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CD1d restricted NKT cells in regulation of pathogenic autoimmunity

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DOCTORAL DISSERTATION

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| Abstract <p>Human autoimmune diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS) are believed to develop when self-tolerance mechanisms are failing, with a chronic destructive inflammation as result. To experimentally study autoimmunity, we have used murine models that mimic their human counterparts; experimental autoimmune encephalomyelitis (EAE) – a model for MS, and collagen induced arthritis (CIA) and antigen induced arthritis (AIA) – models for RA.</p> <p>A sub-population of T cells, termed natural killer T cells (NKT), have been suggested as having immunoregulatory features in pathogenic autoimmunity. How NKT cells are activated to perform this regulation and by which functional pathways they act is not completely understood. A number of natural antigens activating NKT cells via the antigen presenting molecule CD1d have so far been identified as glycolipids originating from either bacteria or self.</p> <p>This thesis investigates the role of NKT cells in autoimmune tissue-specific inflammation. We found that NKT cells can down-regulate both the central nervous system-specific autoimmunity in EAE, and the joint-specific inflammation in experimentally induced arthritis. We also show data indicating that this regulation requires activation in the periphery, and we have identified a natural self-peptide capable of exerting this activation; A CD1d restricted peptide derived from autologous collagen type II. By vaccinating mice with this peptide we were able to ameliorate the inflammation and reduce the disease phenotype in both CIA and EAE. We also found the peptide-specific NKT cells capable of suppressing activated T cells in vitro. Taken together, this thesis show that CD1d restricted NKT cells can be activated to down-regulate pathogenic autoimmunity, and should be investigated as targets for future autoimmune therapies.</p> | | |
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CD1d restricted NKT cells in regulation of pathogenic autoimmunity

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Original papers

This thesis is based on the following papers, which in the text are referred to by their roman numbers (I-III):

- I. Anna Teige*, Ingrid Teige*, Sharam Lavasani, Robert Bockermann, Emma Mondoc, Rikard Holmdahl, & Shohreh Issazadeh-Navikas. **CD1-Dependent Regulation of Chronic Central Nervous System Inflammation in Experimental Autoimmune Encephalomyelitis.** Journal of Immunology 2004, 172(1):186-94.
- II. Anna Teige, Robert Bockermann, Maruf Hasan, Katarina Olofsson, Yawei Liu, & Shohreh Issazadeh-Navikas. **CD1d-Dependent NK T Cells Play an Important Protective Role in Acute and Chronic Arthritis.** Journal of Immunology 2010, 185:345-356
- III. Yawei Liu*, Anna Teige*, Emma Mondoc, Saleh Ibrahim, Rikard Holmdahl, & Shohreh Issazadeh-Navikas. **Endogenous collagen peptide activation of CD1d-restricted NKT cells ameliorates tissue-specific inflammation in mice.** Journal of Clinical Investigations 2011 Jan;121(1):249-64.

*) These authors contributed equally to this work

Abbreviations

| | |
|-------------------------|---|
| APC | Antigen presenting cell |
| AIA | Antigen induced arthritis |
| B10 | C57B110.Q |
| B6 | C57B16 |
| CD | Cluster of differentiation |
| CFA | Complete Freund's adjuvant |
| CIA | Collage induced arthritis |
| CNS | Central nervous system |
| DTH | Delayed-type hypersensitivity |
| EAE | Experimental autoimmune encephalomyelitis |
| i.p | Intra-peritoneal |
| IFN | Interferon |
| IL | Interleukin |
| <i>M.t.</i> | <i>Mycobacterium tuberculosis</i> |
| mBSA | Methylated bovine serum albumin |
| MBP | Myelin basic protein |
| mCII ₇₀₇₋₇₂₁ | Mouse collagen type II peptide 707-721 |
| MHC | Major histocompatibility complex |
| MOG | Myelin oligodendrocyte glycoprotein |
| MS | Multiple sclerosis |
| NK cell | Natural killer cell |
| NKT cell | Natural killer like T cell |
| NOD mice | Non-obese diabetic mice |
| OVA | Ovalbumin |
| PLP | Proteolipoprotein |
| PT | Pertussis toxin |
| RA | Rheumatoid arthritis |
| s.c | Subcutaneous |

| | |
|------------------|--|
| T1D | Type 1 diabetes |
| TAP | Transporter associated with antigen processing |
| TCR | T cell receptor |
| TGF | Transforming growth factor |
| Th1 | T helper 1 |
| Th2 | T helper 2 |
| Th17 | T helper 17 |
| TNF | Tumour necrosis factor |
| α -GalCer | α -galactosyl ceramide |

Summary of original papers

This chapter provides a short summary of the papers included in this thesis. The results are further discussed in the context with other findings and studies in sections *CD1d antigen presentation and NKT cells* and *Conclusions and reflections*.

Paper I;

CD1-dependent regulation of chronic central nervous system inflammation in experimental autoimmune encephalomyelitis

Background

CD1d restricted NKT cells had been implicated in regulating pathogenic autoimmunity, mostly investigated by activating NKT cell populations with α GalCer. In paper I we investigated whether CD1d restricted NKT cells had intrinsic regulatory properties in EAE, and by what means this regulation was carried out.

Method

We immunized wild type and CD1d deficient mice on a B6 background with MOG₃₅₋₅₅ in CFA to induce EAE, and scored for clinical signs. At different time points during the disease development, we sacrificed animals from both groups to do histopathological examinations of the CNS investigating the degree of immune cell infiltration as well as demyelination. The peripheral immune system was also investigated, by measuring MOG₃₅₋₅₅ specific T cell response in the spleen, assessing T cell proliferation and cytokine production after *in vitro* re-challenge with antigen. In addition, an encephalitogenic MOG₃₅₋₅₅ specific T cell line was established, and used to induce EAE in CD1d deficient and wild type mice.

Results

CD1d deficient mice developed a more severe EAE compared to wild type mice when immunized with MOG₃₅₋₅₅. This was correlated with higher degree of immune cell infiltration and demyelination in the CNS. A pronounced TGF- β production in the CNS of wild type mice could be seen, which was virtually absent in the CD1d deficient mice. In addition, MOG₃₅₋₅₅ specific T cells from CD1d deficient mice had an aberrant level of cytokine production when re-challenged with antigen *in vitro*, which was not correlated with elevated proliferative capacity. When EAE was induced by passive T cell transfer, no difference in clinical phenotype between CD1d deficient and wild type mice was observed. By immunizing the mice with CFA prior to passive EAE T cell transfer, the CD1d dependent regulations observed during the active EAE immunization protocol was restored, as wild type mice developed milder EAE compared to CD1d deficient mice.

Conclusion

CD1d restricted NKT cells regulated clinical manifestations of MOG₃₅₋₅₅ induced EAE, and dampened the immune cell infiltration and demyelination of the CNS. This is possibly mediated through induction of TGF- β at the inflammatory lesions in the CNS. CD1d restricted NKT cells also rendered MOG₃₅₋₅₅ specific T cells less capable of cytokine production in response to antigen activation, but did not influence their capacity to proliferate. CD1d restricted NKT cells needed activation in the periphery to be able to suppress encephalitogenic T cells, either in the periphery, or centrally. This could be achieved by immunization with CFA, but the specific mechanism by which this occurred is not clear.

Paper II;

CD1d-dependent NK T cells play an important protective role in acute and chronic arthritis

Background

CD1 dependent NKT cells had been described as regulators of many autoimmune conditions, but their role in experimental arthritis was unclear – reports of NKT cells acting as both regulators and effector cells had been published. Hence it was of importance to clarify this matter by controlling the genetic backgrounds' influence of the congenic knock-out mice strains used in the studies, and investigate different models of experimental arthritis to further investigate the role for intrinsic CD1d dependent NKT cell regulation.

Method

By utilizing two different models for experimental arthritis, collagen induced arthritis (CIA) and antigen induced arthritis (AIA), we investigated CD1 dependent NKT cells role in both chronic and acute arthritis models. As we were interested in distinguishing between NKT cell's role as inherent regulators of immune homeostasis and as active regulators during the priming of an autoimmune inflammation, we investigated the regulatory role of NKT cells using two methods. Firstly, we induced AIA and CIA in CD1 knock out mice to study the arthritis development in mice where NKT cells were lacking from birth. Secondly, we depleted NKT cells at the time of induction of AIA and CIA, by using a depleting antibody directed towards NK1.1, a surface marked expressed on NK and NKT cells, and observed arthritis phenotype development after NK1.1 depletion. Histology evaluation of joints and T cell responses after *in vitro* re-stimulation were also assessed.

Results

CD1d knockout mice lacking NKT cells developed more severe AIA compared to wild type littermates. This was correlated with a higher degree of histopathological changes of inflammation and proteoglycan depletion in affected joints. When depleting N1.1 positive cells at the time of AIA induction, similar aberrant arthritis

was observed. In both experimental systems, peripheral T cells from mice lacking NKT cells responded with a higher IFN- γ production when re-challenged with antigen *in vitro*. T cells from mice depleted of NKT cells at the time of AIA induction also proliferated more vigorously in response to antigen. These findings were confirmed when using the CIA model, assessing chronic arthritis, as both CD1d knockout mice and mice depleted of NK1.1 expressing cells at time of CIA immunizations developed more severe arthritis compared to the control groups. The CD1d deficient mice had higher numbers of splenocytes, and when re-encountering antigen *in vitro*, peripheral T cells produced higher amounts of IFN- γ , but not IL-17 α , TNF- α or IL-13.

Conclusion

As we observed worsened arthritis both in CD1d deficient mice, and in mice depleted of NK1.1⁺ cells at the time of arthritis induction in both arthritis models used, we concluded that NKT cells are indeed functioning as regulatory cells also in autoimmune arthritis, and are capable to suppress a pathogenic inflammation specific for joint tissue. This was also seen as a lowered capacity of antigen specific T cell production of IFN- γ , compared to a maintained IL-17 α production – indicating that NKT cells altered the function of effector T cells, dampening the antigen specific Th1, but not the Th17, response.

Paper III;

Endogenous collagen peptide activation of CD1d-restricted NKT cells ameliorates tissue-specific inflammation in mice

Background

In contrast to heterologous collagen type II (CII), mouse CII is a poor arthritogen. However, several immunogenic epitopes have been identified from mCII. In this paper we aimed at characterizing the response to one of the immunodominant epitope on mouse CII, namely peptide 707-721 (mCII₇₀₇₋₇₂₁).

Method

Mice on B10.Q background were immunized with mCII₇₀₇₋₇₂₁ in CFA and peripheral T cells were re-challenged *in vitro* to investigate the T cell response. The T cell response in a variety of knockout mice on B10.Q background (i.e. TCR $\alpha\beta^{-/-}$, TCR $\gamma\delta^{-/-}$, CD4 $^{-/-}$, and CD8 $^{-/-}$ mice) was characterized to identify the T cell population responding to mCII₇₀₇₋₇₂₁. In addition, the antigen-presenting molecule was identified by using MHCII $^{-/-}$ and TAP $^{-/-}$ mice. The antigen specific cytokine responses and TCR usage of mCII₇₀₇₋₇₂₁ specific cells after *in vitro* antigen re-challenge was evaluated by ELISA and PCR. The TCR signalling and co stimulatory requirements upon mCII₇₀₇₋₇₂₁ stimulation were considered by looking at the phosphotransferase kinase ZAP-70 on the cell surface and by using blocking antibodies towards co stimulatory molecules in the *in vitro* cultures. As CD1d had been identified as a candidate antigen presenting molecule, we also performed a CD1d - mCII₇₀₇₋₇₂₁ binding assay, using plate bound CD1d and biotinylated mCII₇₀₇₋₇₂₁ to detect bound peptide. To further address the qualities of the mCII₇₀₇₋₇₂₁-specific response, we established an antigen specific T cell line by cycled *in vitro* antigen simulation and resting.

The suppressive capabilities of the mCII₇₀₇₋₇₂₁-specific cell line were investigated in a co-culture system where anti-CD3 activated spleenocytes were co-cultured with the mCII₇₀₇₋₇₂₁-specific T cell line and proliferation and apoptosis was assessed.

The mCII₇₀₇₋₇₂₁-specific T cell line was characterized as having immunoregulatory capacity, and hence, different *in vivo* models were used to study the mCII₇₀₇₋₇₂₁-specific population's effect on pathogenic inflammation. B10.Q mice were vaccinated with mCII₇₀₇₋₇₂₁ prior to induction of CIA, EAE, DTH and ovalbumin (OVA) sensitization of the airways. The mCII₇₀₇₋₇₂₁ vaccinated mice were compared with mice vaccinated with a control peptide. EAE and CIA disease phenotype was evaluated, both clinically by scoring the animals for manifested symptoms, as well as by performing histopathological and immunological evaluations of the target tissues. DTH was evaluated by histological examination, and OVA sensitization was evaluated by measuring cytokines and IgE in BAL fluid.

Results

Cells responding to mCII₇₀₇₋₇₂₁ were found to be CD4⁺NK1.1⁺TCRαβ⁺ cells, independent of both MHC I and II, and restricted to CD1d presentation of mCII₇₀₇₋₇₂₁ as showed by blocking of CD1d with antibodies. We also found that the mCII₇₀₇₋₇₂₁ peptide bound to CD1d in a concentration dependent manner. The mCII₇₀₇₋₇₂₁-specific CD1d restricted NKT cells produced IFN-γ, IL-4 and TGF-β in response to antigen, whereas no IL-2 or IL-10 production could be detected. They showed a restricted TCR Vα-Jα18 usage, paired with polyclonal and diverse Vβ chains, and TCR engagement and signalling in antigen activation was observed by ZAP-70 phosphorylation. The CD1d restricted NKT cells were also found to be dependent on functional co stimulation, i.e. B7.1-CD28 and CD40-CD40L, for full activation.

