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FUNCTIONAL AND GENETIC ANALYSES OF THE MHC AND ITS IMPACT ON AUTOIMMUNITY IN THE RAT

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"... one's impression is that this is a slightly truncated PhD thesis"

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ABSTRACT

The major histocompatibility complex (MHC) is an allele-rich and exceptionally gene dense region on human chromosome 6. Over 40% of the genes in this region have immune-related functions, including genes encoding MHCI and MHCII molecules. These molecules, which are found in nearly all vertebrates, present antigenic peptides to CD4 and CD8 T cells. Alleles of MHCI and MHCII are believed to be strong risk factors in autoimmune disorders, such as rheumatoid arthritis (RA), as well as in infectious diseases. However, the differentiation between haplotype and allele associations in the MHC is not straightforward. Strong linkage disequilibrium exists between gene segments throughout the region and impedes identification of disease associated variants. These gene segments can be isolated and studied individually in congenic mice and rats. We produced for this thesis an extensive number of intra-MHC congenic rats to study the association between MHC genes and experimental arthritis, T cell selection and MHC regulation. *Study I* describes a genome-wide approach in heterogenous stock rats to identify quantitative trait loci (QTLs) associated with variations in MHC levels and CD4 and CD8 T cell numbers. A total of 10 QTLs were identified, of which 3 mapped to the MHC. We showed by congenic mapping that two minimal haplotypes of ~0.2 Mb explained the associations to the MHC. We further identified two allelic variants of the gene *Tap2* that contributed to the variation in T cell numbers. *Study II* describes the effect of these minimal haplotypes on arthritis development and positions the MHCII region for the first time in an adjuvant model. We show that genes in the MHCII regulate onset, progression and severity of arthritis but not chronicity. Comparative analyses of different congenic MHCII haplotypes showed an inverse correlation between arthritis severity and proportion of recent thymic emigrants. *Study III* shows an MHCII associated T cell response to the cartilage protein collagen type XI in chronic pristane-induced arthritis (PIA) and the corresponding antibody response to the same antigen in human RA. *Study IV* describes the adoptive transfer of PIA in DA rats and outlines the conditions necessary for the model.

LIST OF PUBLICATIONS

I **Jonatan Tuncel**, Anthony C.Y. Yau, Ulrika Norin, Sabrina Haag, Diana Ekman, Amelie Baud, Erik Lönnblom, Klio Maratou, Soley Thordardottir, Jimmy Ytterberg, Martina Johannesson, Alan Gillett, EURATRANS Consortium, Maja Jagodic, Tomas Olsson, Roman A. Zubarev, Timothy J. Aitman, Richard Mott, Jonathan Flint and Rikard Holmdahl *Interaction between two Conserved Haplotypes in the Major Histocompatibility Complex Determines T cell Selection in the Rat.* Under Review, *PLoS Genetics*

II **Jonatan Tuncel***, Sabrina Haag*, Soley Thordardottir, Daniel E. Mason, Anthony C.Y. Yau, Ulrika Norin, Doreen Dobritzsch, Eric C. Peters, Rikard Holmdahl. *A Comparative Analysis of T cell Priming and Disease Development of Five MHCII Haplotypes in a Chronic Adjuvant Model of Rheumatoid Arthritis* *These authors contributed equally to this work. Manuscript

III **Jonatan Tuncel**, Sabrina Haag, Stefan Carlsén, Anthony C.Y. Yau, Shemin Lu, Harald Burkhardt, Rikard Holmdahl. *Class II major histocompatibility complex-associated response to type XI collagen regulates the development of chronic arthritis in rats.* Arthritis Rheum. 2012 Aug;64(8):2537-47

IV Jens Holmberg, **Jonatan Tuncel**, Hisakata Yamada, Shemin Lu, Peter Olofsson, Rikard Holmdahl *Pristane, a non-antigenic adjuvant, induces MHC class II-restricted, arthritogenic T cells in the rat.* J Immunol. 2006 Jan 15;176(2):1172-9.

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INTRODUCTION

GENETIC MAPPING

Introduction to genetic mapping

There are several strategies to map genes in animals and most, if not all, of these are based on the existence of genetic markers. These markers, which are relatively common (microsatellites) or very common (single nucleotide polymorphisms), are used to position quantitative trait loci (QTLs) within the genome, either by linkage or association. A classical method to position (or map) QTLs is to study genetic segregation by linkage in a cross between two inbred strains. The method is simple but rarely provides information on gene level. However, it does provide a good starting point for a congenic strain, in which a segment from one strain is inserted (or isolated) within the genome of another strain. In contrast to a cross, a congenic strain is genetically stable and therefore suitable for functional studies.

The poor resolution that is obtained in a two-strain segregation study is the major disadvantage of the method. A heterogenous stock is derived from a larger number of founder strains and the allelic diversity is therefore greater than in a conventional cross. By increasing the allelic diversity, the number of phenotypes that can be captured increases and more QTLs can be identified for each phenotype. However, a heterogenous stock does not necessarily lead to increased resolution, as the resolution is dependent on the recombination frequency. More recently, other types of crosses have been developed or are currently under development, such as the collaborative cross[1] and outbred crosses[2].

Regardless of model, mapping genes in animals to explain phenotypes in humans depend on (1) the quality of the phenotype (how relevant the phenotype is for the human trait), and (2) genetic heterogeneity. The quality of the phenotype, for example arthritis, is only valuable if it shares mechanisms relevant to the human trait (rheumatoid arthritis in this case). The second point (genetic heterogeneity) relates to the disadvantage with a conventional cross. Traits associated with rare alleles in humans might be associated with rare alleles also in other species. Hence, the chances of identifying a gene depend on its allele frequency in a population. Fortunately, different genetic variants may produce similar effects and, hence, a gene may be identified in an

animal model even if the causative variant is not the same in humans. Moreover, a gene may be mapped to a pathway that is of relevance in humans even if the gene itself is not.

Below, I describe strategies for genetic mapping that are of relevance for this thesis, in particular the generation of congenic and recombinant strains. First, however, I discuss the evolution of genetic markers and the importance of recombinations, which is the basis of virtually all genetic mapping.

Evolution of genetic markers

The advent of modern agriculture in the 1930s demanded new strategies to control breeding (Figure 1). However, in the beginning of the century, suitable markers were limited to morphological and biochemical phenotypes and it was not until the development of restriction fragment length polymorphisms (RFLPs) in 1974, researchers for the first time could use genetic markers to detect QTLs[3]. Although diallelic, RFLPs were believed to exist throughout the genome, and therefore be suitable for genetic mapping. Indeed, these markers were used to perform the first genome-wide scan, which was conducted on tomatoes by Paterson in 1988[4]. However, since they are diallelic and therefore non-informative (homozygous) in many loci, RFLPs were less suitable for large genetic studies in animal species.

It has been known since the 1960s that a large proportion of the genome consists of repetitive sequences. These sequences, which are mainly dinucleotide repeats, are found almost exclusively in non-coding regions, and at least some of these sequences seem to have regulatory properties[5]. Three groups showed independently in 1989 that these repetitive sequences, which were named microsatellites or short tandem repeats (STRs), if consisting of less than 6 base pairs, were suitable for genetic studies[6-8]. Microsatellites were more frequent than RFLPs and easier to use than these. Most importantly, these new markers were polymorphic, also in animals, and by definition codominant (i.e. heterozygous and homozygous alleles are informative). Hence, microsatellites could be used to generate dense genetic maps for a large number of species. However, their relatively high mutation rate makes them evolutionary instable.

