hedstrom, A.K., et al., *Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis.* Neurology, 2009. **73**(9): p. 696-701.
35. Levin, L.I., et al., *Primary infection with the Epstein-Barr virus and risk of multiple sclerosis.* Ann Neurol, 2010. **67**(6): p. 824-30.
36. Alvarez-Lafuente, R., et al., *Relapsing-remitting multiple sclerosis and human herpesvirus 6 active infection.* Arch Neurol, 2004. **61**(10): p. 1523-7.
37. Challoner, P.B., et al., *Plaque-associated expression of human herpesvirus 6 in multiple sclerosis.* Proc Natl Acad Sci U S A, 1995. **92**(16): p. 7440-4.
38. Ascherio, A. and M. Munch, *Epstein-Barr virus and multiple sclerosis.* Epidemiology, 2000. **11**(2): p. 220-4.
39. Sospedra, M., et al., *Recognition of conserved amino acid motifs of common viruses and its role in autoimmunity.* PLoS Pathog, 2005. **1**(4): p. e41.
40. O'Connor, K.C., A. Bar-Or, and D.A. Hafler, *The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis.* J Clin Immunol, 2001. **21**(2): p. 81-92.
41. Bar-Or, A., *The immunology of multiple sclerosis.* Semin Neurol, 2008. **28**(1): p. 29-45.
42. Krakowski, M.L. and T. Owens, *Naive T lymphocytes traffic to inflamed central nervous system, but require antigen recognition for activation.* Eur J Immunol, 2000. **30**(4): p. 1002-9.
43. Wucherpfennig, K.W. and J.L. Strominger, *Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein.* Cell, 1995. **80**(5): p. 695-705.
44. Ransohoff, R.M., *Mechanisms of inflammation in MS tissue: adhesion molecules and chemokines.* J Neuroimmunol, 1999. **98**(1): p. 57-68.
45. Yong, V.W., et al., *Metalloproteinases in biology and pathology of the nervous system.* Nat Rev Neurosci, 2001. **2**(7): p. 502-11.
46. Babbe, H., et al., *Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction.* J Exp Med, 2000. **192**(3): p. 393-404.

47. Jacobsen, M., et al., *Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients.* Brain, 2002. **125**(Pt 3): p. 538-50.
48. Oukka, M., *Interplay between pathogenic Th17 and regulatory T cells.* Ann Rheum Dis, 2007. **66 Suppl 3**: p. iii87-90.
49. von Budingen, H.C., A. Bar-Or, and S.S. Zamvil, *B cells in multiple sclerosis: connecting the dots.* Curr Opin Immunol, 2011. **23**(6): p. 713-20.
50. Karni, A., et al., *Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis are activated and drive a proinflammatory immune response.* J Immunol, 2006. **177**(6): p. 4196-202.
51. Gandhi, R., A. Laroni, and H.L. Weiner, *Role of the innate immune system in the pathogenesis of multiple sclerosis.* J Neuroimmunol, 2010. **221**(1-2): p. 7-14.
52. Takahashi, K., et al., *Natural killer type 2 bias in remission of multiple sclerosis.* J Clin Invest, 2001. **107**(5): p. R23-9.
53. Fitzgerald, D.C., et al., *Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells.* Nat Immunol, 2007. **8**(12): p. 1372-9.
54. Nagai, T., et al., *Interferon-beta mediates opposing effects on interferon-gamma-dependent Interleukin-12 p70 secretion by human monocyte-derived dendritic cells.* Scand J Immunol, 2007. **65**(2): p. 107-17.
55. McRae, B.L., et al., *Type I IFNs inhibit human dendritic cell IL-12 production and Th1 cell development.* J Immunol, 1998. **160**(9): p. 4298-304.
56. Guo, B., E.Y. Chang, and G. Cheng, *The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice.* J Clin Invest, 2008. **118**(5): p. 1680-90.
57. Martin-Saavedra, F.M., et al., *Beta interferon restricts the inflammatory potential of CD4+ cells through the boost of the Th2 phenotype, the inhibition of Th17 response and the prevalence of naturally occurring T regulatory cells.* Mol Immunol, 2008. **45**(15): p. 4008-19.
58. Toker, A., et al., *Glatiramer Acetate Treatment Directly Targets CD11b+Ly6G− Monocytes and Enhances the Suppression of Autoreactive T cells in Experimental Autoimmune Encephalomyelitis.* Scandinavian Journal of Immunology, 2011. **74**(3): p. 235-243.
59. Weber, M.S., R. Hohlfeld, and S.S. Zamvil, *Mechanism of action of glatiramer acetate in treatment of multiple sclerosis.* Neurotherapeutics, 2007. **4**(4): p. 647-53.
60. Engelhardt, B. and L. Kappos, *Natalizumab: targeting alpha4-integrins in multiple sclerosis.* Neurodegener Dis, 2008. **5**(1): p. 16-22.
61. Miller, D.H., et al., *A controlled trial of natalizumab for relapsing multiple sclerosis.* N Engl J Med, 2003. **348**(1): p. 15-23.
62. Kleinschmidt-DeMasters, B.K. and K.L. Tyler, *Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis.* N Engl J Med, 2005. **353**(4): p. 369-74.
63. Langer-Gould, A., et al., *Progressive multifocal leukoencephalopathy in a patient treated with natalizumab.* N Engl J Med, 2005. **353**(4): p. 375-81.