The mCII₇₀₇₋₇₂₁-specific NKT cells inhibited *in vitro* anti-CD3 activated T cell proliferation in a cell-cell contact dependent manner, and they were capable of inducing apoptosis through Fas-Fas-ligand interaction of a co-cultured anti-CD3 activated T cell line.

Mice vaccinated with mCII₇₀₇₋₇₂₁ were partially protected from both CIA and EAE, showing both lower clinical scores as well as less inflammation and tissue damage at site of inflammation. The alleviation of CIA was not due to a Th2 cytokine shift in inflamed joints, as cells in vaccinated mice produced reduced levels of both IFN-γ and IL-4. In line with this finding, the mCII₇₀₇₋₇₂₁-specific NKT cells also lowered Th1 driven DTH reaction in vaccinated mice, and reduced the Th2 response (measured by IL-4, IL-5, and IL-13 and IgE) in OVA sensitized airways of vaccinated animals compared to control group.

Conclusion

Immunization with mCII₇₀₇₋₇₂₁ induced an immunoregulatory CD1d restricted NKT cell population. The NKT cells responded to mCII₇₀₇₋₇₂₁ via CD1d presentation and were dependent on TCR and co stimulatory signalling for activation. This activation induced a clear cytokine response, but the *in vitro* suppressive functions were shown to be cell-cell contact dependent. The mCII₇₀₇₋₇₂₁-specific NKT cell population was shown to have the capacity to suppress pathogenic autoimmune inflammatory reactions independent of tissue specificity, as ameliorated EAE and CIA was observed after pre-vaccination. In addition, both Th1 and Th2 driven inflammatory conditions (i.e. DTH versus allergic airway inflammation) was suppressed by this NKT cell population. The results suggest that this immunological control is partly exerted via their capacity to induce apoptosis in activated T cells.

General introduction

The immune system is a highly specialized system, whose primary function is to defend its host against infectious microbes. It is generally divided in innate and adaptive immunity, with a distinction in the specificity of the immune reaction. As evident from the nomenclature, innate immunity consists of defence mechanisms that are in place even before an infection has occurred and is fully functioning and not further developed from birth, whereas the adaptive immune system is evolving during lifetime, and dependent on the challenges the individual encounters over time.

The adaptive immune system consists of cells that originate from stem cells in the bone marrow. From here, two types of adaptive immune cells –lymphocytes– emerge, giving rise to humoral immunity mediated via B cells and antibodies, or cellular immunity mediated via T cells. B cells leave the bone marrow as mature lymphocytes, whereas progenitors for T cells leave the bone marrow for the thymus, where the maturation of T cells is completed. From here, mature T cells emerge to circulate via blood and lymph, patrolling through the body. Both B and T lymphocytes carry a diverse set of receptors, generated by somatic gene rearrangements and capable of recognizing a wide variety of structures, antigens, giving rise to a highly antigen specific immune response when activated.

Innate immune cells also originate from stem cells in the bone marrow, but in contrast to adaptive lymphocytes, the innate immune response is elicited by structures shared by groups of microbes, pattern recognition, and provides the early line of defence against pathogens. Another important difference between the two branches of the immune systems is the so-called memory function of the adaptive immune response. Whereas the innate immune system has exactly the same features after an infection, the adaptive immunity evolves from and remembers an antigenic challenge, leaving the host better equipped to respond to the same antigen than before.

Both these two systems are highly integrated, dependent on each other for full activation and termination, and cannot be looked upon as isolated parties. To mediate communication between different immune cells (and with other organs and tissues), cells secrete soluble proteins called cytokines and chemokines. These are immunological mediators important for initiating, maintaining and terminating an immune reaction, and they also localizes the immune cells from the circulation to the tissue where an immune reaction is required.

Tolerance and autoimmunity

Mechanisms for keeping self-tolerance

The immune system is a powerful organ with the ability to kill cells and give rise to harmful inflammations; hence it needs to be under strict regulation. One remarkable feature of the immune system is its ability to recognize and respond to foreign antigens, while not attacking the individual's own tissues. This is called the immune system's capacity to distinguish between non-self and self, or keeping self-tolerance.

Self-tolerance is achieved through several mechanisms; one is through the elimination of T lymphocytes that express receptors capable of recognizing self-antigens presented in the thymus, a process called negative selection and maintaining central tolerance. This is a process carried out during the maturation of T cells in the thymus. As a strong and forceful immune reaction is dependent on specific T cell help, deleting auto reactive T cells is an efficient way to prevent immune responses to self-antigens. The negative selection process is however not complete, as not all antigens expressed in the periphery is presented to the maturing T cell in thymus, and self-reactive T cells do emerge from the thymus. To control these potentially dangerous self-reactive cells, other mechanisms need to be operative in the periphery – to keep peripheral tolerance to self. Peripheral T cell tolerance is maintained mainly by three known mechanisms; induced anergy leaving the T cell in an unresponsive state, deletion and cell death by apoptosis of cells reactive to self, and functional suppression of auto reactive T cells by regulatory T cells.

Regulatory T cells are believed to have the capacity to suppress auto reactivity, either by by-stander mechanisms and secreting anti-inflammatory cytokines and hereby create an anti-inflammatory milieu for further immune cell activation, or by direct cell-cell interactions, affecting both antigen presenting cells (APCs) and auto reactive T cells (1, 2). The most extensively studied regulatory T cells are the cluster of differentiation (CD)4⁺CD25⁺FoxP3⁺ T cells, but other populations clearly exists. One is the CD1d dependent natural killer (NK) like T cell population, which has been the main research focus of this thesis. This cell population is further described in section *CD1d antigen presentation and NKT cells*.

Autoimmune diseases

When tolerance mechanisms are failing, an autoimmune disease can develop. The self-reactive cells of an individual's immune system will unhindered react towards cells and matrix of the own body, which in turn leads to a chronic inflammatory disease. Most often these disorders are tissue specific, meaning that one target organ is under attack rather than the whole body. Examples of this are rheumatoid arthritis (RA) and multiple sclerosis (MS) where epitopes and antigens in the joint and in the central nervous system (CNS) respectively are believed to be the targets for the adverse immune reaction.

Rheumatoid arthritis, RA

About 1% of the world's population is affected by rheumatoid arthritis, with a three times higher prevalence in women than in men. Although rheumatoid arthritis can occur at any age from childhood to old age, onset is most frequent between the ages of 30 and 50 (3, 4). RA is a chronic disease in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function and joint deformation. The pattern of joints affected is usually symmetrical, involving the hands and other peripheral joints. For the patient it is a disabling and painful condition which can eventually lead to complete immobilization and premature mortality. Diagnosis is made mainly on signs and symptoms, complemented with blood tests (screening for rheumatoid antibodies and other markers for inflammation) and imaging examinations such as ultra sound, magnetic resonance imaging analysis, and radiological examinations (5). The etiology of RA is unknown, although a combination of factors including an abnormal autoimmune response, genetic susceptibility, and environmental factors probably triggers the disease.

The disease process leading to RA is believed to begin in the synovium surrounding the joint. An abnormal immune reaction causes a chronic inflammation of the synovium, leading to collagen breakdown of the cartilage in the joint. This narrows the joint space and leads eventually bone damage. In progressive RA, the disease course is accelerated and a pannus is formed. The pannus is a growth composed of synovial tissue, which in turn produces more tissue destructive molecules and enzymes, driving the cartilage destruction, and leads to further inflammation and immune cell infiltration to the joint. Pathogenic T cells producing pro-inflammatory cytokines driving the joint inflammation, are believed to be imperative in the detrimental inflammation, but it is clear that also B-cells and antibodies binding to Fc γ receptors on macrophages are needed to

make the inflammation pathogenic with cartilage and bone destruction as a consequence (6).

Drugs commonly used to treat RA include nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biologic response modifiers, and corticosteroids. The NSAIDs are prescribed to relieve the pain caused by the underlying inflammation, but they do not modify the disease progression. The DMARDs and the biological modifiers are on the other hand indicated to slow down the disease progression by modifying the immune system. Even though the exact mode of action for its use in RA is unknown, the most commonly used DMARD is methotrexate, as this compound is available in generic forms. However, severe side effects have been reported with this drug. A number of newer and more specific biological modifiers are available, with the anti tumor necrosis factor (TNF)- α drugs being the most commonly prescribed. These drugs includes infliximab, etanercept, adalimumab , golimumab, and certolizumab, and acts to modify the immune system and dampen the tissue destruction by blocking the inflammatory cytokine TNF- α . In addition, biological compounds targeting interleukin (IL)-6, IL-1, or the activation of T or B cells are approved to treat RA. Further, the regulatory authorities in US last year approved a new DMARD, tofacitinib, a janus kinase 3 (JAK3) inhibitor, inhibiting the production of inflammatory mediators by suppressing genes regulating cytokine production in joint tissue. Finally, classical immune suppressive corticosteroids are used to dampen the joint inflammation, but the poor safety profile of these drugs prohibits long term use.

Over the past years, biological compounds with disease modifying properties have become standard care for RA in the western world, usually prescribed in addition to classical anti-inflammatory and analgesic drugs, and leading to not only symptom relief but also slower disease progression. However, there is currently no cure for RA, and the underlying cause of the inflammatory attack on the joints cannot be treated.

Multiple sclerosis, MS

MS is a relatively common neurological disease affecting more than 1 million people worldwide. Its prevalence rate varies between ethnicity and region, ranging from more than 100 per 100,000 in Northern and Central Europe to 50 per 100,000 in Southern Europe. It is more common in women and in Caucasians. In MS, the inflammatory process is believed to be triggered by T cells reacting with myelin antigens surrounding the neuronal axons in the central nervous system, which in turn leads to myelin breakdown, axonal disturbances, and impaired neuronal functions and signalling (7, 8). Affected patients go through a series of

neurological disturbances, usually with a remitting-relapsing pattern, which eventually progress to physical and cognitive disability, and increased mortality. As for RA, diagnosis is made from clinical evidence, complemented with analysis of the cerebral spinal fluid for antibodies, and magnetic resonance imaging analysis (8). 82 to 85 % of all patients present with relapsing-remitting MS, which is characterized by unpredictable acute episodes of neurological dysfunction (relapses), followed by variable recovery and periods of clinical stability. Within ten years more than 50% of patients who presented with the relapse remitting form develop sustained deterioration with or without superimposed relapses; this form of MS is known as secondary progressive MS. Approximately 15% of MS patients develop a sustained deterioration of neurological function from the beginning; this MS variant is known as primary progressive MS. Pathophysiological processes involve acute inflammatory focal lesions, gliosis, demyelination, impaired remyelination, axonal loss and neuronal loss which occur at all stages of the disease. The relative contribution of these processes changes during the course of the disease. Relapses are considered to be the clinical expression of acute inflammatory focal lesions, whereas progression is more associated with demyelination, impaired remyelination, axonal loss and neuronal loss. In primary progressive multiple sclerosis, inflammation is cortical and more diffuse. Also very similar to RA, there is no cure available for MS, and the etiology of MS remains unknown. Current therapeutic approaches involve symptomatic treatment, treatment of acute relapses, and disease modifying therapies. Symptomatic treatment refers to all therapies applied to improve symptoms and complications caused by the disease e.g. fatigue, spasticity, ataxia, walking disability, weakness and bladder and bowel disturbances. During the last two decades, interferon (IFN) β -1a and 1b, mitoxantrone, glatiramer acetate and natalizumab have been established as disease modifying treatments; all of these treatments are administered parentally. The first oral treatment for MS, fingolimod, was recently approved by regulatory authorities in EU and US. Patients are given disease-modifying treatments with the aim to adjust or diminish the immune response and inflammatory reactions in the CNS, but classic immune suppressive treatments, such as systemic corticosteroids, are usually only given during acute relapses. Drugs available today have been shown to reduce the frequency of new relapses, and are hence believed to have the capacity to reduce disease development over time.

Both MS and RA are diseases devastating for the individual and families, with only partially effective treatments available. This shows the importance of proper regulation of the immune system and maintenance of self-tolerance. It also highlights the need of increasing the knowledge of how immune regulation can be restored when it does not function as needed and an autoimmune disease develops. As of today, the exact cause of autoimmune diseases is not known. However,

patients are usually treated with drugs targeting inflammatory mechanisms, modulating or suppressing the immune system. These drugs have the capacity to ameliorate and dampen the symptoms and to delay the progression of tissue destruction and disease, but not to completely cure the patient. More knowledge in the field of immune regulation in the context of tolerance to self-antigens will enable the scientific community to develop more effective treatments in the future, and eventually also cures, to affected individuals.

Aim of this thesis

As illustrated, there is a need to learn more about control mechanisms operating in the immune system, and how one can modulate the regulation of auto reactive immune responses.

This thesis investigates a subpopulation of T cells – CD1d dependent NKT cells - in the context of pathogenic autoimmunity. Our interests in these cells originated from studies implicating that they constitute a T cell subpopulation with regulatory capacities. The work is focused on investigating whether NKT cells have the capability to regulate and suppress autoimmune conditions, and how NKT cells are activated to exert such a regulatory function. The studies also included investigations on operating mechanisms in this suppression.