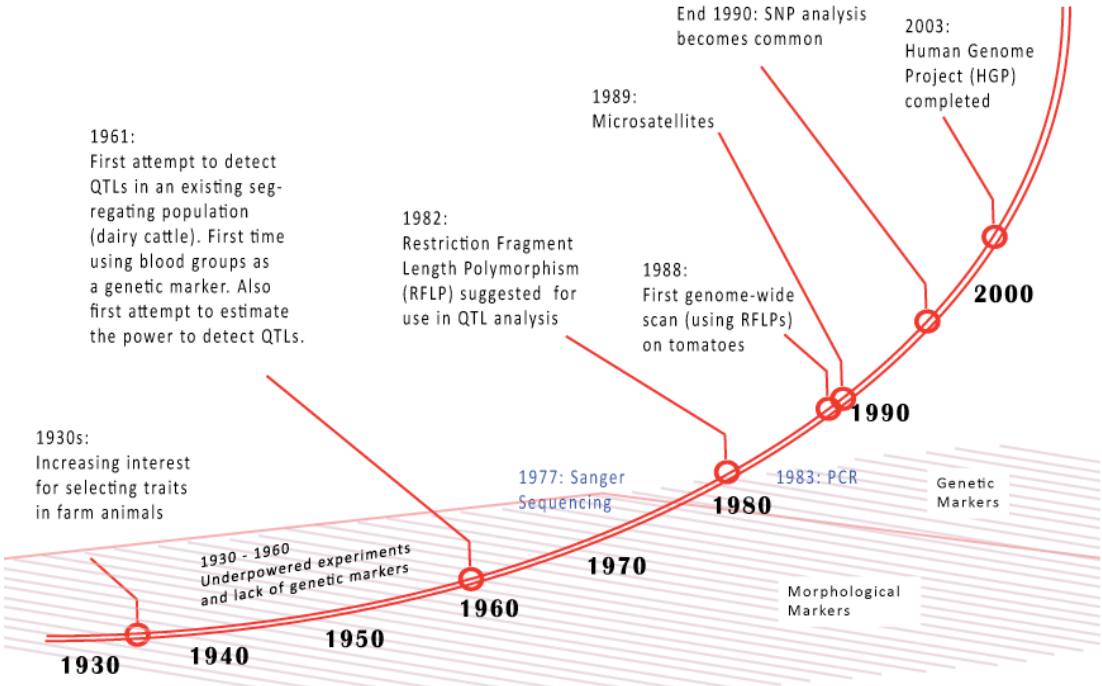

Figure 1: The evolution of genetic markers. The era of modern genetics started with the invention of the restriction fragment length polymorphism (RFLP) and Southern blot techniques in the 1970s. However, the first major step towards a full genome characterization was taken in 1989 by the introduction of microsatellites which was followed by single nucleotide polymorphisms in the 1990s.

Although of minor importance in a conventional cross, this "drift" in genotypes makes them incompatible for tracking ancestry in population studies

Today, single nucleotide polymorphisms (SNPs) have largely replaced microsatellites. The accomplishment of the human genome sequence in 2003 showed a remarkable genetic variation, of which most were polymorphisms in single nucleotides, and by using SNPs, genetic associations could be obtained with a much higher resolutions than with microsatellites.

Recombinations and mapping resolution

Recombinations, or chromosomal crossovers, involve the exchange of genetic material between different chromosomes or, to a less extent, between different regions on the same chromosome. Recombinations and mutations are together responsible for creating the patterns of genetic diversity and variation. Crossovers do not occur uniformly on the chromosomes but rather within narrow intervals of 1-2.5 kb[9-11]. These intervals, which are referred to as recombination hotspots, are scattered throughout the

genome. However, they are more common near the telomeres, and less common on the X-chromosome than on the autosomes, at least in rodents[12]. Moreover, recombination frequencies differ between species, being about two-fold greater in humans (~1.2 cM/Mb) than in rodents (~0.55 cM/mb). They are also more common on smaller chromosomes than on larger[12]. Recombinations are also more frequent in oocytes than in sperms and, hence, the recombination rate is higher in females than in males. An exception seems to be recombinations near the telomeric regions, which are more common in males[13].

Studies in humans have shown that the average distance between hotspots is 50 kb and that roughly 80% of the genome is unaffected by recombinations[14]. No consensus sequence has been found so far to explain the existence of recombination hotspots. However, certain repetitive sequences, in particular those with high GC-content and multiple CpG islands, are positively correlating with the presence of recombination hotspots[15]. That hotspots are sequence related is consistent with studies in mutated yeast cells[16] and from studies in mice, which have shown that recombinations are haplotype dependent[17,18]. Interestingly, hotspots emerge and, mainly, disappear in a rate that is disproportionate to the change in nucleotide sequence, which suggests that epigenetic modifications may contribute to the variation in recombination frequency[19]. In addition, active hotspot alleles are rapidly replaced by inactive, suggesting that the recombination frequency is steadily decreasing in a population[20]. That hotspots are not conserved between humans and chimpanzees, which is our closest living evolutionary relative (humans and chimpanzees share 99% sequence similarity), is therefore not surprising[19]. However, while hotspots evolve rapidly, recombination rates are constrained over larger intervals (several Mb), even between species.

Recombination data are available from pedigrees, linkage analysis and from genotyping of single-sperm cells[21-23]. Sperm typing has been the chief method to detect recombinations in humans as it can be used to screen thousands of haploid cells. However, today recombinations can be inferred indirectly by using high-density sequence polymorphism data. Although based on historical recombinations and estimations, these data are largely consistent with data from sperm typing.

Segregation and linkage analyses

Linkage mapping (or family mapping) uses family data, or pedigrees, to identify genetic regions that are associated with a trait. This information can further be used to establish a congenic strain or an advanced intercross line[24]. The regions that are identified in a linkage study are large, often in the order of 10s of megabases or more[25] and the information that can be obtained is therefore limited. Furthermore, QTLs that consist of multiple linked genes with alleles that have opposite effects on a trait will be difficult to detect with a linkage study. Hence, a linkage study will underestimate the true number of QTLs in a population.

In order to obtain a linkage map, genotype information needs to be collected from a set of genetic markers. However, since the expected number of recombination events is low in a cross or in a small set of family members, a relatively small number of markers need to be typed. Moreover, markers do not need to be physically mapped to the genome to be used in a linkage study since only the linkage (and not the physical distance) between traits and markers is studied.

A schematic illustration showing the principles of linkage mapping is shown in Figure 2. In this example, genetic loci are segregating in a cross between two homozygous strains (represented as blue and red chromosomes in Figure 2A). Naturally, chromosomal crossovers that take place in homozygous individuals will not be informative, and the first generation (F1) therefore consists of genetically identical animals only. Whether markers that are linked in the F1 individual will remain linked after meiosis depends on the distance between them (Figure 2B). Markers that are far apart are less likely to be inherited together and markers that are inherited more often than expected are said to be in linkage disequilibrium (LD). If markers are positioned on different chromosomes, the recombination frequency between them is 50%. To express the linkage between markers and traits or between different markers, geneticists use LOD scores (logarithm of odds; Figure 2C). While a positive LOD score suggests a true linkage, the significant threshold is dependent on each particular experiment.