64. Conway, D. and J.A. Cohen, *Combination therapy in multiple sclerosis.* Lancet Neurol, 2010. **9**(3): p. 299-308.
65. Cohen, J.A., *Corticosteroids in combination therapy for multiple sclerosis.* Lancet Neurol, 2009. **8**(6): p. 502-3.
66. Kappos, L., et al., *A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis.* N Engl J Med, 2010. **362**(5): p. 387-401.
67. Cohen, J.A., et al., *Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis.* N Engl J Med, 2010. **362**(5): p. 402-15.
68. Giovannoni, G., et al., *A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis.* N Engl J Med, 2010. **362**(5): p. 416-26.
69. Rivers, T.M. and F.F. Schwentker, *Encephalomyelitis Accompanied by Myelin Destruction Experimentally Produced in Monkeys.* J Exp Med, 1935. **61**(5): p. 689-702.

70. Eastwood, D., et al., *Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells.* Br J Pharmacol, 2010. **161**(3): p. 512-26.
71. Lebovitz, H.E., *Adjunct therapy for type 1 diabetes mellitus.* Nat Rev Endocrinol, 2010. **6**(6): p. 326-34.
72. Ricordi, C. and T.B. Strom, *Clinical islet transplantation: advances and immunological challenges.* Nat Rev Immunol, 2004. **4**(4): p. 259-68.
73. Michels, A.W. and G.S. Eisenbarth, *Immune intervention in type 1 diabetes.* Semin Immunol, 2011. **23**(3): p. 214-9.
74. Banting, F.G., et al., *Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. 1922.* CMAJ, 1991. **145**(10): p. 1281-6.
75. Sherr, J., et al., *Prevention of type 1 diabetes: the time has come.* Nat Clin Pract Endocrinol Metab, 2008. **4**(6): p. 334-43.
76. Harjutsalo, V., L. Sjöberg, and J. Tuomilehto, *Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study.* The Lancet, 2008. **371**(9626): p. 1777-1782.
77. Todd, J.A., *Etiology of type 1 diabetes.* Immunity, 2010. **32**(4): p. 457-67.
78. Karvonen, M., et al., *Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group.* Diabetes Care, 2000. **23**(10): p. 1516-26.
79. Harjutsalo, V., et al., *Incidence of type 1 diabetes in Finland.* JAMA, 2013. **310**(4): p. 427-8.
80. Ziegler, A.G. and G.T. Nepom, *Prediction and pathogenesis in type 1 diabetes.* Immunity, 2010. **32**(4): p. 468-78.
81. Lernmark, A. and H.E. Larsson, *Immune therapy in type 1 diabetes mellitus.* Nat Rev Endocrinol, 2013. **9**(2): p. 92-103.
82. Bresson, D. and M. von Herrath, *Mechanisms underlying type 1 diabetes.* Drug Discovery Today: Disease Mechanisms, 2004. **1**(3): p. 321-327.
83. Herold, K.C., et al., *Type 1 diabetes: translating mechanistic observations into effective clinical outcomes.* Nat Rev Immunol, 2013. **13**(4): p. 243-56.
84. Boitard, C., *Pancreatic islet autoimmunity.* Presse Med, 2012. **41**(12 P 2): p. e636-50.
85. Hober, D. and P. Sauter, *Pathogenesis of type 1 diabetes mellitus: interplay between enterovirus and host.* Nat Rev Endocrinol, 2010. **6**(5): p. 279-89.
86. Barker, J.M., *Chapter 5 The Pathogenesis of Type 1 Diabetes.* 2008. **9**: p. 45-59.
87. Paterson, A.D., *Genetic epidemiology of type 1 diabetes.* Curr Diab Rep, 2006. **6**(2): p. 139-46.
88. Hyppönen, E., et al., *Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study.* The Lancet, 2001. **358**(9292): p. 1500-1503.
89. Wallet, M.A. and R. Tisch, *Type 1 diabetes, inflammation and dendritic cells.* Drug Discovery Today: Disease Mechanisms, 2006. **3**(3): p. 373-379.
90. Gusdon, A.M., J.A. Corbett, and C.E. Mathews, *Type 1 diabetes: Islet inflammation – the contribution of cytokines and beta cells.* Drug Discovery Today: Disease Mechanisms, 2006. **3**(3): p. 367-372.
91. Cantor, J. and K. Haskins, *Interactions of macrophages and T cells in autoimmune diabetes.* Drug Discovery Today: Disease Mechanisms, 2006. **3**(3): p. 381-385.
92. Eizirik, D.L., M.L. Colli, and F. Ortis, *The role of inflammation in insulitis and beta-cell loss in type 1 diabetes.* Nat Rev Endocrinol, 2009. **5**(4): p. 219-26.
93. Cameron, M.J., et al., *Differential expression of CC chemokines and the CCR5 receptor in the pancreas is associated with progression to type I diabetes.* J Immunol, 2000. **165**(2): p. 1102-10.
94. Santamaria, P., *The long and winding road to understanding and conquering type 1 diabetes.* Immunity, 2010. **32**(4): p. 437-45.
95. Lehuen, A., et al., *Immune cell crosstalk in type 1 diabetes.* Nat Rev Immunol, 2010. **10**(7): p. 501-13.
96. Liu, E. and G.S. Eisenbarth, *Type 1A diabetes mellitus-associated autoimmunity.* Endocrinol Metab Clin North Am, 2002. **31**(2): p. 391-410, vii-viii.

97. Coppieters, K.T., L.C. Harrison, and M.G. von Herrath, *Trials in type 1 diabetes: Antigen-specific therapies.* Clin Immunol, 2013.