As the immune system a complex network, the objective of the work was to use *in vivo* animal models as far as possible, and to complement this with *in vitro* experiments. Hence the main emphasis has been on the regulatory function of NKT cells in animal models for two human autoimmune diseases; MS and RA. Murine models for these diseases were utilized, namely experimental autoimmune encephalomyelitis (EAE) which is a model for MS, and antigen induced arthritis (AIA) and collagen induced arthritis (CIA), experimental models for RA.

The specific aims of the thesis were:

- Investigate the natural function of CD1d dependent NKT cells as regulators in tissue specific autoimmunity, utilizing experimental autoimmune animal models.
- Characterize the T cell response towards a major antigen from mouse cartilage, collagen type II peptide 707-721 (mCII₇₀₇₋₇₂₁), and further investigate the regulatory capacity of this cell subset in tissue specific inflammation.

Animal models for autoimmunity

Animal models provide a useful tool in research by which we can control variability in genetic background and environment. It clearly also offers a mean to perform experiments impossible to do in human studies due to ethical reasons. As the need to learn more about mechanisms operative in pathogenic autoimmunity, and by that finding effective therapeutic target for the treatment of chronic and lethal human autoimmune diseases, is urgent, the benefits of valid animal models are evident.

Experimental autoimmune encephalomyelitis, EAE

MS is believed to be caused by an autoimmune T cell driven assault on the myelin sheets in the CNS, including infiltration of CD4 and CD8 expressing T cells. The most commonly used animal model to experimentally mimic and study autoimmune CNS inflammation and MS is Experimental autoimmune encephalomyelitis (EAE) (9). It was used as early as 1935, when Rivers et al. described experimentally induced demyelinating encephalomyelitis in monkeys (10). Today, most groups use murine models of EAE, induced by immunization with myelin derived proteins or peptides in susceptible mouse strains, resulting in activation of auto reactive, myelin specific, CD4⁺ T cells and the hereby following autoimmune inflammatory attack on the myelin sheets in CNS. Myelin proteolipid protein (PLP), myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG), as well as immunogenic peptides from these, are all commonly used as EAE inducing antigens. The characteristics of the induced disease varies depending on mouse strain and immunizing agent, but typical clinical symptoms are paresis with subsequent paralysis, usually starting in the tail of the animal and moving forward to hind- and fore- legs as the disease progress. In severe cases, the disease results in complete paralysis and death. One notable contrast from human MS, is that in MS, the infiltrates are dominated by cytotoxic CD8⁺ T cells, whereas in the EAE model, CD4⁺ T cells are considered to be the drivers of the disease (11, 12). The realization in the scientific community of this inconsistency has evoked the search for a CD8⁺ T cells driven model to complement the MOG induced EAE, and this development is still in progress (13).

As EAE can be induced by passive transfer of myelin specific CD4⁺ T cells to healthy animals, it has been regarded as a T cell mediated disease.

Encephalitogenic T cells usually exhibit a T helper 1 (Th1) or Th17 phenotype (14-20), and even though there are also reports of T helper 2 (Th2) clones causing an un-typical form of EAE in immunodeficient hosts (21, 22), Th2 cells are generally thought to be protective in EAE (23). Lesion formation in the CNS of EAE affected mice is characterized by perivascular demyelination and immune cell infiltration, where CD4⁺ T cells and macrophages are most abundant (24). In addition to T cells, cells of the monocytic lineage have been shown to play a critical role in driving the disease and generating tissue damage (25, 26).

Different cytokines have been proven important in the regulation of EAE (27, 28). The classical Th1/Th2 paradigm with EAE being a Th1 cytokine driven disease has been shown to have some shortcomings; IFN- γ deficient mice are more susceptible to EAE with an impaired recovery, suggesting that this hallmark Th1 cytokine is not necessarily pathogenic but can even inhibit EAE development (29-31); Mice deficient in the IL-12 p35 subunit were more susceptible to EAE, whereas deficiency in the IL-12 p40 subunit rendered mice completely resistant to EAE, indicating that the Th1 driving cytokine IL-12 in its intact form is not imperative in driving the disease, but its subunit p40 is (32). Even so, Th2 cytokines (IL-4, IL-10 and IL-13) have been shown to be protective in EAE (33), indicating that a Th2 response might be beneficial for the affected host. This paradox has at least partly been explained by the identification of a third T helper cell lineage, the Th17 cells, which has been shown to drive the pathogenic inflammation in EAE (34-36). The Th17 response is characterized and sustained by the cytokines IL-17 and IL-23 (18). As IL-23 shares one subunit with IL-12, the p40, this would explain the paradoxical findings described above, and the redundancy in the IL-12 system in driving pathogenesis in EAE. In addition, it is believed that Th17 response is inhibited by IFN- γ , and hence explains the fact that mice deficient in IFN- γ express and ameliorated EAE (31).

The potent immunoregulatory cytokine transforming growth factor (TGF)- β (37) has also been shown to have the capacity to mediate regulation of EAE. This has been demonstrated both by the administration of TGF- β to EAE affected animals (38, 39), and by an association of resistance to EAE to the expression of TGF- β in the CNS (40, 41). In addition, in paper I we present data linking TGF- β production in inflamed CNS to the regulatory functions of CD1d restricted NKT cells in EAE. Another important cytokine in the context of EAE is IFN- β , as one of the most commonly used treatments for MS today is injections with IFN- β (42-44). IFN- β has been shown by our group to have regulatory properties also in EAE by inhibiting the T cell activating capacity of antigen presenting cells in the CNS (45). The exact mode of action of IFN- β -treatment in MS patients is still under investigation, but recent data points towards several points of impact on the immune system, as IFN- β -treatment in MS patients has been shown to promote

regulatory NKT cells via dendritic cell activation (46), as well as dampen the IL-1 β secretion from monocytes via inhibition of signal transducers and activators of transcription-1 (STAT-1) dependent intracellular signalling (47).

In present work two different mouse models to study EAE were utilized. Firstly, the frequently used MOG peptide 35-55 (MOG₃₅₋₅₅) induced EAE in C57Bl6 (B6) mice, described by Mendel et al., have been used to study the intrinsic role of CD1d restricted NKT cells in EAE. Data from these studies are presented in paper I. In this model, B6 mice are subcutaneously (s.c) immunized with the immunodominant peptide of MOG, MOG₃₅₋₅₅, in complete Freund's adjuvant (CFA), together with an additional adjuvant injection, i.e. pertussis toxin (PT) given intra-peritoneal (i.p) (48). This results in a chronic inflammation of the CNS with infiltrating immune cells and clinical symptoms appearing after approximately 10 days. The disease manifests in nearly 100% of immunized animals and is evident histopathologically by the presence of infiltrating inflammatory cells and relatively large areas of demyelination in the CNS. This model is hence looked upon as a valid model for demyelinating autoimmunity of the CNS. To use a model suitable for mice on the B6 genetic background was important, as the B6 strain is the most characterized mouse strain for NKT cell properties and activity.

However, mice with a major histocompatibility complex (MHC) genotype Q are not susceptible to MOG₃₅₋₅₅ induced EAE. Hence, in paper III, the MOG peptide, MOG₇₉₋₉₆, was used to induce EAE in C57Bl10.Q (B10.Q) mice as described by Abdul-Majid et al (49). As in the MOG₃₅₋₅₅ model, mice are immunized with the MOG₇₉₋₉₆ peptide in CFA and PT is given i.p as an adjuvant, resulting in CNS inflammation with demyelination and clinical manifestations. Although less widely used, this model is also considered valid to study autoimmune inflammation of in the CNS. By utilizing BQ mice in paper III we were able to investigate the effects of mCII₇₀₇₋₇₂₁ vaccination on both EAE and experimental arthritis, keeping the experiments focused to the same mouse strain while investigating different tissue specific inflammations.

Experimentally induced arthritis, CIA and AIA

Collagen induced arthritis, CIA

As in the case of EAE, Collagen induced arthritis (CIA) is provoked in susceptible rodents by immunization with a target tissue antigen, in this case collagen type II (CII). This causes an autoimmune attack on the joint cartilage, which is thought to mimic the events in RA patients. The model was first described in 1977, when Trentham et al. induced arthritis in rats by immunization with CII (50), and a few years later CIA in mice was reported (51). Today, it is one of the most widely used animal models for RA. The clinical phenotype of CIA can be seen in swelling and redness of peripheral joints, and permanent deformity of the affected paws is usually observed once the active inflammation subsides. The disease can be considered as chronic relapsing, as new inflammatory foci appears over time, affecting new joints, whereas others disappears leaving tissue destruction and deformity of the joint. Histopathologically the disease is characterized by infiltration of immune cells to the joints, as well as cartilage and bone destruction.

As for EAE, Th1 and Th17 cells seem to play a critical role in inducing CIA (52-54). T cells are found in the joints of arthritic mice (55), and mice carrying a targeted deletion of the TCR- β loci have been shown to be completely resistant to CIA (56). Importantly, the susceptibility to CIA is closely linked to the MHC genotype (57, 58), indicating that T cells activated by specific antigens presented on MCH drives the disease. Even so, B cells and antibody production are required for a full-blown CIA (59, 60), and in contrast to EAE, CIA cannot be induced by antigenic peptides from collagen, but requires immunization with whole CII. This points towards the importance of B cell receptor recognition of the three dimensional structure of CII in addition to T cell activation. In addition, arthritis can be induced solely by injection of collagen specific antibodies, but is then also enhanced by T cells (61, 62).

Inflammatory cytokines, produced mainly by macrophages, such as tumour necrosis factor (TNF)- α , IL-1, and IL-6, play a key role in driving the autoimmune attack in the joint, and these cytokines are today targets for approved therapeutic drugs in the treatment of RA (63-68).

In addition, IL-17 has been shown to synergistically enhance TNF- α induced pathological processes in experimental arthritis, and has been suggested as a future therapeutic target, in combination with existing drugs targeting TNF- α (69). The imperative role of IL-23 in the development CIA has also been demonstrated, as

IL-23 subunit p19 mice were shown to be resistant to CIA (70). Hence, also IL-23 is today a suggestive target for therapeutic interventions for RA (71).

Interestingly, CIA in mice is most effectively induced by immunization with heterologous CII, e.g. rat, chicken or bovine CII, whereas mouse CII provokes a weaker T and B cell response to the immunodominant epitope and induces a lower incidence of arthritis (72, 73). This could in part be due to the fact that compared to the immunodominant epitope in rat CII at position 260-270, mouse CII differs in two amino acids and binds with a slightly lower affinity to the MHC class II molecule in susceptible mouse strains (74-76). We hypothesized that another explanation, contributing to this finding, could be that an immunogenic epitope in mouse CII was inducing a T cell response with regulatory functions. A major epitope apart from the position 260-270 had been identified in mouse CII (77), and hence we further investigated this hypothesis by characterizing the immune response to this epitope. Data supporting that indeed this epitope is inducing a specific regulatory response is presented in paper III.

Antigen induced arthritis, AIA

Murine antigen induced arthritis have been extensively described by Brackertz back in 1977(78-80). It is a T-cell driven model for arthritis, caused by direct injection of an antigen into the knee joint after a pre-immunization with the antigen of choice to evoke the immune response (81, 82). Most commonly used as inducing antigen is methylated bovine serum albumin (mBSA), which is a cationic molecule allowing for antigen retention in the joint through charge mediated bindings.

The model is not as widely used as CIA to study arthritis, as it does not mimic the self-antigen driven chronic features of human RA as close, but as an acute animal model of joint inflammation targeting the cartilage, it has proven useful. The main advantages are shorter experimental incubation time, and less variability in the disease manifestation than CIA, and hence fewer animals can be used for a shorter period of time. In addition, the arthritis affects only the joint injected with the antigen, which makes it possible to use a contra-lateral joint as control.

We used this model as a mean to complement experiments in CIA, and to address the issue of whether NKT cells work as regulators or pathogenic drivers in arthritis. Reports showing dual functions of NKT cells had been published in the literature, and data indicated that the intrinsic functionality of CD1d dependent NKT cells could vary in different animal models, in different mouse strains, and during different stages of inflammation. We aimed to investigate this further by

using two different animal models for arthritis on the same genetic background (the B6 mouse), that could represent a chronic joint cartilage specific arthritis (CIA), versus a more acutely induced joint inflammation (AIA). Data presented in paper II showed that CD1d dependent NKT cells indeed worked as regulators in both arthritis models.

CD1d antigen presentation and NKT cells

CD1d antigen presentation and NKT cell activating ligands

The CD1 protein family is comprised of antigen presenting molecules, found on the cell surface of mainly hematopoietic-derived cells (83, 84). The human genome encodes five CD1 genes (*CD1A-E*) with four corresponding proteins (CD1a-d) expressed on the cell surface (85), and a fifth (CD1e) expressed as a soluble form in the lysosomal compartments (86). Based on shared sequence homology, the CD1 molecules are divided in group 1 (CD1 a, b and c), and group 2 (CD1d) (87). The group 1 CD1 molecules are found in humans but not mice, whereas group 2, CD1d, is found in both species (88). Several similarities between the classical antigen-presenting molecules MHC class I and II and the CD1 antigen presenting molecules can be found; β 2-microglobulin association is required for the cell surface expression, and the overall structural architecture resembles that for MHC class I (89), whereas its localization to endosomal compartments and predominant expression on APCs are more similar to MHC class II (90, 91).