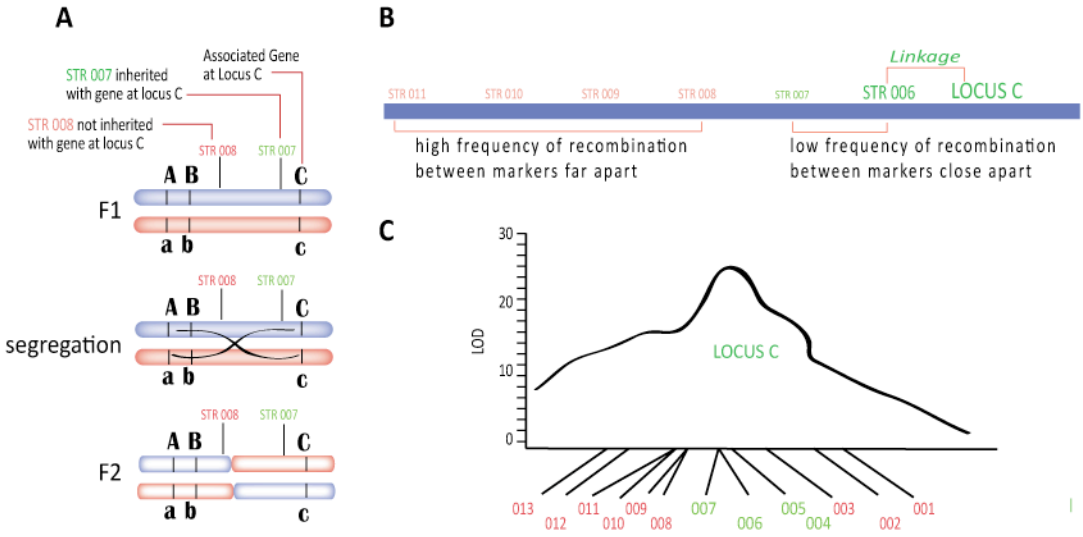

Figure 2: Recombinations and Linkage Analysis. Recombinations do not occur randomly but rather within defined intervals (recombination hotspots). Still, two genetic markers (here represented as microsatellites, STRs) that are far apart are less likely to be inherited together. (A) Segregation of genes and markers following a recombination between locus B and locus C. (B) Microsatellite markers STR 006 and 007 are in linkage with locus C. (C) The probability that markers 001-013 and locus C is significantly associated is expressed as LOD scores.

Congenic strains and recombinant lines

Congenic fine-mapping of loci identified by linkage mapping is a proven strategy to positionally clone genes[26,27]. The resolving power of congenic mapping is determined by the gene density in a particular region of interest and the capacity to generate and screen a sufficiently large number of mice or rats. It is possible to theoretically estimate how many animals that are needed to reduce a region to a fragment of a particular size[28]. For example, to obtain a 0.15 cM fragment from a 4 cM region requires ~1300 mice, whereas ~40.000 mice would be required to achieve the same results by typing a conventional intercross[29]. Thus, a congenic strategy is ideal to fine-map a locus, while a linkage study is necessary to identify suitable QTL candidates. However, promising QTLs with strong and significant linkage may also fractionate into multiple smaller effects or, worse, into no effect at all when genetic interactions are broken[30].

To generate a congenic strain by conventional breeding is time-consuming as it requires at least 10 backcross generations, corresponding to 2.5 - 3 years time, to obtain a 99.9% pure genetic background[31]. By marker-assisted breeding, the same purity can be achieved with half as many backcross generations[32]. However, this speed congenic method requires more genotyping and therefore more hands-on work. A congenic fragment that is isolated by speed congenic or conventional methodologies is on

average 20 cM[32]. A first step towards positional cloning is therefore to generate sub-congenic or recombinant strains. This is accomplished by segregation analysis, using the same principles as outlined in Figure 2. However, since the congenic region is defined by its physical fragment no other markers than those specific for the fragment need to be typed.

A positional cloning approach will rarely lead to a single-gene fragment. More often, genes have to be excluded by other means, such as sequencing and expression analysis. By comparing alleles from different haplotypes, candidate variants can be identified and non-polymorphic gens can be excluded. This strategy, which was used for *Study I* and *Study II* in this thesis, is particularly suitable when dissecting a highly polymorphic region that is constrained by strong LD.

Heterogeneous stock

Heterogeneous stock (HS) animals provide QTLs at a higher resolution than a conventional cross and the mapping is less laborious than with a congenic strain[33]. An HS is derived from eight inbred founder strains that have been semi-randomly bred for 40-50 generations, which should maintain heterozygosity. Thus, in contrast to a conventional cross, in which the mapping resolution is dependent on new recombination events, the recombinations in an HS have accumulated over generations. It is therefore possible to map QTLs with high resolution within a relatively short time period.

Haplotype blocks (i.e. the distance between recombinations) in an HS approaches 2 cM (~3 Mb in mouse) and, hence, the resolution is 10-fold higher in an HS than in a conventional cross. Corresponding haplotype blocks in humans and outbred mice are ~9-18 kb and 100 kb, respectively[34]. Although better resolving power, the fixed strain composition in an HS is a limitation. In a conventional cross, strains are selected specifically for their phenotype. For example, a cross aiming to detect QTLs for diabetes needs to contain both diabetes susceptible and resistant alleles. In an HS, however, the strain composition is fixed and whether the stock is suitable needs to be tested empirically. In mice, two separate but strain-wise similar stocks are available: the Boulder and the Northport stocks[30,35,36]. Thus, there is at least a theoretical possibility to

assess suitability in two different stocks. The only HS that has been developed in the rat was founded at the NIH in 1984, using the inbred strains ACI/N, BN/N, BUF/N, F344/N, M520/N, MR/N, WKY/N and WN/N[37]. We used the NIH-HS to detect QTLs associated with variations in T cell subsets and MHC expression in *Study I*. However, our first intention was to use this stock to map genes controlling pristane-induced arthritis (PIA), a model for rheumatoid arthritis. However, the NIH-HS turned out to lack critical arthritis promoting alleles[38,39], and a pilot study on ~100 HS individuals confirmed that it was not suitable for this purpose (Rintisch, *unpublished data*). Ahlqvist et al. experienced similar problems when using the Northport stock to map arthritis susceptible genes in collagen-induced arthritis (CIA)[40]. Susceptibility to CIA is strictly dependent on H2-A^q and H2-A^r MHCII alleles. Since neither of these MHCII haplotypes were represented in the Northport stock, it was not surprising that the Northport stock would be resistant to CIA[41]. Ahlqvist et al. therefore introduced the arthritis permitting H2-A^q MHC haplotype from the BQ strain in the Northport stock, a strategy originally proposed by Mott and Flint[42]. Homozygous H2-A^q mice were selected to produce an F3 generation with fixed MHC alleles, which they used for arthritis experiments. However, by introducing a disease permitting MHC locus, they also introduced other BQ genes, which contracted the allelic diversity in the stock.

The large number of intercross generations in an HS introduces inbreeding as well as complex family structures (private alleles) that, if not corrected for, lead to confounded associations and an excess of false positives[43,44]. Compared to a fully outbred population, an HS has some distinct advantages, however. Firstly, the allelic variation is controlled as it is limited by the eight founder strains. Thus, by genotyping the recombination breakpoints in the HS individuals, remaining variation can be inferred and instead of sequencing each HS individual, it is enough to sequence the eight founder strains. This concept was indeed proven in the rat HS (Baud et. al, *Nature Genetics*, accepted), which further showed that relatively few QTLs could be explained by a single variant. Secondly, alleles in an HS are not rare, as they are limited to the allele frequency in the founder strains. In an outbred population, common alleles become diluted as new alleles appear (through mutations and possibly recombinations). Hence, the allele frequency increases in an outbred population. Rare alleles that contribute to a trait are very difficult to control for and to map. The absence of rare alleles in an HS (this assumes that no new mutations appear after the stock has been

founded) increases statistical power to detect QTLs, and decreases thereby the requirement for large sample numbers.

In summary, an HS offers superior mapping resolution compared to a conventional cross as well as more power to detect QTLs compared to an outbred population.

MAJOR HISTOCOMPATIBILITY COMPLEX & ANTIGEN PRESENTATION

Introduction to the MHC

The first complete sequence and gene map of the human major histocompatibility complex (MHC) was reported in 1999 by the MHC sequencing consortium[45]. However, the region was described 50 years earlier by George Snell, Jean Dausset and Baruj Benacerraf for which they earned the Nobel prize in 1980, although at that time the MHC was thought to consist of only histocompatibility genes[46-48]. The MHC was early recognized for its extreme diversity[49], and for its association in many autoimmune and infections diseases. It is therefore not surprising that the MHC has remained as one of the most extensively studied regions in the human genome.