98. Diabetes Prevention Trial--Type 1 Diabetes Study, G., *Effects of insulin in relatives of patients with type 1 diabetes mellitus.* N Engl J Med, 2002. **346**(22): p. 1685-91.
99. Vehik, K., et al., *Long-term outcome of individuals treated with oral insulin: diabetes prevention trial-type 1 (DPT-1) oral insulin trial.* Diabetes Care, 2011. **34**(7): p. 1585-90.
100. Skyler, J.S., et al., *Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1.* Diabetes Care, 2005. **28**(5): p. 1068-76.
101. Harrison, L.C., et al., *Pancreatic beta-cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes.* Diabetes Care, 2004. **27**(10): p. 2348-55.
102. Waldron-Lynch, F. and K.C. Herold, *Immunomodulatory therapy to preserve pancreatic beta-cell function in type 1 diabetes.* Nat Rev Drug Discov, 2011. **10**(6): p. 439-52.
103. Tisch, R., et al., *Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice.* Nature, 1993. **366**(6450): p. 72-5.
104. Tian, J., et al., *Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes.* J Exp Med, 1996. **183**(4): p. 1561-7.
105. Ludvigsson, J., *Therapy with GAD in diabetes.* Diabetes Metab Res Rev, 2009. **25**(4): p. 307-15.
106. Agardh, C.D., et al., *Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes.* J Diabetes Complications, 2005. **19**(4): p. 238-46.
107. Herold, K.C., et al., *Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus.* N Engl J Med, 2002. **346**(22): p. 1692-8.
108. Keymeulen, B., et al., *Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes.* N Engl J Med, 2005. **352**(25): p. 2598-608.
109. Herold, K.C., et al., *A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes.* Diabetes, 2005. **54**(6): p. 1763-9.
110. Pescovitz, M.D., et al., *Rituximab, B-lymphocyte depletion, and preservation of beta-cell function.* N Engl J Med, 2009. **361**(22): p. 2143-52.
111. Pescovitz, M.D., et al., *Effect of rituximab on human in vivo antibody immune responses.* J Allergy Clin Immunol, 2011. **128**(6): p. 1295-1302 e5.
112. Orban, T., et al., *Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial.* Lancet, 2011. **378**(9789): p. 412-9.
113. Nepom, G.T., M. Ehlers, and T. Mandrup-Poulsen, *Anti-cytokine therapies in T1D: Concepts and strategies.* Clin Immunol, 2013.
114. Morath, C., et al., *Simultaneous pancreas-kidney transplantation in type 1 diabetes.* Clin Transplant, 2009. **23 Suppl 21**: p. 115-20.
115. Shapiro, A.M., et al., *International trial of the Edmonton protocol for islet transplantation.* N Engl J Med, 2006. **355**(13): p. 1318-30.
116. Aguayo-Mazzucato, C. and S. Bonner-Weir, *Stem cell therapy for type 1 diabetes mellitus.* Nat Rev Endocrinol, 2010. **6**(3): p. 139-48.
117. Hashimoto, D., et al., *Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes.* Immunity, 2013. **38**(4): p. 792-804.
118. Ginhoux, F., et al., *Fate mapping analysis reveals that adult microglia derive from primitive macrophages.* Science, 2010. **330**(6005): p. 841-5.
119. Schulz, C., et al., *A lineage of myeloid cells independent of Myb and hematopoietic stem cells.* Science, 2012. **336**(6077): p. 86-90.
120. Emile, J.F., et al., *Langerhans cell deficiency in reticular dysgenesis.* Blood, 2000. **96**(1): p. 58-62.

121. Wang, Y., et al., *IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia.* Nat Immunol, 2012. **13**(8): p. 753-60.
122. Wynn, T.A., A. Chawla, and J.W. Pollard, *Macrophage biology in development, homeostasis and disease.* Nature, 2013. **496**(7446): p. 445-55.
123. Mosser, D.M. and J.P. Edwards, *Exploring the full spectrum of macrophage activation.* Nat Rev Immunol, 2008. **8**(12): p. 958-69.
124. Mantovani, A., et al., *The chemokine system in diverse forms of macrophage activation and polarization.* Trends Immunol, 2004. **25**(12): p. 677-86.
125. Murray, P.J. and T.A. Wynn, *Obstacles and opportunities for understanding macrophage polarization.* J Leukoc Biol, 2011. **89**(4): p. 557-63.
126. Berahovich, R.D., et al., *Proteolytic activation of alternative CCR1 ligands in inflammation.* J Immunol, 2005. **174**(11): p. 7341-51.
127. Alfano, M., et al., *Macrophage polarization at the crossroad between HIV-1 infection and cancer development.* Arterioscler Thromb Vasc Biol, 2013. **33**(6): p. 1145-52.
128. Stein, M., et al., *Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation.* J Exp Med, 1992. **176**(1): p. 287-92.
129. Sica, A., et al., *Macrophage polarization in tumour progression.* Semin Cancer Biol, 2008. **18**(5): p. 349-55.
130. Cao, L. and C. He, *Polarization of macrophages and microglia in inflammatory demyelination.* Neurosci Bull, 2013. **29**(2): p. 189-98.
131. Yona, S. and S. Jung, *Monocytes: subsets, origins, fates and functions.* Curr Opin Hematol, 2010. **17**(1): p. 53-9.
132. Gordon, S., *Targeting a monocyte subset to reduce inflammation.* Circ Res, 2012. **110**(12): p. 1546-8.
133. Swirski, F.K., et al., *Identification of splenic reservoir monocytes and their deployment to inflammatory sites.* Science, 2009. **325**(5940): p. 612-6.