Unlike the MHC molecules, the CD1 molecules have a deep and hydrophobic antigen binding groove, comprising two pockets, and numerous studies have demonstrated the capacity of CD1 to present lipid antigens from bacterial pathogens, mounting a T cell response thought to be of importance in clearance of infections (92-98). This is in contrast to the classical MHC class I and II molecules, which present peptides to CD8⁺ and CD4⁺ T cells respectively. The findings that a specific immune response can be elicited to bacterial lipidic

two subsets recognize different sets of antigens, and studies have revealed that they also have different functional attributes (132). Tumour immunity studies have revealed that α -GalCer responsive type I NKT cells promote the immune response, whereas the type II variant NKT cells negatively regulate the tumour immunosurveillance (133-135). It is also evident that in certain infectious diseases, invariant type I NKT cells play an immune enhancing role, helping clearing the infection (136, 137), whereas the CD1d restricted response to the myelin-derived antigen sulfatide, mediated by type II NKT cells, is of negative regulatory nature (105, 138). In addition it has been shown that tolerogenic dendritic cells are acting via type II NKT cells, not involving invariant type I NKTs (139). This could indicate a natural duality between the variant and invariant NKT cells, suppressing and enhancing an immune reaction respectively. It has however been shown that also type I invariant NKT cells suppress and protect against autoimmune conditions, specifically EAE and Type 1 diabetes (T1D), (140-142) (143), indicating that this duality in function is not as clear cut as one could first imagine.

CD1d restricted NKT cells have been shown to be dependent on the expression of CD1d in the thymus to develop, and therefore this population is missing in CD1d deficient mice (144, 145). They leave the thymus as mature cells, and have been described as fast potent cytokine producers; when stimulated *in vitro*, they rapidly release large quantities of cytokines, IL-4, IFN- γ and IL-17 included (146, 147). Due to their rapid cytokine secretion potential, they have been suggested as regulators of the delicate balance between Th1 and Th2 immune responses, but research has showed that mice deficient in CD1d are capable of developing as good a Th2 response as wild type mice (144, 145). Nevertheless, they have been implicated as regulators and suppressors in pathogenic Th1 and Th17 driven inflammatory autoimmunity, both in human diseases such as T1D (148), MS (149-152) och RA (153-156), as well as in the animal model counterparts (157-161). Specifically, patients with MS has been reported to have a numerically altered NKT cell populations in the periphery, with higher numbers of circulating invariant NKT cells, but this population was shown to be unresponsive to stimulation with α -GalCer and IFN- γ , which would be consistent with an anergic state induced by previous exposure to antigen (152). In addition, it has been shown that CD4+ NKT cells expanded from MS patients in remission produced higher amounts of IL-4 compared to cells from healthy subjects or MS patients in relapse, indicating a regulatory role for these cells in MS (149). In patients with RA, reports have shown that the number of circulating NKT cells are lower compared to healthy controls (155, 162), and after treatment with rituximab, clinical remission was correlated with an increase in NKT cell frequency (162). Further, it has been shown that NKT cells are present in the affected synovia of RA patients. NKT cell lines from peripheral blood from these RA patients showed a significantly reduced number of IL-4 producing cells, whereas this was not the

case for NKT cells isolated from synovial fluid (152, 153). This shows that NKT cells might be functional in the progression of RA, and the authors suggested that providing a local boost to the regulatory potential of NKT cells might represent a useful candidate therapy for RA.

In papers I, and II, we show data that strengthens the idea of CD1d restricted NKT cells as regulators of pathogen autoimmunity and as having an intrinsic capacity to suppress and ameliorate the clinical and pathological course of both EAE and experimental arthritis (data is further discussed in sections *NKT cells in the regulation of EAE* and *NKT cells in the regulation of experimental arthritis*).

Further showing the immune regulatory capacity of CD1d restricted type II NKT cells, paper III presents the characterization of a murine NKT cell population capable of recognizing an immunodominant epitope of mouse CII (mCII₇₀₇₋₇₂₁) in the context of CD1d, which upon activation showed immunoregulatory properties on a variety of tissue specific inflammations. The recognition of the immunodominant peptide of mouse CII by CD1d restricted NKT cells is a novel finding as it is the first report of a self-peptide presented by CD1d to NKT cells. The mCII₇₀₇₋₇₂₁ specific CD1d restricted NKT cell population was shown to be TCR $\alpha\beta^+$ and CD4 $^+$, and dependent on both TCR and co-stimulatory signalling for its activation in response to CD1d and mCII₇₀₇₋₇₂₁. Further adding importance to this finding, we observed that the NKT cells elicited in response to immunization with the CII-peptide were capable of down regulating pathogenic inflammatory autoimmunity. This was shown by an alleviated clinical course of both CIA and EAE after pre-vaccination with the peptide, with corresponding decreased inflammation in joints and CNS respectively. A Th2 cytokine shift of the inflammatory cells in vaccinated mice was not evident, but rather a decrease in both IFN- γ and IL-4 producing cells when investigating arthritic joints from vaccinated mice compared to control group. In addition, a lowered delayed-type hypersensitivity (DTH) reaction - a model for cell-mediated Th1 immunity - as well as a decreased immunoglobulin (Ig) E response in bronchoalveolar lavage fluid (BAL) from sensitized mice - a model for humoral Th2 immunity - was found after vaccination with the peptide. This showed that the CD1d restricted NKT cell population specific for mCII₇₀₇₋₇₂₁ had suppressing effects on inflammatory responses regardless of the Th1 / Th2 dichotomy. Attempting to disclose the mechanisms behind this regulation, we found that the mCII₇₀₇₋₇₂₁ specific NKT cells were suppressing T cells via cell-cell contact and were capable of inducing Fas-ligand dependent apoptosis in T cells, similar to the killing of tumour cells by a-GalCer activated NKT cells previously reported (163), and their regulatory capacity was independent on cytokines. Fas-ligand depended induced apoptosis has also been described for another subset of CD1d restricted cells; CD1d restricted $\gamma\delta$ T cells are during virus infections capable of killing regulatory T cells

through Fas-dependent apoptosis (164). It has been suggested that the balance between CD1d restricted NKT cells and CD1d restricted $\gamma\delta$ T cells activation can be an influencing factor between self-tolerance and autoimmunity.

Our findings indicate that the NKT cell population characterized in paper III have immunomodulatory qualities. Hence activation hereof by the CII-peptide could potentially be used as treatment for harmful immune reactions, such as tissue specific inflammatory autoimmunity. It has been described that there is a high degree of conservation between man and mouse regarding the recognition of CD1d by NKT cells. Human NKT cells recognise mouse CD1d and vice versa (88, 97). This indicates that the mechanisms described above could be applicable to treat human autoimmune diseases. Clearly, paper III gives just a first hint on the therapeutic potential of this antigen, but one well worth investigating further. Future studies should include a thorough investigation on how the collagen peptide binds to mouse CD1d and interacts with the NKT cell TCR, which signalling pathways this interaction triggers, and also studies to elucidate whether human CD1d can present the mouse collagen peptide to elicit a similar response in human NKT cells, and later if such a response could have regulatory capacities on pathogenic inflammation in man.

NKT cells in the regulation of EAE

A number of studies on CD1d dependent NKT cells and their regulatory function in EAE in mice have been published to date. The majority of these studies have focused on the activation of invariant type I NKT cells by α -GalCer as a mean to regulate pathogenesis in CNS (103, 165-168). A few others, including a study from our group (paper I), have looked at NKT cell's inherited capacity to regulate EAE without α -GalCer activation (141, 142, 157, 169).

Induced NKT cell activity to regulate EAE

Studies with somewhat conflicting results have been published on the ability of α -GalCer-activated NKT cells to protect mice from EAE (103, 165-168). Pál et al. was using IL-4 and IFN- γ knockout B6 mice to show that activation of CD1d restricted NKT cells by α -GalCer could either enhance or suppress EAE respectively, whereas no effect could be seen when using wild type mice (167). From these data they concluded that IL-4 triggered by α -GalCer protected from EAE, whereas the opposite was true for IFN- γ . Supportive of this was a study

from Miyamoto et al., where a α -GalCer injection to B6 mice did not confer any protection from EAE (103). On the other hand, when using an analogue of α -GalCer which directed the NKT cell response towards producing more IL-4 and less IFN- γ , OCH, a clear effect could be seen on the disease course, with a milder EAE as outcome. In contrast to this, several groups have showed that treatment with α -GalCer protected B6 wild type mice from EAE in a CD1d dependent manner (165, 166, 168, 170), and it was demonstrated that both IFN- γ , IL-4 and IL-10 was important for the α -GalCer-mediated protection. In the above studies, the time point for injection of α -GalCer in relation to immunization for EAE, the number of injections, and the route of administration has all been shown to be of importance for conferring protection, possibly explaining the conflicting results. The protective effects of α -GalCer-activated NKT cells have been shown to be mediated by the peripheral induction of myeloid-derived suppressor cells that later migrate to the CNS to suppress the pathogenic inflammation in MOG induced EAE (170).

The above mentioned studies give some hope that a possible future pharmaceutical treatment for MS could be α -GalCer – or a Th2 inducing analogue of this compound. In addition, the data presented in paper III and discussed in previous section, show that NKT cells can be activated by the mCII₇₀₇₋₇₂₁ peptide, to become functional regulators in EAE, as well as other autoimmune and inflammatory conditions.

Inherited capacity of NKT cells to regulate EAE

Fritz et al. published in 2001 a study showing that passive transfer EAE in B6 TCR knockout mice could be partially inhibited by an additional transfer of NK1.1⁺ T cells, indicating that an excessive number of NKT cells could protect from EAE (169). Mars et al. also showed in a study using V α 14-J α 18 transgenic non-obese diabetic (NOD) mice, that an increase in the number of type I NKT cells could significantly reduce the severity of EAE induced by direct immunization (142). The clinical phenotype correlated with histopathological findings in CNS, and the effect was independent of IL-4. In the same publication, they also looked at the severity of EAE in CD1d deficient NOD mice, without observing any difference as compared to wild type littermates. They concluded that this was not unexpected, as NOD mice are known to have a defective NKT cell population, both qualitatively and quantitatively. The same group later showed that the protection from EAE in the type I NKT cell enriched mice was associated with an infiltration of cytotoxic NKT cells to the CNS. Even though the regulation was independent on expression of CD1d outside the thymus, an increased level of CD1d was found on CNS resident APCs during the EAE inflammation (141). *In*

vitro recall experiments with MOG peptide suggested that the type I NKT cells prevented EAE by the inhibition of autoaggressive Th1 and Th17 response. In addition, Oh et al (157) showed that type I NKT cells suppress Th1 and Th17 responses in the CNS during EAE.

In paper I we showed that CD1d deficient mice, lacking CD1d restricted NKT cells, developed a more severe and chronic EAE as compared to wild type mice when immunized with MOG₃₅₋₅₅. This correlated with a higher degree of demyelination and immune cell infiltration in the CNS. These findings illustrated that CD1d restricted NKT cells can be of importance in immune regulation of pathogenic autoimmunity. We also observed that activation of CD1d restricted NKT cells in the periphery was required for them to exert their regulatory function, as when an encephalitogenic T cell transfer was used as EAE inducing protocol, the regulation was absent. However, the regulation could be restored by an immunization with CFA of recipient mice, and we concluded that this immunization activated the regulatory pathway. Trying to reveal the mechanisms behind how NKT cells exerted this regulation, we found that the specific MOG₃₅₋₅₅ response differed in characteristics between the CD1d deficient and wild type mice. Autoantigen specific T cells from mice lacking CD1d had an enhanced cytokine production when investigating both IL-4 and IFN- γ . This indicated that CD1d restricted NKT cells can control the level of cytokine production from autoantigen specific T cells, without skewing them towards a Th2 or Th1 profile (at the time point for this study, the importance of the Th17 lineage in EAE was unknown, and hence could not be addressed). We could also show that TGF- β production in the CNS, which was prominent in wild type mice, was virtually absent in the CD1d deficient CNS, suggesting that TGF- β could be one pathway by which NKT cells regulate EAE. One of the interesting findings in our study was that CD1d dependent NKT cells regulated EAE without activation by α -GalCer. We instead hypothesised on other possible activation pathways; either in an antigen specific manner by a bacterial antigen in CFA, or via non-specific, innate mechanisms in inflammation, both of which are operative during a normal “working day” for the immune system. Indeed it has later been shown that the activation of type I NKT cells *in vivo* can be CD1d and TCR independent and instead accomplished by the raised cytokine levels in an inflammatory milieu (171-173).

To further add light on the function of CD1d dependent NKT cells in EAE, Jahng et al. 2004 published their findings of a NKT cell population reactive to a myelin derived self glycolipid, sulfatide, presented by CD1d (105). These NKT cells were shown not to use the described invariant TCR, and their antigen specific activation prevented EAE. In concordance with our findings, the disease protection correlated with a decrease in both IFN- γ and IL-4 production by the MOG₃₅₋₅₅

specific T cells, and the regulation did not appear to be dependent on IL-4. This study interestingly showed that CD1d restricted NKT cells could be regulatory in EAE without operating through an exogenous ligand. It was further shown that multiple tissue specific isoforms of sulfatide, present in both myelin and pancreatic β -cells, could activate type II CD1d restricted NKT cells, showing that this is one of the naturally occurring antigens for NKT cells (138). In addition, CD1d-dependent NKT cells have been shown to be crucial mediators for conferring protection to EAE via tolerogenic dendritic cells, without the addition of an exogenous NKT cell ligand (139, 174).