Here, I discuss the genomic organization of the MHC, the distance between haplotype blocks as well as MHC class I and II antigen presentation, whereas the MHC association in autoimmune diseases will be discussed in a later chapter. First, however, I will introduce the somewhat confusing MHC nomenclature.

The MHC nomenclature

The MHC nomenclature is not straightforward. The term MHC is used in diverse ways, although typically referring to the continuous sequence that in humans spans ~4 Mb. Indeed, the word 'complex' in MHC refers to the early findings that genes in this region were clustered together and related by function[50]; however, this is not a universal feature of MHC in all species[51]. Dawkins et al. suggested, however, that in order to keep genes, gene families and region apart, the region should be referred to as the 'MHC region' rather than the shorter 'MHC[52]. However, since the latter is more common, I will use MHC here when referring to the region and MHCI and MHCII when referring to those particular subregions.

Both classical and non-classical MHC genes in the rat are prefixed RT1, which is also the name of the region. What the abbreviation RT1 formally stands for is unclear, although Rat Transplantation locus 1 seems reasonable. In humans and mice, these genes (and the region) are called HLA (Human Leukocyte Antigen) and H2 or H-2 (Histocompatibility locus 2), respectively. Moreover, according to standard nomenclature, all gene names are depicted in italic whereas regular fonts are used for protein names. Human gene names are spelled in capital letters (e.g. *PSMB9*) whereas rat genes names only have capital initials (*Psmb9*). Unfortunately, this consistency was not adopted in the manuscripts and papers included in this thesis, in particular not in *Study IV*, which uses 'chimeric' human and rat nomenclatures such as *RT1-DQ* and *RT1-DR*.

Origin and organization of the MHC

The MHC sequencing consortium determined the size of the human MHC to 3.6 Mb, with an average gene density of 1 gene per 16 kb[45]. This should be compared to 1 gene per 60 kb in the rest of the genome and 1 gene per 0.13 kb in the prokaryote *E. coli*[53]. This 'classical MHC' is flanked by extended MHCI (xMHCI) and xMHCII subregions, which together with MHCIII and the classical MHCI and MHCII subregions make the entire 'extended MHC' span about 7.6 Mb[54]. Approximately 420 genes are encoded within the extended MHC, of which 60% are expressed and 22% have immune-related functions. Within the classical MHC, roughly 40% have immune-related functions whereas all genes in the classical MHCII except *RING3* encode components of the adaptive immune system.

The second MHC sequence to be completed was the chickens' B locus[55]. The 92 kb B locus is far more simple than the HLA locus with only 19 genes encoded, but still more complex than the MHC in frogs and sharks. MHC class I and II genes have been found in all jawed vertebrates examined so far but not in jawless vertebrates (e.g. hagfish, lamprey and sea urchin) or in invertebrates[56]. Sharks possess the simplest form of MHC, which have emerged some 520 million years ago. Sharks are also the oldest living species that have an adaptive immune system and a functional MHC-T cell

receptor (TCR) system, including the recombination-activating gene (RAG) that is necessary for immunoglobulin (Ig) and TCR rearrangement.

Matsunaga et al. and others have suggested that the first prototypic MHC evolved in fish that have started to develop jaw-like structures and that the adaptive immune system evolved in the gastrointestinal area of these animals[57]. Hence, jaws, which probably have evolved as a consequence of a predatory life style, may have triggered the development of an adaptive immune system, which served to protect the animals from gastrointestinal infections[57]. However, the MHC as a region is older than the adaptive immune system and therefore does not seem to have evolved around class I and II genes. MHC genes such as *NOTCH4* and *RXRB* are present also in organisms that lack an adaptive immune system, while other genes with apparently no immunological functions, such as *RING3*[58], have coevolved with class I and II genes since the speciation of jawed vertebrates. Hence, it is possible that the histocompatibility function of MHC is an adapted feature while the original complex had other functions, possibly as a system for sexual selection and avoidance of inbreeding[59].

Linked and unlinked MHC

The MHCI and MHCII subregions are linked in most species but not in all. In the teleost fish, the MH class I, II and III loci (since the major histocompatibility genes in the fish are not part of a complex, the region is simply referred to as MH) are found on different chromosomes[60,61]. Interestingly, framework genes, such as *TAP*, *LMP* and *RXRB*, that in other species are located in the MHCII but functionally associated with MHC class I presentation are in pufferfish and zebrafish linked with the classical MHC class I genes[62]. In other species, such as the chicken and the quail[55,63], MHC is separated into two clusters on the same chromosome, the B locus and the restriction fragment pattern-Y (Rfp-Y). It is likely that these two loci reflect a duplication of a larger segment in the avian MHC. Comparative studies have shown that both loci encode functional and polymorphic MHC class I genes and that these genes share a large degree of sequence homology. However, genes in the Rfp-Y locus appear to have more in common with MHC class Ib (non-classical) than class Ia genes[64]. This would argue more for an organization similar to the RT1 in the rat, where class Ia (RT1-A) and class

Ib (RT1-C/E/M) are separated by the MHCII and MHCIII loci. Also the swine has an unlinked MHC. The swine MHC, known as SLA, is positioned on chromosome 7 but MHCII and MHCI are separated by the location of the centromere. The SLA locus appears to be the only mammalian MHC locus that has unlinked MHCI and MHCII subregions[65].

The class I region

Centromeric collinearity in the MHC begins with *Kifc1* in rodents and humans[66]. This gene is located in the z block, which is proximal (near the centromere) to RT1-A and H2-K in rats and mice, respectively. The z block in humans accommodates the xMHCII locus, which, except for *RXRB* and *TAPASIN* (Tap binding protein, *TAPBP*), contains no immune related genes. MHC class I genes in rats and mice vary largely in copy numbers between different haplotypes[67]. The rat encodes 8 class I gene clusters, which are depicted RT1-A, -CE, -N, -M1, -M4, -M3A, -M3B, and -M2[68]. The classical RT1-A locus, which consists of three genes (*A1*, *A2* and *A3*), is the only MHCI locus that is located proximal to MHCII and MHCIII. The number of functional RT1-A genes vary between haplotypes, and the current assembly (RGSC 5.0), which is based on the $RT1^n$ haplotype, excludes *RT1-A3*. All remaining class I genes in the rat are non-classical MHCIb genes.

The rodent MHC has a block of olfactory receptor genes at the distal (telomeric) end, which is substantially contracted in primates. In contrast, rodents lack the class I linked MIC genes that are found in primates, although a MIC related gene family called MILL has been mapped to chromosome 7 in mice[69].

The MHCI genes demonstrate a large degree of plasticity between species, which is not as evident for MHCII genes. In humans and chimpanzees, class I and MIC genes are organized as repeating units within duplicated blocks (so called evolutionary or frozen blocks). These blocks are serially connected as modules that have undergone significant diversifications, which have resulted in the immense heterogeneity that characterizes this locus. This diversification has further generated a large number of defective genes, which possibly explains why more than half of the genes in the MHCI are pseudogenes[70]. However, the module hypothesis does not fit with the expansion of

class I genes in the rat. Hurt et al., who published the first rat MHC sequence in 2004, noted similarities between genes in the RT1-A and the first telomeric RT1-CE clusters but not with other MHCIb genes[66]. Their data suggested that MHCIb genes in the rat were not generated through module duplications and they speculated that MHCI genes in rodents might have evolved independently following the speciation of mouse and rat.