134. Robbins, C.S., et al., *Extramedullary hematopoiesis generates Ly-6C(high) monocytes that infiltrate atherosclerotic lesions.* Circulation, 2012. **125**(2): p. 364-74.
135. Gordon, S. and P.R. Taylor, *Monocyte and macrophage heterogeneity.* Nat Rev Immunol, 2005. **5**(12): p. 953-64.
136. Ziegler-Heitbrock, L., *The CD14+ CD16+ blood monocytes: their role in infection and inflammation.* J Leukoc Biol, 2007. **81**(3): p. 584-92.
137. Aguilar-Ruiz, S.R., et al., *Human CD16+ and CD16- monocyte subsets display unique effector properties in inflammatory conditions in vivo.* J Leukoc Biol, 2011. **90**(6): p. 1119-31.
138. Grage-Griebenow, E., H.D. Flad, and M. Ernst, *Heterogeneity of human peripheral blood monocyte subsets.* J Leukoc Biol, 2001. **69**(1): p. 11-20.
139. Cros, J., et al., *Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors.* Immunity, 2010. **33**(3): p. 375-86.
140. Rossol, M., et al., *The CD14(bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population.* Arthritis Rheum, 2012. **64**(3): p. 671-7.
141. Wong, K.L., et al., *Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets.* Blood, 2011. **118**(5): p. e16-31.
142. Skrzeczynska-Moncznik, J., et al., *Peripheral blood CD14high CD16+ monocytes are main producers of IL-10.* Scand J Immunol, 2008. **67**(2): p. 152-9.
143. Gille-Johnson, P., et al., *Circulating monocytes are not the major source of plasma cytokines in patients with sepsis.* Shock, 2012. **38**(6): p. 577-83.
144. Ziegler-Heitbrock, L., et al., *Nomenclature of monocytes and dendritic cells in blood.* Blood, 2010. **116**(16): p. e74-80.
145. Auffray, C., et al., *CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation.* J Exp Med, 2009. **206**(3): p. 595-606.

146. Nahrendorf, M. and F.K. Swirski, *Monocyte and macrophage heterogeneity in the heart.* Circ Res, 2013. **112**(12): p. 1624-33.
147. Geissmann, F., et al., *Development of monocytes, macrophages, and dendritic cells.* Science, 2010. **327**(5966): p. 656-61.
148. Geissmann, F., et al., *Blood monocytes: distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses.* Immunol Cell Biol, 2008. **86**(5): p. 398-408.
149. Auffray, C., M.H. Sieweke, and F. Geissmann, *Blood monocytes: development, heterogeneity, and relationship with dendritic cells.* Annu Rev Immunol, 2009. **27**: p. 669-92.
150. Shechter, R., et al., *Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice.* PLoS Med, 2009. **6**(7): p. e1000113.
151. Nahrendorf, M., et al., *The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions.* J Exp Med, 2007. **204**(12): p. 3037-47.
152. Ginhoux, F., et al., *Origin and differentiation of microglia.* Front Cell Neurosci, 2013. **7**: p. 45.
153. Perry, V.H., *A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation.* J Neuroimmunol, 1998. **90**(2): p. 113-21.
154. Eglitis, M.A. and E. Mezey, *Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice.* Proc Natl Acad Sci U S A, 1997. **94**(8): p. 4080-5.
155. Chan, W.Y., S. Kohsaka, and P. Rezaie, *The origin and cell lineage of microglia: new concepts.* Brain Res Rev, 2007. **53**(2): p. 344-54.
156. Gomez Perdiguero, E., C. Schulz, and F. Geissmann, *Development and homeostasis of "resident" myeloid cells: the case of the microglia.* Glia, 2013. **61**(1): p. 112-20.
157. Neumann, H. and H. Wekerle, *Brain microglia: watchdogs with pedigree.* Nat Neurosci, 2013. **16**(3): p. 253-5.
158. Herber, D.L., et al., *Microglial activation is required for Abeta clearance after intracranial injection of lipopolysaccharide in APP transgenic mice.* J Neuroimmune Pharmacol, 2007. **2**(2): p. 222-31.
159. Lu, W., M. Bhasin, and S.E. Tsirka, *Involvement of tissue plasminogen activator in onset and effector phases of experimental allergic encephalomyelitis.* J Neurosci, 2002. **22**(24): p. 10781-9.
160. Bhasin, M., M. Wu, and S.E. Tsirka, *Modulation of microglial/macrophage activation by macrophage inhibitory factor (TKP) or tuftsin (TKPR) attenuates the disease course of experimental autoimmune encephalomyelitis.* BMC Immunol, 2007. **8**: p. 10.
161. Luo, X.G. and S.D. Chen, *The changing phenotype of microglia from homeostasis to disease.* Transl Neurodegener, 2012. **1**(1): p. 9.
162. van Rossum, D. and U.K. Hanisch, *Microglia.* Metab Brain Dis, 2004. **19**(3-4): p. 393-411.
163. Hanisch, U.K. and H. Kettenmann, *Microglia: active sensor and versatile effector cells in the normal and pathologic brain.* Nat Neurosci, 2007. **10**(11): p. 1387-94.
164. Biber, K., et al., *Neuronal 'On' and 'Off' signals control microglia.* Trends Neurosci, 2007. **30**(11): p. 596-602.