Together, these studies indicate that CD1d restricted NKT cells have an inherited capacity to regulate EAE.

NKT cells in the regulation of experimental arthritis

During the last decade, studies have been published on NKT cell's role in experimental arthritis. Some of them have focused on the possible therapeutic effects of treatment with α -GalCer, whereas others have worked with different knock out animals to elucidate NKT cells' intrinsic role in arthritis. Confusingly enough, the results from these studies showed great discrepancies, some results pointing towards a suppressive role for NKT cells also in experimental arthritis (175-177), while others suggesting that NKT cells are actually drivers of the arthritogenic inflammation (178-181). Dissecting the field a bit closer, it becomes evident that results obtained are greatly dependent on the experimental protocols used, i.e. chosen arthritis model, genetic background of mice utilized, and for studies on α -GalCer, timing of administration.

NKT cells have been implicated as effector cells in antibody-induced arthritis, as Kim et al (178, 179) used serum transfers to induce arthritis in both J α 281^{-/-} NKT cell deficient mice and CD1d^{-/-} mice and showed that compared to wild type B6 mice, these knock-outs developed less ankle swelling. As the authors conclude, the serum transfer model does probably not include adoptive immune responses, but solely immune-complex induced inflammation, and hence the suppressive role of NKT cells described from other adoptive immune models was not found in the antibody induced arthritis model. Indeed, it has been shown that antibody-induced arthritis is using effector pathways independent of T-cells (178), and might therefore be unaffected by regulatory NKT cells.

In contrast, activation of NKT cells using α -GalCer or its analogues α -C-GalCer and OCH has been reported to protect mice from CIA (176, 180). Chiba et. al

activated NKT cells using both α -GalCer and OCH, the analogue known to stimulate NKT cells to produce mainly IL-4 in contrast to α -GalCer which induces both IL-4 and IFN- γ production from NKT cells. From these experiments they report reduced CIA and protection against disease via IL-4 and IL-10 when NKT cells were stimulated.

However, also utilizing the CIA model, $J\alpha 281^{-/-}$ mice lacking the invariant NKT cells have been shown to manifest a decreased clinical arthritis score compared to wild type B6 mice (180). When using $CD1d^{-/-}$ mice (181), Ohnishi et al. also observed a decrease in arthritis scores compared to wild type, whereas Chiba et al. could only see this when using an antibody induced arthritis protocol. These reports would, in contrast to the studies activating NKT cells with α -GalCer, support the notion that NKT cells are instead pathogenic effector cells driving arthritic inflammation.

These results could, taken together, indicate that NKT cells have dual functions in arthritis; In the immune induction phase working as regulators of pathogenic self reactive T cells, and in the effector phase, where B-cells leading to antibody mediated cartilage and bone destruction is prominent, functioning as disease drivers.

To test this hypothesis further, we investigated the role of NKT cells in acute joint inflammation, using AIA as an experimental model, versus chronic arthritis, utilizing the CIA model. Results reported in paper II show that $CD1d^{-/-}$ mice lacking NKT cells develop more arthritis, both AIA and CIA, compared to wild type mice. Hence, in our lab, NKT cells seemed to be regulatory both in acute and chronic arthritis, and not responsible for driving pathogenesis. In addition, wt mice where depleted of NK1.1 expressing cells by injection with a depleting antibody at the time of arthritis induction. Paper II shows that also these mice exhibited a more severe arthritis compared to control injected mice, indicating that the regulatory mechanism whereby NKT cells exert their function needs to be active during disease initiation, and a functional pool of NKT cells for keeping the inherent immune homeostasis before disease induction might not be enough. This was further strengthened by the results previously discussed and reported in paper III, where we showed that a $CD1d$ restricted NKT cell population could be activated by immunization with a specific collagen peptide. When vaccinating mice to evoke the NKT cell response prior to immunization for CIA, an ameliorated CIA developed compared to the control group. The collagen peptide-specific NKT cells produced IFN- γ , as well as IL-4 and TGF- β , and where capable of inducing Fas ligand dependent apoptosis of activated T cells. This indicates that the mechanisms by which this population of NKT cells exerted regulatory functions on T cells might be different from the α -GalCer-analogue activated NKT cells,

where regulatory instructions via dendritic cells in addition to regulatory cytokines are believed to play the main role (182), even though cell contact dependent regulation of B-cells has been described also for this population (183).

The conflicting results described from studies on NKT cell function in experimental arthritis could possibly in part be explained by the fact that different inbred mouse strains were used, in addition to different CIA protocols. It highlights the need for more studies to elucidate CD1d restricted NKT cell's biology in CIA.

Conclusions and reflections

As autoimmune diseases are devastating for affected individuals, the need for knowledge on their regulation is crucial. In this thesis we investigated the role of CD1d restricted NKT cells in this context. We were able to show that in the autoimmune models EAE, CIA and AIA, CD1d restricted NKT cells have a suppressive role on disease course, and tissue inflammation and degradation. This suppression is an active process, occurring during disease initiation, and requires immunological activation to be functional. In addition, we identified a CD1d restricted type II NKT cell population with specificity for a mouse CII peptide (mCII₇₀₇₋₇₂₁), and we demonstrated that this NKT cell population has a general immunomodulatory and regulatory effect on inflammatory conditions, EAE and CIA included. All these studies support the idea that NKT cells have regulatory functions in autoimmunity and should be regarded as potential targets when developing pharmaceutical treatments for human autoimmune diseases.

An important novel finding from the studies on the mCII₇₀₇₋₇₂₁ specific NKT cells was the notion that CD1d have the capacity to present a self-peptide antigen to NKT cells. That NKT cells are reactive to self-antigens has been known for some time, and recently has their capacity to regulate immune reactions been suggested to be attributed this capacity (123, 184). However, CD1d has in general been looked upon as a lipid antigen-presenting molecule, but in this thesis we show that this is not always the case, and that peptide self-antigens should be further investigated for specificity to CD1d and NKT cell activation. Whether the peptide identified in paper III binds into the hydrophobic antigen-binding cleft of the CD1d molecule is not clear, even though it was able to partly outcompete the ligand α -GalCer, and this should be one of the aims of future studies with this immunoregulatory peptide. Nevertheless, our study shows that the field might

need to revise its view on CD1d antigen presentation and the structures that could have the potential to activate regulatory CD1d restricted NKT cells. Clearly, identifying specific antigens able to enhance regulatory NKT cells function should be important in finding interesting candidates for therapeutic disease modifying interventions in autoimmune diseases. However, to develop this further, one should better investigate the capacity of these NKT cells to overcome an on-going autoimmune process. In our studies, mice were immunized with the immunoregulatory peptide prior to disease inductions. These protocols should be repeated, but with the peptide immunization performed during the disease course, where a chronic pathogenic inflammation has already been established. One should also better characterize the influence of the dose of the peptide, and the adjuvant used for enhancing the response, to optimize the induction of regulatory NKT cells. It is clear from the studies using α -GalCer to activate type I NKT cells that the protocol used for administration is strongly influencing the type of NKT cell response elicited. Treatment with α -GalCer could induce both prevention and acceleration of disease, depending on the timing of administrations, the number of administrations, and the route of administration, and this should be studied for mCII₇₀₇₋₇₂₁ activation as well. In addition, the capacity of human CD1 molecules to present the studied mouse collagen peptide to human NKT cells, and the type of response it would evoke, should be investigated. Even though there is a high degree of conservation amongst species in terms of NKT cells capacity to recognize CD1, there are by no means any guarantees of cross reactivity between the species in terms of mCII₇₀₇₋₇₂₁ recognition and cell activation, and this needs to be clarified. However, should no cross reactivity be confirmed, molecular modification of the mCII₇₀₇₋₇₂₁ might still prove to be a viable antigen also in a human system.

Elucidating the mechanisms by which CD1d restricted NKT cells regulate autoimmunity and inflammation will further facilitate the understanding of their role in the immune system. It has earlier been suggested that NKT cells mainly suppress autoimmunity by secreting large amounts of Th2 cytokines, in particular IL-4 and IL-10, and thereby shifting the immune response to a status regarded as less pathogenic in the autoimmune context. In our studies we found data indicating that this is not necessarily the case, and we could not show a classical Th1/Th2 shift during the manifested regulations in any of the investigated models. Instead, our results show that NKT cells might be able to induce apoptosis via Fas-FasL and suppress T cells in a cell-cell contact dependent manner; as has been described for NKT cells killing of tumour cells and $\gamma\delta$ cells killing of regulatory T cells. This finding opens up a new view on how NKT cell mediated regulation of autoimmunity can be carried out, and should be investigated further. However, the role for cytokines and bystander suppression is still relevant, and might very well be a part of the regulatory pathway by which NKT cells act. For example, we

found TGF- β to be produced by the mCII₇₀₇₋₇₂₁ activated immunoregulatory NKT cells, and we also saw a parallel between lack of TGF- β expression in the inflamed CNS of CD1d deficient mice and worsened EAE. This indicates that TGF- β could be of importance when CD1d restricted NKT cells suppress autoimmunity, either by direct production by activated NKT cells, or as a down-stream mechanism. In addition, we showed that the mCII₇₀₇₋₇₂₁ specific NKT cells have a capacity of producing high levels of cytokines, and others have described the cytokine dependent nature of the NKT cell driven control in autoimmune models. However, modulation of the immune response through vigorous cytokine production is not the complete picture for how NKT cells exert control, and we show here that it should be complemented by the capacity of these cells to induce apoptosis in activated pathogenic T cells, and thereby end an immune reaction. Studies by other groups have also showed that NKT cells can act via tolerogenic instructions to dendritic cells. This pathway was not the focus of our work, but it should be interesting to study the peptide-specific NKT cells in this context, and investigate any possible influences on dendritic cell capacity to induce pathogenic or tolerogenic T cells. In addition, the NKT cell subset responding to the mCII₇₀₇₋₇₂₁ should be further described in terms of surface markers, cytokine profiling, and intracellular signalling pathways upon activation.

Taken together, our studies show that properly activated, NKT cells can indeed be beneficial in protecting against autoimmune diseases. The fact that we were able to show that NKT cells can be regulatory cells in both CIA, AIA, and EAE, as well as in other inflammatory conditions such as DTH and antigen induced airway inflammation points to the important notion that this regulation is neither restricted to a certain tissue, nor restricted to the Th1/Th2 paradigm, but it is operative during a variety of immune driven reactions. These studies provide a step on the way towards identifying a role for NKT cells for therapeutic purposes in the future, and specifically for self-peptides as possible activators of regulation through CD1 restricted NKT cells. Without doubt, more knowledge of the nature of this response and its possible translation to a human setting is needed, but the data presented here in should provide the start of such studies. Hopefully this will lead to beneficial treatments for patients with autoimmune diseases in the future.

Populärvetenskaplig sammanfattning

Immunsystemets främsta uppgift är att bekämpa infektioner och tumörer samt att läka vävnadsskador. För att göra detta har det i däggdjur utvecklats ett komplicerat system av celler och molekyler som samspelar i ett stort nätverk – immunsystemet. Läran om detta system kallas immunologi, och syftar till att öka vår kunskap om hur immunsystemet fungerar i så väl en frisk kropp som i en sjuk.

En invasion av skadliga bakterier eller andra mikroorganismer, eller en okontrollerad tillväxt av tumörceller får fatala följder för individen. Därför krävs det en kraftig reaktion för att bekämpa detta, och immunsystemet är utrustat för att kunna döda andra celler vid behov. Alla har nog känt av detta kraftfulla maskineri i form av en smärtsam inflammation, immunsystemets svar på provokation och skada. Ett så kraftfullt system kräver nogsam reglering, och för att immunsystemet i våra kroppar inte skall löpa amok finns det reglerande funktioner som kontrollerar dess aktivitet. En viktig sådan är immunsystemets förmåga att se skillnad på den egna kroppens celler och andra främmande typer av celler. Därigenom undviks att den egna kroppen angrips och dödas, utan bara sådana celler som är främmande för kroppen, ex bakterier eller tumörceller. När denna kontrollmekanism inte fungerar som den ska kan en så kallad autoimmun sjukdom uppstå. Då angriper immunsystemet felaktigt den egna kroppens vävnad, vilket kan leda till allvarliga följder för individen. Exempel på sådana sjukdomar är multipel skleros (MS), reumatoid artrit (RA) samt ungdomsdiabetes, vilka alla är allvarliga och livslånga sjukdomar. Idag finns ingen bot för dessa sjukdomar, utan man behandlar patienter med läkemedel för att försöka mildra och dämpa sjukdomens förlopp och för att lindra dess symptom. Många av de läkemedel som idag används påverkar immunsystemet relativt oprecist, och ger därför ofta oönskade bieffekter utan tillräckligt bra önskad effekt. Om vi visste mer om immunsystemet, skulle vi kunna få fram bättre mediciner mot dessa allvarliga autoimmuna sjukdomar, och vi kan även på sikt hoppas på att kunna hitta botemedel. Därför är det viktigt att studera immunsystemet och dess reglering i autoimmunitet.