The class II region

Duplicated modules are also apparent in MHCII; however, the orthologous relationships between mice, rats and humans for genes in MHCII and, in particular, MHCIII are more conserved in terms of gene order. Two larger blocks, known as epsilon (DP and DM) and delta (DQ and DR) have been identified in HLA[52]. Moreover, MHC class II alpha and beta genes appear in pairs except for DO, where the alpha and beta genes are separated by the location of *RING3*, *DMA*, *DMB*, *PSMB8*, *PSMB9* and the *TAP* genes. The human MHCII contains 19 genes, of which 8 are pseudogenes. The number of pseudogenes differs between haplotypes[71] and they appear to have evolved through duplications (mostly tandem) and have since then undergone diversifications and lost their function. It is possible that pseudogenes may have been maintained in this way to provide a source for generating new alleles by gene conversions[72].

However, not all module duplications lead to a loss of function. Paralogs have been shown for *DQA*, *DQB* and, in particular, *DRB*, while there is only one copy of *DRA*. This gene (*DRA*) is also monomorphic in both rodents and humans, which certainly reduces the total variation in the HLA-D locus substantially. It is known that certain DQ beta chains form very instable dimers with certain DQ alpha chains and these *DQA* and *DQB* alleles are therefore rarely found together on the same haplotype[73]. Since DQ and DR genes are in strong LD, a non-polymorphic *DRA* gene may therefore have been preferred in order to reduce the complexity in the MHCII by limiting the number of possible allele combinations.

At least four of the *DRB* genes are functional: *DRB1*, *DRB3*, *DRB4* and *DRB5*. While little allelic diversity has been shown for the paralogous *DRB3*, *DRB4* and *DRB5*, more than 100 alleles of *DRB1* have been characterized and grouped into haplotypes DR1-DR10. These DR haplotypes combine different *DRB1* alleles with specific paralogs

of *DRB3* (DR3, 5 and 6), *DRB4* (DR4, 7 and 9) and *DRB5* (DR2), while there is no second *DRB* gene for haplotypes DR1, 8 and 10 [74]. The *DRB* loci are highly similar and it is therefore likely that they represent recent duplications. Both MHCII paralogs and pseudogenes are fewer in the rat, which to some extent reflect the lack of sequencing efforts. It is clear however that rats lack functional DP genes, and so far only a single MHCII paralog, *RT1-Db2*, has been reported[66] and *Study I*.

The class III region

The centrally located MHCIII region is the most gene dense region in the human genome with 62 genes or 1 gene per 11 kb[45]. This region appears to have no pseudogenes, which makes it markedly different from MHCI and II. MHCIII contains further a number of highly conserved genes, such as *NOTCH4*, *complement C4* and genes of the tumour necrosis factor (TNF) family that have members on other chromosomes[66].

Sequence heterogeneity in the MHC

Early characterization of sequence polymorphisms in the MHC suggested that heterogeneity was focused to the hypervariable exonic sites (e.g. the second exons of the HLA-DR and HLA-DQ genes), and that non-coding heterogeneity was merely bi-products of these highly variable exons. However, more recent genome sequencing has shown that polymorphism is prevalent throughout the region, albeit to a lesser extent. Why MHC display such diversity in non-coding DNA is still not clear. Certainly, this may reflect redundant sequence polymorphism, but the heterogeneity may also co-localize with transcription regulatory sites. These sites may be close to (cis) or several kilobases away (trans) from the nearest gene. Significant efforts to map regulatory sites on a genome-wide level are currently ongoing[75].

The class I genes are the most polymorphic genes in the MHC and in the genome. The MH cluster of the fish seems to be the only exception where class II genes are more heterogenous than class I genes. In addition to the allelic variation in the MHC,

frequent gene duplications and copy number variations add to the overall heterogeneity[76]. Since also pathogens evolve rapidly, this extreme diversity may serve to increase the heterozygosity in the entire region and thereby the probability to clear an infection.

Mapping recombination hotspots in the MHC

The high gene density in combination with an extensive LD impair identification of causative variants in the MHC. However, the notion that LD is much stronger in the MHC than elsewhere in the genome is partially incorrect[77]. Strong LD exists in segments of approximately 22 kb throughout the genome[78], including the MHC[79]. However, a complex pattern of LD between different segments seems to be unique for the MHC. These segments were described by Dawkins in 1982 as frozen haplotype blocks[80,81]. Dawkins postulated that these blocks acted as transposable elements that could form new haplotypes. Although recombinations occur between the blocks, they rarely, or never, occur within them. Thus, these blocks remained in the genome as small restricted units[82], possibly conserved by a marked reduction in recombination activity. Such decline in recombination activity may result from mutations as discussed previously. Several of these blocks are apparent in the MHC, for example between *HLA-KE* and *HLA-DMA* in the junction of xMHCII and MHCII, as well as between *HLA-DQB2* and *HLA-DRB9*. Why certain segments appear in LD is not known but cis-interactions between linked segments may have had selective advantages.

In a number of studies published in the beginning of the 20th century, Cullen et al. and Jeffreys et al. described the recombination rate in the HLA using single-sperm cell typing[21,83,84]. Their studies showed that recombination hotspots in the MHC were unevenly distributed and that the recombination rate varied greatly between different individuals. Their data are therefore consistent with the notion that specific sequence motifs may accelerate the recombination activity. Cullen et al. identified six major hotspots within an interval of 3.3 Mb as well as several 'colder' regions, which largely overlapped with the distribution of frozen haplotype blocks as postulated by Dawkins[80]. The MHCIII showed overall lower recombination activity than MHCI and MHCII, which is in keeping with the conserved gene order that characterizes this region in

many species. Intervals with particularly high recombination activity were found within *DPB1* to *RING3* (*Brd2* in rat), *DRA* to *TSBP* (*Tesb* in rat) and *BAT2* to *LTA*[21].

MHC class II expression is controlled by CIITA

The transcription factor CIITA (class II transactivator) is a chief regulator ofMHC class II transcription[85]. CIITA belongs to the NOD-like receptor (NLR) protein family, which trigger innate immune responses, for example upon exposure to bacterial cells wall components[86]. CIITA was the first NLR to be characterized, and while certain NLRs, such as NLRP3, are constituents of the inflammasome[87], the function of CIITA appears to be specific for MHC regulation.

Mutations in the *MHC2TA* gene that severely affect the expression or function of CIITA result in a rare form of immunodeficiency known as bare lymphocyte syndrome (BLS)[88], a disorder that has served as a prototypic model for the study of MHC regulation in humans[85]. Mutations in MHCII-transcription factors *RFXANK*, *RFX5*, *RFXAP* are also associated with BLS, while genes in the MHCII locus itself are not associated.

That CIITA is a master regulator of MHCII expression is demonstrated by its wide role in both constitutive and inducible MHCII expression[73], as well as by its impact on both classical and non-classical (DM and DO) MHC genes[88]. CIITA does not possess a DNA-binding domain itself and is therefore dependent on other transcription factors[89]. Cis-regulating promoter regions in both MHCI and MHCII genes share distinct consensus sequences containing W/S, X1 and X2 motifs as well as a Y-box, which is a canonical CCAAT box, which promote binding of transcription factors RFX, CREB/ATF1 and NF-Y[90]. These form together with CIITA the MHC enhanceosome[91]. The regulation of CIITA expression occurs mainly on transcript level, which is controlled by four cell-specific promoters entitled P1-P4. The P1 promoter is dendritic cell (DC) specific and drives the expression of the longest CIITA isoform[92]. The P1 promoter is also associated with the highest expression of the *MHC2TA* gene and the expression of MHCII in DCs, at least in immature cells, is therefore higher than in any other MHCII bearing cell[93].