165. Streit, W.J., et al., *Dystrophic microglia in the aging human brain.* Glia, 2004. **45**(2): p. 208-12.
166. Flanary, B.E., et al., *Evidence that aging and amyloid promote microglial cell senescence.* Rejuvenation Res, 2007. **10**(1): p. 61-74.
167. Aguzzi, A., B.A. Barres, and M.L. Bennett, *Microglia: scapegoat, saboteur, or something else?* Science, 2013. **339**(6116): p. 156-61.
168. Saederup, N., et al., *Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice.* PLoS One, 2010. **5**(10): p. e13693.

169. Butovsky, O., et al., *Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS.* J Clin Invest, 2012. **122**(9): p. 3063-87.
170. Remington, L.T., et al., *Microglial recruitment, activation, and proliferation in response to primary demyelination.* Am J Pathol, 2007. **170**(5): p. 1713-24.
171. Hussain, S.F., et al., *The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses.* Neuro Oncol, 2006. **8**(3): p. 261-79.
172. Greter, M., et al., *Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia.* Immunity, 2012. **37**(6): p. 1050-60.
173. Bedard, A., et al., *Identification of genes preferentially expressed by microglia and upregulated during cuprizone-induced inflammation.* Glia, 2007. **55**(8): p. 777-89.
174. Cella, M., et al., *Impaired differentiation of osteoclasts in TREM-2-deficient individuals.* J Exp Med, 2003. **198**(4): p. 645-51.
175. Schmid, C.D., et al., *Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia.* J Neurochem, 2002. **83**(6): p. 1309-20.
176. Feldmann, M. and L. Steinman, *Design of effective immunotherapy for human autoimmunity.* Nature, 2005. **435**(7042): p. 612-9.
177. Kopf, M., M.F. Bachmann, and B.J. Marsland, *Averting inflammation by targeting the cytokine environment.* Nat Rev Drug Discov, 2010. **9**(9): p. 703-18.
178. Lipsky, P.E., 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): p. 1594-602.
179. Klareskog, L., et al., *Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial.* The Lancet, 2004. **363**(9410): p. 675-681.
180. Keystone, E.C., et al., *Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial.* Arthritis Rheum, 2004. **50**(5): p. 1400-11.
181. *TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group.* Neurology, 1999. **53**(3): p. 457-65.
182. van Oosten, B.W., et al., *A phase II trial of anti-CD4 antibodies in the treatment of multiple sclerosis.* Mult Scler, 1996. **1**(6): p. 339-42.
183. De Bandt, M., *Lessons for lupus from tumour necrosis factor blockade.* Lupus, 2006. **15**(11): p. 762-7.
184. Steinman, L., *Immune therapy for autoimmune diseases.* Science, 2004. **305**(5681): p. 212-6.
185. Keane, J., et al., *Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent.* N Engl J Med, 2001. **345**(15): p. 1098-104.
186. Day, R., *Adverse reactions to TNF-alpha inhibitors in rheumatoid arthritis.* Lancet, 2002. **359**(9306): p. 540-1.
187. Bongartz, T., et al., *Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials.* JAMA, 2006. **295**(19): p. 2275-85.
188. Eugster, H.P., et al., *IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis.* Eur J Immunol, 1998. **28**(7): p. 2178-87.

189. Samoilova, E.B., et al., *IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: roles of IL-6 in the activation and differentiation of autoreactive T cells.* J Immunol, 1998. **161**(12): p. 6480-6.
190. Alonzi, T., et al., *Interleukin 6 is required for the development of collagen-induced arthritis.* J Exp Med, 1998. **187**(4): p. 461-8.
191. Eriksson, U., et al., *Interleukin-6-deficient mice resist development of autoimmune myocarditis associated with impaired upregulation of complement C3.* Circulation, 2003. **107**(2): p. 320-5.
192. Yamamoto, M., et al., *IL-6 is required for the development of Th1 cell-mediated murine colitis.* J Immunol, 2000. **164**(9): p. 4878-82.
193. Smolen, J.S., et al., *Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial.* The Lancet, 2008. **371**(9617): p. 987-997.
194. Emery, P., et al., *IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial.* Ann Rheum Dis, 2008. **67**(11): p. 1516-23.
195. Jones, G., et al., *Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study.* Ann Rheum Dis, 2010. **69**(1): p. 88-96.
196. Yokota, S., et al., *Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial.* The Lancet, 2008. **371**(9617): p. 998-1006.
197. Hooks, J.J., et al., *Immune interferon in the circulation of patients with autoimmune disease.* N Engl J Med, 1979. **301**(1): p. 5-8.
198. Axtell, R.C., et al., *T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis.* Nat Med, 2010. **16**(4): p. 406-12.
199. Voorthuis, J.A., et al., *Suppression of experimental allergic encephalomyelitis by intraventricular administration of interferon-gamma in Lewis rats.* Clin Exp Immunol, 1990. **81**(2): p. 183-8.
200. Billiau, A., et al., *Immunomodulatory properties of interferon-gamma. An update.* Ann N Y Acad Sci, 1998. **856**: p. 22-32.
201. Steinman, L., *A rush to judgment on Th17.* J Exp Med, 2008. **205**(7): p. 1517-22.
202. Mandrup-Poulsen, T., L. Pickersgill, and M.Y. Donath, *Blockade of interleukin 1 in type 1 diabetes mellitus.* Nat Rev Endocrinol, 2010. **6**(3): p. 158-66.
203. Genovese, M.C., et al., *LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-of-concept study.* Arthritis Rheum, 2010. **62**(4): p. 929-39.
204. Uyttenhove, C. and J. Van Snick, *Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis.* Eur J Immunol, 2006. **36**(11): p. 2868-74.