Genom att använda djurmodeller som efterliknar människors autoimmuna sjukdomar kan man ställa, och få svar på, frågor som inte går att undersöka i människor. Vi kan till exempel ha bättre kontroll över gens inflytande och miljöpåverkan i våra djurexperiment. Vi kan titta på hur immunsystemet ser ut i ett djur som vi vet kommer att bli sjukt, innan sjukdomen har brutit ut, vilket är omöjligt i människor. Viktigt är även att vi kan titta på immunsystemets celler och komponenter i detalj. Genom att ta ut organ från djur och undersöka med metoder

för cellodling, eller granska organet i mikroskop, kan vi se vilka delar av immunsystemet som är aktivt och på vilket sätt det agerar. På så sätt får vi mer kunskap om hur varje del av immunsystemet fungerar, och kan förhoppningsvis på sikt utveckla läkemedel som påverkar exakt den mekanism som är fel i en autoimmun sjukdom.

I mitt arbete har jag undersökt en typ av celler i immunsystemet som kallas NKT celler. Detta är en specialiserad celltyp som man tror kan ha betydelse för hur immunsystemets andra celler uppträder och reagerar. Bland annat så har man sett att djur som inte har NKT celler har lättare för att utveckla olika typer autoimmuna sjukdomar, och därför har man dragit slutsatsen att NKT celler på något sätt kan reglera immunsystemet. Jag har tittat på NKT cellers funktion, främst i olika musmodeller för autoimmunitet. Två modeller som liknar RA, som kallas AIA och CIA, och en som liknar MS och kallas EAE, har använts i mina studier. I båda modellerna gör man mössen sjuka genom att injicera ett protein som kommer från det organet som sedan blir angripet av immunsystemet. I CIA ger man mössen en injektion av kollagen som bygger upp brosk, vilket gör att ledernas brosk blir inflammerat och bryts ned av immunceller, och mössen får svårt att gå. I EAE ger man en injektion av det ämne som ligger runt nervcellerna för att isolera och skydda dessa, myelin, vilket gör att hjärnan och ryggmärgen blir inflammerade och mössen får neurologiska symptom som t ex förlamning. När jag har undersökt om NKT celler är viktiga i dessa modeller har jag sett att dessa celler till viss del kan reglera sjukdomarna. I en studie såg jag att om möss har NKT celler utvecklas mindre EAE än om dom har blivit genetiskt modifierade till att inte ha NKT celler, vilket tyder på att NKT celler kan kontrollera sjukdomsförloppet. Det samma gäller för CIA och AIA, möss som saknar NKT celler utvecklar mer artrit än möss med ett normal fungerande immunsystem. Jag har även hittat en del av ett protein - en peptid - som man kan använda för att aktivera NKT celler. Om man vaccinerar möss med den peptiden svarar NKT celler genom att blir fler och mer aktiva. Vi upptäckte att om man aktiverade NKT celler med peptiden i möss innan vi inducerade EAE eller CIA, så blev inte mössen lika sjuka som icke-vaccinerade möss. Detta tyder återigen på att NKT celler har kapacitet att reglera autoimmuna sjukdomar. Ur ett behandlingsperspektiv är detta särskilt viktigt, eftersom man kanske skulle kunna aktivera NKT celler i sjuka människor på samma sätt. NKT celler i möss och människor liknar varandra väldigt mycket, och kan troligtvis aktiveras av ungefär samma saker. Vad man däremot inte vet så mycket om, är om NKT celler är viktiga i regleringen av autoimmuna sjukdomar i människor också, inte bara i djur. Även om det finns studier som tyder på att det kan vara så, och djurmodeller kan användas för att ge information om människans sjukdomar, behövs det mer kunskap från människor innan man vet med säkerhet. Min avhandling ger information som jag tror kan vara användbar i framtiden till att utveckla läkemedel mot autoimmuna sjukdomar.

References

1. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nature immunology*. 11(1):7-13.
2. Jiang H, Chess L. Regulation of immune responses by T cells. *The New England journal of medicine*. 2006;354(11):1166-76.
3. Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. *The American journal of medicine*. 2007;120(11):936-9.
4. Firestein GS. Pathogenesis of rheumatoid arthritis: how early is early? *Arthritis research & therapy*. 2005;7(4):157-9.
5. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Annals of the rheumatic diseases*. 69(9):1580-8.
6. Nandakumar KS, Holmdahl R. Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis. *Arthritis research & therapy*. 2006;8(6):223.
7. Prat A, Antel J. Pathogenesis of multiple sclerosis. *Current opinion in neurology*. 2005;18(3):225-30.
8. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008;372(9648):1502-17.
9. Steinman L. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. *Neuron*. 1999;24(3):511-4.
10. Rivers TM, Schwentker FF. Encephalomyelitis Accompanied by Myelin Destruction Experimentally Produced in Monkeys. *J Exp Med*. 1935;61(5):689-702.
11. Wekerle H, Krishnamoorthy G. Brain autoimmunity: the CD8 question(s). *Immunity*. 2012;37(1):8-10.
12. Friese MA, Fugger L. Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? *Brain : a journal of neurology*. 2005;128(Pt 8):1747-63.
13. Na SY, Hermann A, Sanchez-Ruiz M, Storch A, Deckert M, Hunig T. Oligodendrocytes enforce immune tolerance of the uninfected brain by purging the peripheral repertoire of autoreactive CD8+ T cells. *Immunity*. 2012;37(1):134-46.
14. Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA, Jr. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J Exp Med*. 1993;177(1):57-68.
15. Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunology today*. 1995;16(1):34-8.

16. van der Veen RC, Stohlman SA. Encephalitogenic Th1 cells are inhibited by Th2 cells with related peptide specificity: relative roles of interleukin (IL)-4 and IL-10. *Journal of neuroimmunology*. 1993;48(2):213-20.
17. van der Veen RC, Kapp JA, Trotter JL. Fine-specificity differences in the recognition of an encephalitogenic peptide by T helper 1 and 2 cells. *Journal of neuroimmunology*. 1993;48(2):221-6.
18. El-behi M, Rostami A, Ciric B. Current views on the roles of Th1 and Th17 cells in experimental autoimmune encephalomyelitis. *Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology*. 2010;5(2):189-97.
19. Langrish CL, McKenzie BS, Wilson NJ, de Waal Malefyt R, Kastelein RA, Cua DJ. IL-12 and IL-23: master regulators of innate and adaptive immunity. *Immunological reviews*. 2004;202:96-105.
20. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature*. 2003;421(6924):744-8.
21. Lafaille JJ, Keere FV, Hsu AL, Baron JL, Haas W, Raine CS, et al. Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *J Exp Med*. 1997;186(2):307-12.
22. Lafaille JJ, Keere FV, Hsu AL, Baron JL, Haas W, Raine CS, et al. Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *Journal of Experimental Medicine*. 1997;186(2):307-12.
23. Kuchroo VK, Anderson AC, Waldner H, Munder M, Bettelli E, Nicholson LB. T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annual review of immunology*. 2002;20:101-23.
24. Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD. An important role for the chemokine macrophage inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J Immunol*. 1995;155(10):5003-10.
25. Huitinga I, Damoiseaux JG, Dopp EA, Dijkstra CD. Treatment with anti-CR3 antibodies ED7 and ED8 suppresses experimental allergic encephalomyelitis in Lewis rats. *Eur J Immunol*. 1993;23(3):709-15.
26. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J Exp Med*. 2000;192(7):1075-80.
27. Chitnis T, Khoury SJ. Cytokine shifts and tolerance in experimental autoimmune encephalomyelitis. *Immunologic research*. 2003;28(3):223-39.

28. McGeachy MJ, Anderton SM. Cytokines in the induction and resolution of experimental autoimmune encephalomyelitis. *Cytokine*. 2005;32(2):81-4.
29. Wheeler RD, Owens T. The changing face of cytokines in the brain: perspectives from EAE. *Current pharmaceutical design*. 2005;11(8):1031-7.
30. Willenborg DO, Fordham SA, Staykova MA, Ramshaw IA, Cowden WB. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. *J Immunol*. 1999;163(10):5278-86.
31. Petermann F, Korn T. Cytokines and effector T cell subsets causing autoimmune CNS disease. *FEBS letters*. 2011;585(23):3747-57.
32. Gran B, Zhang GX, Yu S, Li J, Chen XH, Ventura ES, et al. IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. *J Immunol*. 2002;169(12):7104-10.
33. Young DA, Lowe LD, Booth SS, Whitters MJ, Nicholson L, Kuchroo VK, et al. IL-4, IL-10, IL-13, and TGF-beta from an altered peptide ligand-specific Th2 cell clone down-regulate adoptive transfer of experimental autoimmune encephalomyelitis. *J Immunol*. 2000;164(7):3563-72.
34. Jager A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J Immunol*. 2009;183(11):7169-77.
35. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*. 2005;201(2):233-40.
36. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *Journal of autoimmunity*. 2008;31(3):252-6.
37. Aoki CA, Borchers AT, Li M, Flavell RA, Bowlus CL, Ansari AA, et al. Transforming growth factor beta (TGF-beta) and autoimmunity. *Autoimmunity reviews*. 2005;4(7):450-9.
38. Johns LD, Flanders KC, Ranges GE, Sriram S. Successful treatment of experimental allergic encephalomyelitis with transforming growth factor-beta 1. *J Immunol*. 1991;147(6):1792-6.
39. Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ. Protective effect of transforming growth factor beta 1 on experimental autoimmune diseases in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1991;88(7):2918-21.
40. Issazadeh S, Mustafa M, Ljungdahl A, Hojeberg B, Dagerlind A, Elde R, et al. Interferon gamma, interleukin 4 and transforming growth factor beta in experimental autoimmune encephalomyelitis in Lewis rats: dynamics of cellular

mRNA expression in the central nervous system and lymphoid cells. *Journal of neuroscience research*. 1995;40(5):579-90.

41. Issazadeh S, Lorentzen JC, Mustafa MI, Hojeberg B, Mussener A, Olsson T. Cytokines in relapsing experimental autoimmune encephalomyelitis in DA rats: persistent mRNA expression of proinflammatory cytokines and absent expression of interleukin-10 and transforming growth factor-beta. *Journal of neuroimmunology*. 1996;69(1-2):103-15.
42. Espejo C, Brieva L, Ruggiero G, Rio J, Montalban X, Martinez-Caceres EM. IFN-beta treatment modulates the CD28/CTLA-4-mediated pathway for IL-2 production in patients with relapsing-remitting multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)*. 2004;10(6):630-5.
43. Fernandez O. Interferons in relapsing-remitting multiple sclerosis: are there benefits from long-term use? *CNS drugs*. 2004;18(15):1057-70.
44. Patti F, Reggio E, Palermo F, Fiorilla T, Politi G, Nicoletti A, et al. Stabilization of rapidly worsening multiple sclerosis for 36 months in patients treated with interferon beta plus cyclophosphamide followed by interferon beta. *Journal of neurology*. 2004;251(12):1502-6.
45. Teige I, Treschow A, Teige A, Mattsson R, Navikas V, Leanderson T, et al. IFN-beta gene deletion leads to augmented and chronic demyelinating experimental autoimmune encephalomyelitis. *J Immunol*. 2003;170(9):4776-84.
46. Gigli G, Caielli S, Cutuli D, Falcone M. Innate immunity modulates autoimmunity: type I interferon-beta treatment in multiple sclerosis promotes growth and function of regulatory invariant natural killer T cells through dendritic cell maturation. *Immunology*. 2007;122(3):409-17.
47. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Forster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity*. 2011;34(2):213-23.
48. Mendel I, Kerlero de Rosbo N, Ben-Nun A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur J Immunol*. 1995;25(7):1951-9.
49. Abdul-Majid KB, Jirholt J, Stadelmann C, Stefferl A, Kjellen P, Wallstrom E, 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. *Journal of neuroimmunology*. 2000;111(1-2):23-33.
50. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. *J Exp Med*. 1977;146(3):857-68.
51. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature*. 1980;283(5748):666-8.
52. Holmdahl R, Andersson M, Enander I, Goldschmidt T, Jansson L, Larsson P, et al. Nature of the type II collagen autoimmunity in mice susceptible

- to collagen-induced arthritis. *International reviews of immunology*. 1988;4(1):49-64.
53. Lubberts E, van den Berg WB. Cytokines in the pathogenesis of rheumatoid arthritis and collagen-induced arthritis. *Advances in experimental medicine and biology*. 2003;520:194-202.
54. El Azreq MA, Boisvert M, Cesaro A, Page N, Loubaki L, Allaey I, et al. alpha2beta1 Integrin Regulates Th17 Cell Activity and Its Neutralization Decreases the Severity of Collagen-Induced Arthritis. *J Immunol*. 2013;191(12):5941-50.
55. Arai K, Yamamura S, Hanyu T, Takahashi HE, Umezu H, Watanabe H, et al. Extrathymic differentiation of resident T cells in the joints of mice with collagen-induced arthritis. *J Immunol*. 1996;157(11):5170-7.
56. Corthay A, Johansson A, Vestberg M, Holmdahl R. Collagen-induced arthritis development requires alpha beta T cells but not gamma delta T cells: studies with T cell-deficient (TCR mutant) mice. *International immunology*. 1999;11(7):1065-73.
57. Holmdahl R. Genetics of susceptibility to chronic experimental encephalomyelitis and arthritis. *Curr Opin Immunol*. 1998;10(6):710-7.
58. Wooley PH, Luthra HS, Stuart JM, David CS. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J Exp Med*. 1981;154(3):688-700.
59. Bajtner E, Nandakumar KS, Engstrom A, Holmdahl R. Chronic development of collagen-induced arthritis is associated with arthritogenic antibodies against specific epitopes on type II collagen. *Arthritis research & therapy*. 2005;7(5):R1148-57.
60. Svensson L, Jirholt J, Holmdahl R, Jansson L. B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clinical and experimental immunology*. 1998;111(3):521-6.
61. Nandakumar KS, Holmdahl R. Efficient promotion of collagen antibody induced arthritis (CAIA) using four monoclonal antibodies specific for the major epitopes recognized in both collagen induced arthritis and rheumatoid arthritis. *Journal of immunological methods*. 2005;304(1-2):126-36.
62. Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM. Induction of arthritis with monoclonal antibodies to collagen. *J Immunol*. 1992;148(7):2103-8.
63. Cohen SB. The use of anakinra, an interleukin-1 receptor antagonist, in the treatment of rheumatoid arthritis. *Rheumatic diseases clinics of North America*. 2004;30(2):365-80, vii.
64. Finckh A, Simard JF, Duryea J, Liang MH, Huang J, Daneel S, et al. The effectiveness of anti-tumor necrosis factor therapy in preventing progressive radiographic joint damage in rheumatoid arthritis: a population-based study. *Arthritis and rheumatism*. 2006;54(1):54-9.