It is unclear to which extent CIITA also can regulate the expression of MHCI genes, although a few studies suggest that it does[88,94]. However, since the expression of CIITA is confined to lymphocytes and APCs, it is unlikely that CIITA is responsible for the constitutive expression of MHCI[73]. That CIITA is essential for MHCI expression is further challenged by the normal level of MHCI expression in BLS patients[85]. Recently, another NLR protein, NLRC5, was shown to operate as a regulator of MHCI expression[95]. This proteins, which has no influence on MHCII expression, has further been shown to control the expression of the non-classical MHCIb gene *HLA-E* as well as genes involved in class I antigen presentation and processing, such as *β2M*, *LMP2* (*Psmb8*) and *TAP1*[95]. *RXRB* is another transcription factor that regulates MHC class I gene expression. This gene is, in contrast to NLRC5, encoded within the MHCI subregion[96].

Antigen presentation: MHC class II

Professional antigen-presenting cells (APCs), which include DCs, macrophages and B cells, express MHCII constitutively and possess efficient mechanisms for internalizations of proteins and other macromolecules. In contrast, these mechanisms are relatively poor in non-professional APCs, which are cells of epithelial origin, such as keratinocytes, that express MHCII only after activation.

MHCII molecules exit the endoplasmic reticulum (ER) in a nonameric complex consisting of three MHCII molecules surrounding a core of three glycoproteins known as invariant chains (li)[97,98]. The association with li has at least three purposes; firstly, the li protects the empty pocket from being pre-maturely loaded until the complex reaches the late endosomal compartment; secondly, a domain in the li directs the complex to the endosomal compartment and, thirdly, li stabilizes the complex and prevents it from degradation[99]. li:MHCII complexes enter late endosomal compartments known as MIICs (MHCII compartments), after which the li is degraded by resident MIIC lysosomal proteases such as cathepsin S and cathepsin L[100]. Within the peptide binding groove stays a small li-derived peptide called CLIP (class II-associated invariant chain)[101]. This peptide competes with other peptides in the MIIC for binding to MHCII. Hence, the purpose of CLIP is to ensure that low-affinity peptides are excluded from

the binding groove. The non-classical MHCII molecule HLA-DM is essential for the exchange of CLIP peptides in the MIIC[102,103]. DM stabilizes the open conformation of the pocket until a stable interaction with a new peptide can form[104,105]. Indeed, the exchange of CLIP peptides is markedly reduced in DCs knocked-down for HLA-DMB, which results in increased levels of CLIP:MHCII complexes on the cell surface[106]. A second non-classical MHCII chaperone known as HLA-DO associates with DM during the transport from ER to the MIIC[107]. Similarly to Ii, DO prevents pre-mature loading of MHCII until the complex has reached the MIIC. Although different functions have been ascribed to DO, its major role is to block the function of DM and, hence, regulate the exchange of CLIP peptides. It has been postulated that DO looses its inhibiting capacity in the slightly acidic environment that is present in the MIICs[108]. However, in two recent studies, Guce et al. and Yoon et al., showed by X-ray crystallography and mutagenesis that HLA-DO in fact acts as a competitive inhibitor of HLA-DM for the substrate MHCII[109,110].

Peptides bind differently to MHCII and MHCI molecules. The peptides that fill the grooves of MHCII molecules protrude from each end, while peptides are entirely contained within the groove of MHCI molecules. The MHCII pocket is shaped by the polymorphic external domains α1 and β1, which form two antiparallel helical loops as well as a floor of eight beta strands. Amino acids in the groove form hydrogen-bonds (as well as other chemical interactions) with the backbone and side chains of the encompassed peptide[111,112]. The nine amino acid residues of the peptide that fit within the groove are numbered (from the N-terminal end) p1-p9. The stability of the interaction is mainly dependent on peptide side chains at position p1, p4, p6 and p9 that can bind strongly to structures (pockets) within the groove. The MHCII residues that occupy these positions can help to identify specific binding motifs, i.e. peptides that fit particularly well into the pocket[113,114].

Antigen presentation: MHC class I

Proteins of intracellular origin are degraded by cytosolic and nuclear proteases for transport to the ER and subsequent loading onto MHCI molecules. Although the process shares several features with MHCII presentation, the process of loading and

the compartment, in which this takes place are entirely different. Many of the peptides that are presented in the context of MHCI are derived from proteins that are at the end of their life-cycle. However, a system entirely devoted to presentation of only 'worn-out' proteins would be very vulnerable to viral infections. Therefore, many, if not most, of the peptides that are presented by MHCI derive from proteins that are degraded immediately after synthesis. Thus, the MHCI peptides that are displayed on the cell surface are derived from proteins that are currently being translated in the cell. A part of the rapidly degraded proteins are DRIPS (defective ribosomal products), which result from defective transcription or translation or from protein mis-assembly. MHCI peptides may also be generated by unconventional transcriptional mechanisms. Such peptides may stem from frame-shifted sequences, introns, and 5' and 3' untranslated regions. CD8 T cells must therefore screen an extreme diversity of peptides in order to identify the few that are non-self.

Peptides are funnelled from the cytosol to the ER via the transporter associated with antigen processing (TAP)[115,116]. Inside the ER, TAP associates with an additional four members of the peptide-loading complex (PLC): tapasin, calreticulin, ERp57 and the MHC class I molecule. Although peptides are trimmed by the proteasome before entering the ER, an additional aminopeptidase within the ER known as ERAAP is important for the finale shaping of the class I cargo. ERAAP was first identified by Serwold et al. who showed that class I expression is greatly reduced on the cell surface in the absence of this enzyme[117]. Peptides that do not fit the binding groove of MHCI after trimming is shuttled back via the translocon Sec61p for destruction in the cytosol[118] or reentering into the ER via TAP[119].

Tapsin influences the stability of the MHCI:peptide complex, the translocation of peptides to the ER and the levels of Tap in the ER membrane[120], albeit the dependancy of tapasin varies greatly between different MHCI alleles[121]. The stoichiometric relationship between tapasin and MHCI in the PLC is 2:1[122] and each MHCI molecule is associated with one molecule of β2-microglobulin (β2m). The tapasin molecules associate with TAP via their ER transmembran domain whereas they are covalently bound to ERp57 via a disulfide bridge[123]. The role of ERp57 in the PLC appears to be to stabilize the interaction with MHCI. The recruitment of MHCI to the PLC is normal in B cells deficient for ERp57[124], while in the absence MHC class I dissociates more rapidly from the PLC. Hence, several components of the PLC are responsible for bringing the

MHCI molecule into close contact with the TAP transporter and to retain it there long enough for peptides to be loaded. Once a stable interaction between peptide and MHCI has formed, the loaded MHCI dissociates from the PLC and translocates to the cell surface.

AUTOIMMUNITY

Introduction to autoimmunity

MHC is, without comparison, the strongest genetic risk factor for autoimmune and infectious diseases. Albeit some diseases, such as ankylosing spondylitis and psoriasis vulgaris have been convincingly associated with class I genes and yet others with class II genes, a large number of these diseases appear to be associated with multiple alleles in several different subregions of the MHC. In rheumatoid arthritis, as well as in other common autoimmune diseases, alleles in MHCII appear to be the major risk components, which suggests that a breakdown in T cell tolerance is a major trigger in the pathogenesis of these diseases. Here I discuss the role of MHC genes in autoimmunity and the etiology and pathology of rheumatoid arthritis.

MHC genes in autoimmunity

The complexity of the MHC hampers identification of disease causing variants. The reason for this complexity is the high degree of genetic variation and extensive LD that exists between alleles, or between segments of alleles. The large proportion of rare MHC alleles, copy number variants, and the diversification of paralogous genes have a great negative impact on mapping resolution and the differentiation between haplotype and allele associations. Nevertheless, a number of predisposing variants have been proposed for a large number of autoimmune diseases.