205. Ikeuchi, H., et al., *Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine.* Arthritis Rheum, 2005. **52**(4): p. 1037-46.
206. Beyeen, A.D., et al., *IL-22RA2 associates with multiple sclerosis and macrophage effector mechanisms in experimental neuroinflammation.* J Immunol, 2010. **185**(11): p. 6883-90.
207. Wolk, K., et al., *IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not.* J Mol Med (Berl), 2009. **87**(5): p. 523-36.
208. Sainathan, S.K., et al., *Granulocyte macrophage colony-stimulating factor ameliorates DSS-induced experimental colitis.* Inflamm Bowel Dis, 2008. **14**(1): p. 88-99.

209. Cua, D.J., et al., *Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain.* Nature, 2003. **421**(6924): p. 744-8.
210. Murphy, C.A., et al., *Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation.* J Exp Med, 2003. **198**(12): p. 1951-7.
211. Papp, K.A., et al., *Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2).* The Lancet, 2008. **371**(9625): p. 1675-1684.
212. Gottlieb, A., et al., *Ustekinumab, a human interleukin 12/23 monoclonal antibody, for psoriatic arthritis: randomised, double-blind, placebo-controlled, crossover trial.* Lancet, 2009. **373**(9664): p. 633-40.
213. Sandborn, W.J., et al., *A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease.* Gastroenterology, 2008. **135**(4): p. 1130-41.
214. Kohler, G. and C. Milstein, *Continuous cultures of fused cells secreting antibody of predefined specificity.* Nature, 1975. **256**(5517): p. 495-7.
215. Lonberg, N., *Human antibodies from transgenic animals.* Nat Biotechnol, 2005. **23**(9): p. 1117-25.
216. Reichert, J.M., et al., *Monoclonal antibody successes in the clinic.* Nat Biotechnol, 2005. **23**(9): p. 1073-8.
217. Leader, B., Q.J. Baca, and D.E. Golan, *Protein therapeutics: a summary and pharmacological classification.* Nat Rev Drug Discov, 2008. **7**(1): p. 21-39.
218. Ransohoff, R.M., *Natalizumab for multiple sclerosis.* N Engl J Med, 2007. **356**(25): p. 2622-9.
219. Hauser, S.L., et al., *B-cell depletion with rituximab in relapsing-remitting multiple sclerosis.* N Engl J Med, 2008. **358**(7): p. 676-88.
220. Plevy, S., et al., *A phase I study of visilizumab, a humanized anti-CD3 monoclonal antibody, in severe steroid-refractory ulcerative colitis.* Gastroenterology, 2007. **133**(5): p. 1414-22.
221. Nussenblatt, R.B., et al., *Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial.* Proc Natl Acad Sci U S A, 1999. **96**(13): p. 7462-6.
222. Rose, J.W., J. Foley, and N. Carlson, *Monoclonal antibody treatments for multiple sclerosis.* Curr Neurol Neurosci Rep, 2008. **8**(5): p. 419-26.
223. Hansel, T.T., et al., *The safety and side effects of monoclonal antibodies.* Nat Rev Drug Discov, 2010. **9**(4): p. 325-38.
224. Hemmer, B., et al., *Immunopathogenesis and immunotherapy of multiple sclerosis.* Nat Clin Pract Neurol, 2006. **2**(4): p. 201-11.
225. Becher, B., B.G. Durell, and R.J. Noelle, *Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12.* J Clin Invest, 2002. **110**(4): p. 493-7.
226. Hueber, W., et al., *Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis.* Sci Transl Med, 2010. **2**(52): p. 52ra72.
227. Hueber, W., et al., *Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial.* Gut, 2012. **61**(12): p. 1693-700.
228. Hayosh, N.S. and R.H. Swanborg, *Autoimmune effector cells. IX. Inhibition of adoptive transfer of autoimmune encephalomyelitis with a monoclonal antibody specific for interleukin 2 receptors.* J Immunol, 1987. **138**(11): p. 3771-5.
229. Ochi, H., et al., *Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4+ CD25- LAP+ T cells.* Nat Med, 2006. **12**(6): p. 627-35.
230. Ishikawa, H., et al., *Inhibition of autoimmune diabetes by oral administration of anti-CD3 monoclonal antibody.* Diabetes, 2007. **56**(8): p. 2103-9.
231. Wu, H.Y., F.J. Quintana, and H.L. Weiner, *Nasal anti-CD3 antibody ameliorates lupus by inducing an IL-10-secreting CD4+ CD25- LAP+*

regulatory T cell and is associated with down-regulation of IL-17+ CD4+ ICOS+ CXCR5+ follicular helper T cells. J Immunol, 2008. **181**(9): p. 6038-50.
232. Faria, A.M. and H.L. Weiner, *Oral tolerance: therapeutic implications for autoimmune diseases.* Clin Dev Immunol, 2006. **13**(2-4): p. 143-57.
233. Warren, K.G., et al., *Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment.* Eur J Neurol, 2006. **13**(8): p. 887-95.
234. Weiner, H.L., et al., *Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis.* Science, 1993. **259**(5099): p. 1321-4.
235. Hohlfeld, R. and H. Wiendl, *The ups and downs of multiple sclerosis therapeutics.* Ann Neurol, 2001. **49**(3): p. 281-4.
236. Miyara, M., et al., *Human FoxP3+ regulatory T cells in systemic autoimmune diseases.* Autoimmun Rev, 2011. **10**(12): p. 744-55.