65. Lange U, Teichmann J, Muller-Ladner U, Strunk J. Increase in bone mineral density of patients with rheumatoid arthritis treated with anti-TNF-alpha antibody: a prospective open-label pilot study. *Rheumatology (Oxford, England)*. 2005;44(12):1546-8.
66. Mavropoulos JC, Cuchacovich M, Llanos C, Aguillon JC, Gatica H, Pizzo SV, et al. Anti-tumor necrosis factor-alpha therapy augments dipeptidyl peptidase IV activity and decreases autoantibodies to GRP78/BIP and phosphoglucose isomerase in patients with rheumatoid arthritis. *J Rheumatol*. 2005;32(11):2116-24.
67. Waugh J, Perry CM. Anakinra: a review of its use in the management of rheumatoid arthritis. *BioDrugs*. 2005;19(3):189-202.
68. Patel AM, Moreland LW. Interleukin-6 inhibition for treatment of rheumatoid arthritis: a review of tocilizumab therapy. *Drug design, development and therapy*. 2010;4:263-78.
69. Koenders MI, Marijnissen RJ, Devesa I, Lubberts E, Joosten LA, Roth J, et al. Tumor necrosis factor-interleukin-17 interplay induces S100A8, interleukin-1beta, and matrix metalloproteinases, and drives irreversible cartilage destruction in murine arthritis: rationale for combination treatment during arthritis. *Arthritis and rheumatism*. 2011;63(8):2329-39.
70. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med*. 2003;198(12):1951-7.
71. Rong C, Hu W, Wu FR, Cao XJ, Chen FH. Interleukin-23 as a potential therapeutic target for rheumatoid arthritis. *Molecular and cellular biochemistry*. 2012;361(1-2):243-8.
72. Holmdahl R, Jansson L, Gullberg D, Rubin K, Forsberg PO, Klareskog L. Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II. *Clinical and experimental immunology*. 1985;62(3):639-46.
73. Holmdahl R, Jansson L, Larsson E, Rubin K, Klareskog L. Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthritis and rheumatism*. 1986;29(1):106-13.
74. Brunsberg U, Gustafsson K, Jansson L, Michaelsson E, Ahrlund-Richter L, Pettersson S, et al. Expression of a transgenic class II Ab gene confers susceptibility to collagen-induced arthritis. *Eur J Immunol*. 1994;24(7):1698-702.
75. Michaelsson E, Andersson M, Engstrom A, Holmdahl R. Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection. *Eur J Immunol*. 1992;22(7):1819-25.
76. Michaelsson E, Malmstrom V, Reis S, Engstrom A, Burkhardt H, Holmdahl R. T cell recognition of carbohydrates on type II collagen. *J Exp Med*. 1994;180(2):745-9.

77. Bayrak S, Holmdahl R, Travers P, Lauster R, Hesse M, Dolling R, et al. T cell response of I-A_q mice to self type II collagen: meshing of the binding motif of the I-A_q molecule with repetitive sequences results in autoreactivity to multiple epitopes. *International immunology*. 1997;9(11):1687-99.
78. Brackertz D, Mitchell GF, Vadas MA, Mackay IR. Studies on antigen-induced arthritis in mice. III. Cell and serum transfer experiments. *J Immunol*. 1977;118(5):1645-8.
79. Brackertz D, Mitchell GF, Vadas MA, Mackay IR, Miller JF. Studies on antigen-induced arthritis in mice. II. Immunologic correlates of arthritis susceptibility in mice. *J Immunol*. 1977;118(5):1639-44.
80. Brackertz D, Mitchell GF, Mackay IR. Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. *Arthritis and rheumatism*. 1977;20(3):841-50.
81. Walker JM, Cope AP, van den Berg WB, Joosten LAB, van Lent PLEM. Murine Antigen-Induced Arthritis. *Arthritis Research*2007. p. 243.
82. van den Berg WB, Joosten LA, van Lent PL. Murine antigen-induced arthritis. *Methods in molecular medicine*. 2007;136:243-53.
83. Brossay L, Jullien D, Cardell S, Sydora BC, Burdin N, Modlin RL, et al. Mouse CD1 is mainly expressed on hemopoietic-derived cells. *J Immunol*. 1997;159(3):1216-24.
84. Teitell M, Holcombe HR, Brossay L, Hagenbaugh A, Jackson MJ, Pond L, et al. Nonclassical behavior of the mouse CD1 class I-like molecule. *J Immunol*. 1997;158(5):2143-9.
85. Porcelli SA, Modlin RL. The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annual review of immunology*. 1999;17:297-329.
86. de la Salle H, Mariotti S, Angenieux C, Gilleron M, Garcia-Alles LF, Malm D, et al. Assistance of microbial glycolipid antigen processing by CD1e. *Science (New York, NY)*. 2005;310(5752):1321-4.
87. Calabi F, Jarvis JM, Martin L, Milstein C. Two classes of CD1 genes. *Eur J Immunol*. 1989;19(2):285-92.
88. Brossay L, Chioda M, Burdin N, Koezuka Y, Casorati G, Dellabona P, et al. CD1d-mediated recognition of an alpha-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. *J Exp Med*. 1998;188(8):1521-8.
89. Zeng Z, Castano AR, Segelke BW, Stura EA, Peterson PA, Wilson IA. Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove. *Science (New York, NY)*. 1997;277(5324):339-45.
90. De Libero G, Mori L. Recognition of lipid antigens by T cells. *Nat Rev Immunol*. 2005;5(6):485-96.
91. Sullivan BA, Nagarajan NA, Kronenberg M. CD1 and MHC II find different means to the same end. *Trends in immunology*. 2005;26(5):282-8.

92. Gilleron M, Stenger S, Mazorra Z, Wittke F, Mariotti S, Bohmer G, et al. Diacylated sulfoglycolipids are novel mycobacterial antigens stimulating CD1-restricted T cells during infection with *Mycobacterium tuberculosis*. *J Exp Med*. 2004;199(5):649-59.
93. Moody DB, Ulrichs T, Muhlecker W, Young DC, Gurcha SS, Grant E, et al. CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. *Nature*. 2000;404(6780):884-8.
94. Kinjo Y, Tupin E, Wu D, Fujio M, Garcia-Navarro R, Benhnia MR, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nature immunology*. 2006;7(9):978-86.
95. Mattner J, Debord KL, Ismail N, Goff RD, Cantu C, 3rd, Zhou D, et al. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature*. 2005;434(7032):525-9.
96. Sriram V, Du W, Gervay-Hague J, Brutkiewicz RR. Cell wall glycosphingolipids of *Sphingomonas paucimobilis* are CD1d-specific ligands for NKT cells. *Eur J Immunol*. 2005;35(6):1692-701.
97. Fischer K, Scotet E, Niemeyer M, Koebernick H, Zerrahn J, Maillet S, et al. Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(29):10685-90.
98. Wu D, Xing GW, Poles MA, Horowitz A, Kinjo Y, Sullivan B, et al. Bacterial glycolipids and analogs as antigens for CD1d-restricted NKT cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(5):1351-6.
99. Moody DB, Young DC, Cheng TY, Rosat JP, Roura-Mir C, O'Connor PB, et al. T cell activation by lipopeptide antigens. *Science (New York, NY)*. 2004;303(5657):527-31.
100. Van Rhijn I, Young DC, Im JS, Lavery SB, Illarionov PA, Besra GS, et al. CD1d-restricted T cell activation by nonlipidic small molecules. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(37):13578-83.
101. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science (New York, NY)*. 1997;278(5343):1626-9.
102. Yamaguchi Y, Motoki K, Ueno H, Maeda K, Kobayashi E, Inoue H, et al. Enhancing effects of (2S,3S,4R)-1-O-(alpha-D-galactopyranosyl)-2-(N-hexacosanoylamino) -1,3,4-octadecanetriol (KRN7000) on antigen-presenting function of antigen-presenting cells and antimetastatic activity of KRN7000-pretreated antigen-presenting cells. *Oncology research*. 1996;8(10-11):399-407.
103. Miyamoto K, Miyake S, Yamamura T. A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature*. 2001;413(6855):531-4.

104. Yu KO, Im JS, Molano A, Dutronc Y, Illarionov PA, Forestier C, et al. Modulation of CD1d-restricted NKT cell responses by using N-acyl variants of alpha-galactosylceramides. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(9):3383-8.
105. Jahng A, Maricic I, Aguilera C, Cardell S, Halder RC, Kumar V. Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. *J Exp Med*. 2004;199(7):947-57.
106. Gumperz JE, Roy C, Makowska A, Lum D, Sugita M, Podrebarac T, et al. Murine CD1d-restricted T cell recognition of cellular lipids. *Immunity*. 2000;12(2):211-21.
107. Zhou D, Mattner J, Cantu C, 3rd, Schrantz N, Yin N, Gao Y, et al. Lysosomal glycosphingolipid recognition by NKT cells. *Science (New York, NY)*. 2004;306(5702):1786-9.
108. Christiansen D, Milland J, Mouhtouris E, Vaughan H, Pellicci DG, McConville MJ, et al. Humans lack iGb3 due to the absence of functional iGb3-synthase: implications for NKT cell development and transplantation. *PLoS Biol*. 2008;6(7):e172.
109. Porubsky S, Speak AO, Luckow B, Cerundolo V, Platt FM, Grone HJ. Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(14):5977-82.
110. Speak AO, Salio M, Neville DC, Fontaine J, Priestman DA, Platt N, et al. Implications for invariant natural killer T cell ligands due to the restricted presence of isoglobotrihexosylceramide in mammals. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(14):5971-6.
111. Rossjohn J, Pellicci DG, Patel O, Gapin L, Godfrey DI. Recognition of CD1d-restricted antigens by natural killer T cells. *Nat Rev Immunol*. 2012;12(12):845-57.
112. Pellicci DG, Clarke AJ, Patel O, Mallevaey T, Beddoe T, Le Nours J, et al. Recognition of beta-linked self glycolipids mediated by natural killer T cell antigen receptors. *Nature immunology*. 2011;12(9):827-33.
113. Yu ED, Girardi E, Wang J, Zajonc DM. Cutting edge: structural basis for the recognition of beta-linked glycolipid antigens by invariant NKT cells. *J Immunol*. 2011;187(5):2079-83.
114. Castano AR, Tangri S, Miller JE, Holcombe HR, Jackson MR, Huse WD, et al. Peptide binding and presentation by mouse CD1. *Science (New York, NY)*. 1995;269(5221):223-6.
115. Tangri S, Brossay L, Burdin N, Lee DJ, Corr M, Kronenberg M. Presentation of peptide antigens by mouse CD1 requires endosomal localization and protein antigen processing. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;95(24):14314-9.

116. Lee DJ, Abeyratne A, Carson DA, Corr M. Induction of an antigen-specific, CD1-restricted cytotoxic T lymphocyte response *In vivo*. *J Exp Med*. 1998;187(3):433-8.
117. Sykes M. Unusual T cell populations in adult murine bone marrow. Prevalence of CD3+CD4-CD8- and alpha beta TCR+NK1.1+ cells. *J Immunol*. 1990;145(10):3209-15.
118. Sykes M, Hoyles KA, Romick ML, Sachs DH. *In vitro* and *in vivo* analysis of bone marrow-derived CD3+, CD4-, CD8-, NK1.1+ cell lines. *Cellular immunology*. 1990;129(2):478-93.
119. Fowlkes BJ, Kruisbeek AM, Ton-That H, Weston MA, Coligan JE, Schwartz RH, et al. A novel population of T-cell receptor alpha beta-bearing thymocytes which predominantly expresses a single V beta gene family. *Nature*. 1987;329(6136):251-4.
120. Bendelac A, Lantz O, Quimby ME, Yewdell JW, Bennink JR, Brutkiewicz RR. CD1 recognition by mouse NK1+ T lymphocytes. *Science (New York, NY)*. 1995;268(5212):863-5.
121. Exley M, Garcia J, Balk SP, Porcelli S. Requirements for CD1d recognition by human invariant Valpha24+ CD4-CD8- T cells. *J Exp Med*. 1997;186(1):109-20.
122. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol*. 2004;4(3):231-7.
123. Gapin L, Godfrey DI, Rossjohn J. Natural Killer T cell obsession with self-antigens. *Current Opinion in Immunology*. 2013(0).
124. Gumperz JE, Miyake S, Yamamura T, Brenner MB. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J Exp Med*. 2002;195(5):625-36.
125. Hammond KJ, Pelikan SB, Crowe NY, Randle-Barrett E, Nakayama T, Taniguchi M, et al. NKT cells are phenotypically and functionally diverse. *Eur J Immunol*. 1999;29(11):3768-81.
126. Hammond KJ, Pellicci DG, Poulton LD, Naidenko OV, Scalzo AA, Baxter AG, et al. CD1d-restricted NKT cells: an interstrain comparison. *J Immunol*. 2001;167(3):1164-73.
127. Coquet JM, Chakravarti S, Kyparissoudis K, McNab FW, Pitt LA, McKenzie BS, et al. Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105(32):11287-92.
128. Cardell S, Tangri S, Chan S, Kronenberg M, Benoist C, Mathis D. CD1-restricted CD4+ T cells in major histocompatibility complex class II-deficient mice. *J Exp Med*. 1995;182(4):993-1004.
129. Behar SM, Podrebarac TA, Roy CJ, Wang CR, Brenner MB. Diverse TCRs recognize murine CD1. *J Immunol*. 1999;162(1):161-7.