In 1967, Hodgkin's lymphoma became the first disease to be associated with MHC, when HLA-B antigens were found to be at increased frequency in these patients[125]. The MHC association in multiple sclerosis (MS), a chronic inflammatory demyelinating disorder, remains the most reproducible association that has been identified in an autoimmune disease so far[126]. This association, which is directed to the HLA-DR2 haplotype, was discovered as early as in 1972[127]. However, the extensive LD in the DR2 haplotype has made it difficult to determine whether the risk is conferred by *DQB1*0602* or *DRB1*1501*, although recent studies have favoured the latter[128]. Linked

MHCI alleles, in particular *HLA-A*0201* and *HLA-A3*, may further contribute to the disease association in MS. In addition, recent meta-analysis have shown an association for haplotypes DR3 and DR4[129].

In rheumatoid arthritis (RA), which is distinguished by chronic inflammation in small and large joints (see below), the total variance explained by heritability is about 50%, of which HLA explains 36%[130,131]. Markers in HLA-DRB1, in particular at position 11, which correspond to the β-sheet floor of the antigen-binding groove, account for the strongest association[130,132]. Significant associations have also been found for position 71 in this protein when adjusting for the effect in position 11 and for 74 when adjusting for the effects in both 11 and 71. The association to positions 71 and 74 in DRB1 is consistent with Gregersens' shared epitope hypothesis[133], which stated that a sequence of amino acids in the third hypervariable region of DRB1 (70 Q/R-K/R-R-A-A 74) was common in patients with RA. When all effects associated with DRB1 are neutralized, Eyre et al. and Raychaudhuri et al. found significant associations for HLA-B (position 9) and, after adjusting for HLA-B, also HLA-DPB (position 9). The combined effect of these five positions account for most of the MHC association in a subset of patients with antibodies to citrullinated peptides[132].

A few MHCII alleles appear to be overlapping between different types of autoimmunity; however, the vast majority of associated alleles are not. This disease specificity may argue for tissue-specific antigen presentation in autoimmunity. It has therefore been of major interest to characterize the ligandome of different MHCII alleles [134-137].

Rheumatoid Arthritis - a heterogenous disease

The strong HLA association in RA to *DRB1* as well as to other MHCII genes and to MHCI, provide important insights into the disease etiology. Other genetic associations to *PTPN22*, *STAT4*, *IL6R*, *PADI4* and TRAF1-C5[130,138-141] have also contributed to uncover disease related mechanisms, although their effects on disease are relatively small (odds ratio, OR<2) compared to MHCII (OR>6). The most strongly associated SNP in *IL6R*, for example, correlates with the levels of circulating IL-6R, a cytokine receptor that is a promising therapeutic target in RA. The associated genes highlight

molecular pathways in both the adaptive and innate immune system, and the association to PADI4, a citrullinating enzyme, suggests an important role for antigen modifications in RA. Accumulating data suggest that the presence of anti-citrulline protein antibodies (ACPAs) as well as IgM and IgA rheumatoid factors (RFs), which are directed against the Fc fragment of IgG, are highly correlating with shared epitope alleles of *DRB1* and disease associated variants of *PTPN22*[142,143]. This suggests that seropositive RA, which is the most common and most severe form of RA[144], constitute a distinct disease subset with an etiology that is more related to defective adaptive responses[145]. That patients respond differently to treatments with biological drugs, in particular the cytokine or cytokine receptor inhibitors directed to TNF (etanercept and others), IL-1 (anakinra) and IL-6R (tocilizumab), but also to B cells (CD20 [rituximab]), support the notion that RA is a heterogenous disease[146]. Hence, the genetic makeup and/or environmental stimuli may trigger different disease mechanisms in different patients and to use genetic typing to personalize the treatment for these patient may be a plausible scenario in the future.

T cells are expendable in established RA

The strong association to *DRB1* agues for an important role of MHCII:TCR interactions in RA, and highlights the relevance of CD4 T cells in the disease. That T cells infiltrate the synovia in RA was shown nearly 40 years ago[147]. Infiltrating T cells consists of CD4 and CD8 subsets. However, while CD8 cells are relatively sparse in the synovia, the CD4 cells are numerous (~50% of the cells in the RA synovia) and appear to be pre-activated, as demonstrated by their defective IL-2 response[148,149]. However, to which extent these T cells contribute to the pathogenicity in RA is still unclear. It seems reasonable that T cells play an important role in undifferentiated RA (before diagnosis), which would be consistent with the presence of ACPAs of IgG isotype years before the clinical disease onset[150,151]. Furthermore, studies by Moreland et al. and others[152,153] have shown that patients treated with chimeric antibodies to human CD4 (cM-T412) in combination with methotrexate (the most commonly used DMARD in RA) show no or minor clinical effects of the treatment, despite being severely lymphopenic. The outcome of the clinical trails with alemtuzumab, a CD52-specific monoclonal antibody,

were also disappointing in RA despite showing promising effects in MS[154]. These studies may suggest that T cells do not contribute to the perpetuation of RA.

The articular disease

The synovial lining, which encompasses the joint cavity, consists of macrophage-like synovial cells and synovial lining fibroblasts, also known as fibroblast-like synoviocytes (FLS). This thin hypocellular layer, which has no epithelium or basement membrane, becomes hyperplastic in the inflamed joint. Hyperplasia triggers terminally differentiated macrophage-like synovial cells to produce cytokines, chemokines and growthfactors and to upregulate MHCII and co-stimulatory molecules. This, in turn, stimulates FLS to produce matrix metalloproteinases, which promote articular damage and cartilage destruction[155]. These activated FLS display a transformed phenotype. When isolated from RA, but not from osteoarthritis patients (OA), FLS demonstrate increased invasiveness after implantation along with human cartilage in SCID mice[156]. The FLS share other features with tumour cells, in particular the ability to grow in suspension[157] and the resistance to apoptosis. Importantly, these activated FLS can spread between joints and thereby transmit inflammation to unaffected tissue, which may explain the polyarthritic manifestation that is typical for RA. However, the disease might also emerge from the bone as a consequence of osteoclast activation leading to bone erosion and then spread to the joint to initiate an activation of FLS[158]. Inhibition of osteoclast activation may prevent bone destruction, although it has little or no effect on joint inflammation[159]. Moreover, the subcortical bone marrow is subjected to T cell and B cell infiltrates, suggesting that the bone marrow is an additional compartment in the disease process of RA[160].

Breaking tolerance

While the series of events that take place in the arthritic joint (i.e. overproduction of cytokines that sustain the chronic synovitis and infiltration of immune cells) are fairly well delineated, it is still unclear why T cell and B cell tolerance is breached in the first

place. The association to *PTPN22* suggests that the threshold for T cell activation may be affected. The associated variant (R620W) in *PTPN22* is a gain of function mutation that may promote negative selection of autoreactive T cell clones in the thymus[161]. The fact that *PTPN22* show a similar association in a number of autoimmune diseases further supports that this gene regulates T cells on a general level rather than acting in a disease specific manner[162]. It is not clear whether T cells in fact are primed within the synovial compartment. The synovial milieu contains certainly all cytokines necessary to activate T cells, such as such as IL-1, IL-6, IL-12, IL-23p19 and TGF-β, as well as both myeloid and plasmacytoid DCs necessary for the activation of naive T cells[163]. Once the cartilage damage is initiated, new epitopes provide a rich source of immune stimuli for T cell priming. However, it remains to be shown which autoantigens these are. T cell response to both cartilage (collagen type II) and ubiquitous protein antigens have been described in RA but to which extent these antigens are important for disease priming is not known[164-166].

Autoimmunity ensues when both central and peripheral tolerance mechanisms fail. Expression of tissue specific antigens in the thymic medullary epithelium ensures that T cells are properly tolerized when leaving the thymus. Those autoreactive T cells that escape negative selection are maintained in a non-responsive state by peripheral regulatory T cells.