237. Costantino, C.M., C. Baecher-Allan, and D.A. Hafler, *Multiple sclerosis and regulatory T cells.* J Clin Immunol, 2008. **28**(6): p. 697-706.
238. Long, S.A. and J.H. Buckner, *CD4+FOXP3+ T regulatory cells in human autoimmunity: more than a numbers game.* J Immunol, 2011. **187**(5): p. 2061-6.
239. Noris, M., et al., *Regulatory T cells and T cell depletion: role of immunosuppressive drugs.* J Am Soc Nephrol, 2007. **18**(3): p. 1007-18.
240. Battaglia, M. and M.G. Roncarolo, *Immune intervention with T regulatory cells: past lessons and future perspectives for type 1 diabetes.* Semin Immunol, 2011. **23**(3): p. 182-94.
241. Komatsu, N., et al., *Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity.* Proc Natl Acad Sci U S A, 2009. **106**(6): p. 1903-8.
242. Hoffmann, P., et al., *Loss of FOXP3 expression in natural human CD4+CD25+ regulatory T cells upon repetitive in vitro stimulation.* Eur J Immunol, 2009. **39**(4): p. 1088-97.
243. Edwards, J.C., et al., *Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis.* N Engl J Med, 2004. **350**(25): p. 2572-81.
244. Browning, J.L., *B cells move to centre stage: novel opportunities for autoimmune disease treatment.* Nat Rev Drug Discov, 2006. **5**(7): p. 564-76.
245. Zappia, E., et al., *Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy.* Blood, 2005. **106**(5): p. 1755-61.
246. Zhang, J., et al., *Bone marrow stromal cells reduce axonal loss in experimental autoimmune encephalomyelitis mice.* J Neurosci Res, 2006. **84**(3): p. 587-95.
247. Kassis, I., et al., *Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis.* Arch Neurol, 2008. **65**(6): p. 753-61.
248. Constantin, G., et al., *Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis.* Stem Cells, 2009. **27**(10): p. 2624-35.
249. Yamout, B., et al., *Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study.* J Neuroimmunol, 2010. **227**(1-2): p. 185-9.
250. Sun, L., et al., *Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans.* Stem Cells, 2009. **27**(6): p. 1421-32.
251. Augello, A., et al., *Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis.* Arthritis Rheum, 2007. **56**(4): p. 1175-86.
252. Boumaza, I., et al., *Autologous bone marrow-derived rat mesenchymal stem cells promote PDX-1 and insulin expression in the islets, alter T cell cytokine*

pattern and preserve regulatory T cells in the periphery and induce sustained normoglycemia. J Autoimmun, 2009. **32**(1): p. 33-42.
253. Bernardo, M.E. and W.E. Fibbe, *Safety and efficacy of mesenchymal stromal cell therapy in autoimmune disorders.* Ann N Y Acad Sci, 2012. **1266**: p. 107-17.
254. Wyllie, S., P. Seu, and J.A. Goss, *The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages.* Microbes Infect, 2002. **4**(3): p. 351-9.
255. Vidal, S.M., et al., *Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg.* Cell, 1993. **73**(3): p. 469-85.
256. O'Brien, B.A., et al., *Association of SLC11A1 promoter polymorphisms with the incidence of autoimmune and inflammatory diseases: a meta-analysis.* J Autoimmun, 2008. **31**(1): p. 42-51.
257. Blackwell, J.M., et al., *SLC11A1 (formerly NRAMP1) and disease resistance.* Cell Microbiol, 2001. **3**(12): p. 773-84.
258. Gazouli, M., et al., *NRAMP1 polymorphism and viral factors in Sardinian multiple sclerosis patients.* Can J Neurol Sci, 2008. **35**(4): p. 491-4.
259. Ikegami, H., T. Fujisawa, and T. Ogihara, *Mouse models of type 1 and type 2 diabetes derived from the same closed colony: genetic susceptibility shared between two types of diabetes.* ILAR J, 2004. **45**(3): p. 268-77.
260. Anderson, M.S. and J.A. Bluestone, *The NOD mouse: a model of immune dysregulation.* Annu Rev Immunol, 2005. **23**: p. 447-85.
261. Thayer, T.C., S.B. Wilson, and C.E. Mathews, *Use of nonobese diabetic mice to understand human type 1 diabetes.* Endocrinol Metab Clin North Am, 2010. **39**(3): p. 541-61.
262. Atkinson, M.A. and E.H. Leiter, *The NOD mouse model of type 1 diabetes: as good as it gets?* Nat Med, 1999. **5**(6): p. 601-4.
263. Driver, J.P., D.V. Serreze, and Y.G. Chen, *Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease.* Semin Immunopathol, 2011. **33**(1): p. 67-87.
264. Van Belle, T.L., P. Taylor, and M.G. von Herrath, *Mouse Models for Type 1 Diabetes.* Drug Discov Today Dis Models, 2009. **6**(2): p. 41-45.
265. Acharjee, S., et al., *Understanding Type 1 Diabetes: Etiology and Models.* Canadian Journal of Diabetes, 2013. **37**(4): p. 269-276.
266. Rayat, G., *Immunization with streptozotocin-treated NOD mouse islets inhibits the onset of autoimmune diabetes in NOD mice.* Journal of Autoimmunity, 2003. **21**(1): p. 11-15.
267. Takayama, Y., T. Ichikawa, and T. Maki, *Effect of STZ administration on islet isograft and allograft survival in NOD mice.* Diabetes, 1993. **42**(2): p. 324-9.