130. Makowska A, Kawano T, Taniguchi M, Cardell S. Differences in the ligand specificity between CD1d-restricted T cells with limited and diverse T-cell receptor repertoire. *Scandinavian journal of immunology*. 2000;52(1):71-9.
131. Park SH, Chiu YH, Jayawardena J, Roark J, Kavita U, Bendelac A. Innate and adaptive functions of the CD1 pathway of antigen presentation. *Semin Immunol*. 1998;10(5):391-8.
132. Rolf J, Berntman E, Stenstrom M, Smith EM, Mansson R, Stenstad H, et al. Molecular profiling reveals distinct functional attributes of CD1d-restricted natural killer (NK) T cell subsets. *Molecular immunology*. 2008;45(9):2607-20.
133. Berzofsky JA, Terabe M. The contrasting roles of NKT cells in tumor immunity. *Curr Mol Med*. 2009;9(6):667-72.
134. Berzofsky JA, Terabe M. A novel immunoregulatory axis of NKT cell subsets regulating tumor immunity. *Cancer Immunol Immunother*. 2008;57(11):1679-83.
135. Berzofsky JA, Terabe M. NKT cells in tumor immunity: opposing subsets define a new immunoregulatory axis. *J Immunol*. 2008;180(6):3627-35.
136. Diana J, Lehuen A. NKT cells: friend or foe during viral infections? *Eur J Immunol*. 2009;39(12):3283-91.
137. Manfred B, Michael BB. Review: How invariant natural killer T cells respond to infection by recognizing microbial or endogenous lipid antigens. *Seminars in Immunology*. 22:79-86.
138. Blomqvist M, Rhost S, Teneberg S, Lofbom L, Osterbye T, Brigl M, et al. Multiple tissue-specific isoforms of sulfatide activate CD1d-restricted type II NKT cells. *Eur J Immunol*. 2009;39(7):1726-35.
139. Brandl C, Ortler S, Herrmann T, Cardell S, Lutz MB, Wiendl H. B7-H1-deficiency enhances the potential of tolerogenic dendritic cells by activating CD1d-restricted type II NKT cells. *PloS one*. 5(5):e10800.
140. Oh SJ, Chung DH. Invariant NKT Cells Producing IL-4 or IL-10, But Not IFN- γ , Inhibit the Th1 Response in Experimental Autoimmune Encephalomyelitis, Whereas None of These Cells Inhibits the Th17 Response. *The Journal of Immunology*. 186(12):6815-21.
141. Mars LT, Gautron AS, Novak J, Beaudoin L, Diana J, Liblau RS, et al. Invariant NKT cells regulate experimental autoimmune encephalomyelitis and infiltrate the central nervous system in a CD1d-independent manner. *J Immunol*. 2008;181(4):2321-9.
142. Mars LT, Laloux V, Goude K, Desbois S, Saoudi A, Van Kaer L, et al. Cutting edge: V alpha 14-J alpha 281 NKT cells naturally regulate experimental autoimmune encephalomyelitis in nonobese diabetic mice. *J Immunol*. 2002;168(12):6007-11.
143. Lehuen A, Diana J, Zacccone P, Cooke A. Immune cell crosstalk in type 1 diabetes. *Nat Rev Immunol*. 10(7):501-13.

144. Chen Y-H, Chiu NM, Mandal M, Wang N, Wang C-R. Impaired NK1+ T Cell Development and Early IL-4 Production in CD1-Deficient Mice. *Immunity*. 1997;6(4):459-67.
145. Smiley ST, Kaplan MH, Grusby MJ. Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science (New York, NY)*. 1997;275(5302):977-9.
146. Michel ML, Mendes-da-Cruz D, Keller AC, Lochner M, Schneider E, Dy M, et al. Critical role of ROR-gammat in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105(50):19845-50.
147. Yoshimoto T, Paul WE. CD4pos, NK1.1pos T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. *The Journal of Experimental Medicine*. 1994;179(4):1285-95.
148. Kent SC, Chen Y, Clemmings SM, Viglietta V, Kenyon NS, Ricordi C, et al. Loss of IL-4 secretion from human type 1a diabetic pancreatic draining lymph node NKT cells. *J Immunol*. 2005;175(7):4458-64.
149. Araki M, Kondo T, Gumperz JE, Brenner MB, Miyake S, Yamamura T. Th2 bias of CD4+ NKT cells derived from multiple sclerosis in remission. *International immunology*. 2003;15(2):279-88.
150. Demoullins T, Gachelin G, Bequet D, Dormont D. A biased Valpha24+ T-cell repertoire leads to circulating NKT-cell defects in a multiple sclerosis patient at the onset of his disease. *Immunology letters*. 2003;90(2-3):223-8.
151. Illes Z, Shimamura M, Newcombe J, Oka N, Yamamura T. Accumulation of Valpha7.2-Jalpha33 invariant T cells in human autoimmune inflammatory lesions in the nervous system. *International immunology*. 2004;16(2):223-30.
152. O'Keeffe J, Gately CM, Counihan T, Hennessy M, Leahy T, Moran AP, et al. T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to alpha-galactosylceramide are impaired. *Journal of the neurological sciences*. 2008;275(1-2):22-8.
153. Linsen L, Thewissen M, Baeten K, Somers V, Geusens P, Raus J, et al. Peripheral blood but not synovial fluid natural killer T cells are biased towards a Th1-like phenotype in rheumatoid arthritis. *Arthritis research & therapy*. 2005;7(3):R493-502.
154. Kojo S, Tsutsumi A, Goto D, Sumida T. Low expression levels of soluble CD1d gene in patients with rheumatoid arthritis. *The Journal of Rheumatology*. 2003;30(12):2524-8.
155. Yanagihara, Shiozawa, Takai, Kyogoku, Shiozawa. Natural killer (NK) T cells are significantly decreased in the peripheral blood of patients with rheumatoid arthritis (RA). *Clinical & Experimental Immunology*. 1999;118(1):131-6.

156. Parietti Vr, Chiffлот Hln, Sibia J, Muller S, Monneaux F. Rituximab treatment overcomes reduction of regulatory iNKT cells in patients with rheumatoid arthritis. *Clinical Immunology*.134(3):331-9.
157. Oh SJ, Chung DH. Invariant NKT cells producing IL-4 or IL-10, but not IFN-gamma, inhibit the Th1 response in experimental autoimmune encephalomyelitis, whereas none of these cells inhibits the Th17 response. *J Immunol*. 2011;186(12):6815-21.
158. Cardell SL. The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. *Clinical and experimental immunology*. 2006;143(2):194-202.
159. Hammond KJ, Kronenberg M. Natural killer T cells: natural or unnatural regulators of autoimmunity? *Curr Opin Immunol*. 2003;15(6):683-9.
160. Wu L, Van Kaer L. Natural killer T cells and autoimmune disease. *Curr Mol Med*. 2009;9(1):4-14.
161. Simoni Y, Diana J, Ghazarian L, Beaudoin L, Lehuen A. Therapeutic manipulation of natural killer (NK) T cells in autoimmunity: are we close to reality? *Clinical and experimental immunology*.171(1):8-19.
162. Reis EA, Athanazio DA, Lima I, Oliveira e Silva N, Andrade JC, Jesus RN, et al. NK and NKT cell dynamics after rituximab therapy for systemic lupus erythematosus and rheumatoid arthritis. *Rheumatology international*. 2009;29(4):469-75.
163. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Sato H, et al. Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Valpha14 NKT cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;95(10):5690-3.
164. Liu W, Huber SA. Cross-talk between cd1d-restricted nkt cells and gammadelta cells in t regulatory cell response. *Virology journal*. 2011;8:32.
165. Furlan R, Bergami A, Cantarella D, Brambilla E, Taniguchi M, Dellabona P, et al. Activation of invariant NKT cells by alphaGalCer administration protects mice from MOG35-55-induced EAE: critical roles for administration route and IFN-gamma. *Eur J Immunol*. 2003;33(7):1830-8.
166. Jahng AW, Maricic I, Pedersen B, Burdin N, Naidenko O, Kronenberg M, et al. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. *J Exp Med*. 2001;194(12):1789-99.
167. Pal E, Tabira T, Kawano T, Taniguchi M, Miyake S, Yamamura T. Costimulation-dependent modulation of experimental autoimmune encephalomyelitis by ligand stimulation of V alpha 14 NK T cells. *J Immunol*. 2001;166(1):662-8.
168. Singh AK, Wilson MT, Hong S, Olivares-Villagomez D, Du C, Stanic AK, et al. Natural killer T cell activation protects mice against experimental autoimmune encephalomyelitis. *J Exp Med*. 2001;194(12):1801-11.

169. Fritz RB, Zhao ML. Regulation of experimental autoimmune encephalomyelitis in the C57BL/6J mouse by NK1.1+, DX5+, alpha beta+ T cells. *J Immunol.* 2001;166(6):4209-15.
170. Parekh VV, Wu L, Olivares-Villagomez D, Wilson KT, Van Kaer L. Activated invariant NKT cells control central nervous system autoimmunity in a mechanism that involves myeloid-derived suppressor cells. *J Immunol.* 2013;190(5):1948-60.
171. Novak J, Beaudoin L, Park S, Griseri T, Teyton L, Bendelac A, et al. Prevention of type 1 diabetes by invariant NKT cells is independent of peripheral CD1d expression. *J Immunol.* 2007;178(3):1332-40.
172. Wesley JD, Tessmer MS, Chaukos D, Brossay L. NK cell-like behavior of Valpha14i NK T cells during MCMV infection. *PLoS pathogens.* 2008;4(7):e1000106.
173. Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *J Immunol.* 2007;178(5):2706-13.
174. Wiethe C, Schiemann M, Busch D, Haeberle L, Kopf M, Schuler G, et al. Interdependency of MHC class II/self-peptide and CD1d/self-glycolipid presentation by TNF-matured dendritic cells for protection from autoimmunity. *J Immunol.* 2007;178(8):4908-16.
175. Chiba A, Oki S, Miyamoto K, Hashimoto H, Yamamura T, Miyake S. Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosine-truncated analog of alpha-galactosylceramide. *Arthritis and rheumatism.* 2004;50(1):305-13.
176. Coppieters K, Van Beneden K, Jacques P, Dewint P, Vervloet A, Vander Cruyssen B, et al. A single early activation of invariant NK T cells confers long-term protection against collagen-induced arthritis in a ligand-specific manner. *J Immunol.* 2007;179(4):2300-9.
177. Miellot A, Boissier MC, Bessis N, Zhu R, Diem S, Herbelin A. Activation of invariant NK T cells protects against experimental rheumatoid arthritis by an IL-10- dependent pathway. *European Journal of Immunology.* 2005;35(12):3704-13.
178. Kim HY, Kim HJ, Min HS, Kim S, Park WS, Park SH, et al. NKT cells promote antibody-induced joint inflammation by suppressing transforming growth factor beta1 production. *J Exp Med.* 2005;201(1):41-7.
179. Kim HY, Kim S, Chung DH. FcgammaRIII engagement provides activating signals to NKT cells in antibody-induced joint inflammation. *The Journal of clinical investigation.* 2006;116(9):2484-92.
180. Chiba A, Kaieda S, Oki S, Yamamura T, Miyake S. The involvement of V(alpha)14 natural killer T cells in the pathogenesis of arthritis in murine models. *Arthritis and rheumatism.* 2005;52(6):1941-8.
181. Ohnishi Y, Tsutsumi A, Goto D, Itoh S, Matsumoto I, Taniguchi M, et al. TCR Valpha14 natural killer T cells function as effector T cells in mice with

collagen-induced arthritis. *Clinical and experimental immunology*. 2005;141(1):47-53.

182. Liu TY, Uemura Y, Suzuki M, Narita Y, Hirata S, Ohyama H, et al. Distinct subsets of human invariant NKT cells differentially regulate T helper responses via dendritic cells. *Eur J Immunol*. 2008;38(4):1012-23.

183. Yang JQ, Wen X, Kim PJ, Singh RR. Invariant NKT cells inhibit autoreactive B cells in a contact- and CD1d-dependent manner. *J Immunol*. 186(3):1512-20.

184. Issazadeh-Navikas S. NKT cell self-reactivity: evolutionary master key of immune homeostasis? *J Mol Cell Biol*. 2012;4(2):70-8.

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