268. Hugues, S., et al., *Tolerance to islet antigens and prevention from diabetes induced by limited apoptosis of pancreatic beta cells.* Immunity, 2002. **16**(2): p. 169-81.
269. Biswas, S.K. and A. Mantovani, *Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm.* Nat Immunol, 2010. **11**(10): p. 889-96.
270. Weber, M.S., et al., *Type II monocytes modulate T cell-mediated central nervous system autoimmune disease.* Nat Med, 2007. **13**(8): p. 935-43.
271. Hanson, L.R. and W.H. Frey, 2nd, *Strategies for intranasal delivery of therapeutics for the prevention and treatment of neuroAIDS.* J Neuroimmune Pharmacol, 2007. **2**(1): p. 81-6.
272. Hanson, L.R. and W.H. Frey, 2nd, *Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease.* BMC Neurosci, 2008. **9 Suppl 3**: p. S5.
273. Pan, C., et al., *Comparative proteomic phenotyping of cell lines and primary cells to assess preservation of cell type-specific functions.* Mol Cell Proteomics, 2009. **8**(3): p. 443-50.
274. Andersson, A., et al., *Differential macrophage expression of IL-12 and IL-23 upon innate immune activation defines rat autoimmune susceptibility.* J Leukoc Biol, 2004. **76**(6): p. 1118-24.

275. Teuscher, C., et al., *A common immunoregulatory locus controls susceptibility to actively induced experimental allergic encephalomyelitis and experimental allergic orchitis in BALB/c mice.* J Immunol, 1998. **160**(6): p. 2751-6.
276. Okuda, Y., M. Okuda, and C.C. Bernard, *Gender does not influence the susceptibility of C57BL/6 mice to develop chronic experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein.* Immunol Lett, 2002. **81**(1): p. 25-9.
277. Hurwitz, A.A., et al., *Cytotoxic T lymphocyte antigen-4 (CTLA-4) limits the expansion of encephalitogenic T cells in experimental autoimmune encephalomyelitis (EAE)-resistant BALB/c mice.* Proc Natl Acad Sci U S A, 2002. **99**(5): p. 3013-7.
278. Searle, S. and J.M. Blackwell, *Evidence for a functional repeat polymorphism in the promoter of the human NRAMP1 gene that correlates with autoimmune versus infectious disease susceptibility.* J Med Genet, 1999. **36**(4): p. 295-9.
279. Moussaud, S. and H.J. Draheim, *A new method to isolate microglia from adult mice and culture them for an extended period of time.* J Neurosci Methods, 2010. **187**(2): p. 243-53.
280. Roep, B.O., et al., *The problems and promises of research into human immunology and autoimmune disease.* Nat Med, 2012. **18**(1): p. 48-53.
281. Lucchinetti, C., et al., *Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination.* Ann Neurol, 2000. **47**(6): p. 707-17.
282. Roep, B.O., M. Atkinson, and M. von Herrath, *Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes.* Nat Rev Immunol, 2004. **4**(12): p. 989-97.
283. Abdul-Majid, K.B., et al., *Screening of several H-2 congenic mouse strains identified H-2(q) mice as highly susceptible to MOG-induced EAE with minimal adjuvant requirement.* J Neuroimmunol, 2000. **111**(1-2): p. 23-33.
284. Jiang, Y., et al., *Intranasal delivery of stem cells to the brain.* Expert Opin Drug Deliv, 2011. **8**(5): p. 623-32.
285. Danielyan, L., et al., *Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease.* Rejuvenation Res, 2011. **14**(1): p. 3-16.
286. Ross, T.M., et al., *Intranasal administration of interferon beta bypasses the blood-brain barrier to target the central nervous system and cervical lymph nodes: a non-invasive treatment strategy for multiple sclerosis.* J Neuroimmunol, 2004. **151**(1-2): p. 66-77.
287. Hoves, S., et al., *Monocyte-derived human macrophages mediate anergy in allogeneic T cells and induce regulatory T cells.* J Immunol, 2006. **177**(4): p. 2691-8.
288. Tiemessen, M.M., et al., *CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages.* Proc Natl Acad Sci U S A, 2007. **104**(49): p. 19446-51.
289. Lammertse, D.P., et al., *Autologous incubated macrophage therapy in acute, complete spinal cord injury: results of the phase 2 randomized controlled multicenter trial.* Spinal Cord, 2012. **50**(9): p. 661-71.
290. Mikita, J., et al., *Altered M1/M2 activation patterns of monocytes in severe relapsing experimental rat model of multiple sclerosis. Amelioration of clinical status by M2 activated monocyte administration.* Mult Scler, 2011. **17**(1): p. 2-15.
291. Ranganathan, P.V., C. Jayakumar, and G. Ramesh, *Netrin-1-treated macrophages protect the kidney against ischemia-reperfusion injury and suppress inflammation by inducing M2 polarization.* Am J Physiol Renal Physiol, 2013. **304**(7): p. F948-57.
292. Hino, M., et al., *Intrathecal transplantation of autologous macrophages genetically modified to secrete proenkephalin ameliorated hyperalgesia and allodynia following peripheral nerve injury in rats.* Neurosci Res, 2009. **64**(1): p. 56-62.

293. Gratchev, A., et al., *Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals.* Immunobiology, 2006. **211**(6-8): p. 473-86.
294. Davis, M.J., et al., *Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in Cryptococcus neoformans infection.* MBio, 2013. **4**(3): p. e00264-13.
295. Erwig, L.P., et al., *Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines.* J Immunol, 1998. **161**(4): p. 1983-8.