Self-peptides that provoke an autoreactive T cell response may have either high or low affinity. High affinity is consistent with the finding that most, if not all, of the MHCII association in RA can be explained by a few key residues in the MHCII binding groove. These high-affinity peptides may be absent from the ligandome of thymic epithelial cells but exist in the periphery or emerge as consequent of tissue damage. Peptides that bind strongly to MHCII may also increase the risk of triggering low-affinity autoreactive T cells that have escaped negative selection.

Alternatively, since central tolerance to peptides that have been modified in the periphery might be poor or absent, such peptides may provoke T cell activation despite binding weakly to MHCII. Moreover, since these peptides are not part of the natural peptide repertoire, they may be recognized by high-affinity T cells in the periphery as truly foreign antigens. Compared to high affinity peptides, these peptides may be relatively common, at least in the tissue from which they are derived. One such tissue-specific peptide that is modified in its peripheral compartment is the immunodominant

T cell epitope on collagen type II (CII). This peptide is immunogenic in mice in its glycosylated but not in its non-glycosylated form. However, whether glycosylation is a post translational modification that occurs only in the periphery on collagen is not known, nor is it known whether CII at all is presented in the thymus, although the tolerance to non-glycosylated peptides would suggest so[165].

Th effector cells

RA has recently changed from being a prototypic Th1 disease to be more associated with a Th17 phenotype. This change is largely reflecting the pathogenic role of Th17 cells in rodents (discussed below), while there is still little support for either subset in human RA. IFN-γ, a signature cytokine for Th1, which is indispensable for clearing intracellular pathogens, is found in very small quantities in the RA synovial fluid, suggesting that IFN-γ may not be necessary for the activation of synovial macrophages[167]. Moreover, administration of recombinant IFN-γ to patients with RA does not significantly change the disease course[168]. However, the association to STAT4 in RA, which is a Th1 associated transcription factor, does implicate a role for Th1 cells in the disease. The first study on Th17 cells in RA was performed by Chabaud et al. in 1999. They demonstrated that synovial membrane tissue from RA, but not OA, produced large amounts of IL-17. They further showed expression of IL-17 in perivascular T cells, although they did not quantify Th17 cells directly[169]. Shen et al. measured Th17 and Th1 cells in the SF from patients and found ~1% Th17 cells and ~10% Th1 cells. They could further show that Th17 cells were increased in SF of patients and that the frequency of Th17 in the PBMC compartment correlated with CRP, an acute-phase protein that is increased in RA[170]. Church et al. compared Th1 and Th17 cells in the SF, synovial membrane and blood from patients and controls and found comparable levels of Th17 cells in SF and blood (~1-2%) and significantly more Th17 PBMCs in the blood of healthy controls compared to patients. However, they detected very few Th17 cells in the synovial membrane, which is inconsistent with Chabauds' study[171]. Finally, Yamada and colleges showed that the frequency of Th17 cells in the blood was similar in patients and controls and did not correlate with the DAS28 (a measure of disease activity in RA). In addition, they showed that the

frequency of Th17 cells was decreased in the SF compared to blood, while the frequency of Th1 cells was increased in the SF[172]. It is reasonable to assume that much of the reported discrepancy for Th1 and Th17 cells in RA pertains to antirheumatic therapy, in particular the use of TNF inhibitors and other biological drugs.

ANIMAL MODELS OF RA

Introduction to animal models

Animal models provide tools to identify disease mechanisms and targets for clinical interventions in humans. However, it is debated to which extent they manage to do so. Experimental models for RA have been criticized for not reflecting the diversity of human disease. These arguments have gained relevance in the post-genomic era when patients can be divided into disease subsets according to MHCII alleles and, more recently, anti-citrulline response. However, an experimental model needs to be reproducible and present a phenotype with reasonable frequency (i.e. the disease needs to be penetrant). The vast majority of models therefore use active immunizations with tissue-specific antigens in combination with aggressive adjuvants and, often, supplements of bacterial components. The stability is further reinforced by an extreme genetic homogeneity. Moreover, the microbiome of rodents, in particular in those that are born and raised in a clean environment, differ substantially from humans. Next to the genetic makeup, the microbiome is probably the most important conditioner of the immune system.

To combine information from different animal models can, at least partially, compensate for these flaws. In general, models that are independent on exogenous antigens (i.e. adjuvant models) and spontaneous models, such as the K/B×N and SKG models in the mouse[173-175], are to be preferred over models that are actively induced with tissue-antigens and strong adjuvants. Moreover, models that are not strictly strain dependent are more likely to capture more features of human autoimmunity and models that operate across species is of course more suitable than models that are species-specific. I discuss here three models in rats and mice; pristane-induced arthritis (PIA), PIA transfer and collagen-induced arthritis. The first model is an adjuvant model, which is free from exogenous antigens. The second model is induced by adjuvant primed and ex-vivo restimulated T cells while the third model is antigen induced.

Intraperitoneal injections of pristane induce lupus and arthritis in mice

Adjuvants containing mineral oils, mineral oils themselves and certain alkenes, such as pristane (2,6,10,14 tetramethylpentadecane) are suitable for induction of plasmacytomas (malignant plasma cells) in mice. These agents, which cannot be cleared efficiently by the immune system, induce chronic granulomatous reactions that stimulate tumour growth. The tumour inducing effect of these agents is dependent on IL-6, a cytokine that is found in high concentrations in the circulation of patients with RA[176]. Indeed, in addition to tumours, Potter and Wax reported in 1981 that repeated intraperitoneal injections of large volumes (0.5 mL or more) of pristane induced arthritis in Balb/c mice. The arthritis appeared several months after the first injection in ~60-80% of the mice but rarely spread beyond ankle and wrist joints[177]. Even if a relapsing disease was observed in a few mice, the arthritis was in general self-remitting. Histological analyses of affected joints in mice injected with pristane have shown neutrophilic infiltration, cartilage erosion and pannus formation[178] and serological analyses have shown increased and disease correlating levels of RF[179]. Pristane-induced arthritis in mice is associated with haplotypes $H2^q$, $H2^d$ and $H2^r$, as well as with non-MHC genes[179]. Injection of pristane may also induce the formation of ectopic lymphoid tissue and production of lupus antibodies[180-182] and the model has therefore been suitable for the study of lupus (SLE) in mice.

Intradermal injection of pristane induces chronic arthritis in rats

The rat model of pristane-induced arthritis (PIA) shares several features with the mouse model; however, in the rat protocol pristane is administrated intradermally as a single small dose (typically 100-200 µl) rather than intraperitoneally. The model was described for the first time in 1996[183], although at the time it was known that both complete and incomplete Freund's adjuvant (i.e. with and without *Mycobacterium tuberculosis*, respectively) induced arthritis in rats[184,185]. However, PIA, in contrast to these models, is a chronic relapsing disease, which makes it significantly more useful as a model for RA[186].

The DA and LEW strains are susceptible to PIA while the E3, ACI and F344 strains are resistant[187]. A large number of highly significant QTLs have been shown to be associated with arthritis onset, severity and chronicity in PIA, and many of these overlap with QTLs identified in collagen-induced arthritis[38,39,188-191]. However, with a few exceptions (*Pia4*, harbouring the *Ncf1* gene, being the most prominent[192]), these loci represent large chromosomal intervals with many plausible gene candidates. As discussed in a previous chapter, congenic mapping to position causative genes has been extremely laborious, in particular in regions with many genes and extensive LD. Hence, to which extent susceptibility genes in PIA overlap with identified loci in RA is still not known. An association to the MHC, similar to the one found in mouse PIA, has been described also in the rat[38]; however, an association with MHCII genes has so far not been published (*Study II*).

Immunization with pristane in DA rats leads to elevated levels of acute phase proteins, in particular α1-acid glycoprotein (AGP), as well as RF[193], IL-6[27] and IL-1β[194]. Both IL-6 and AGP levels decrease during the chronic phase of the disease. A correlation has been shown in RA for CRP and IL-6, which is one of several cytokines that can promote synthesis of acute phase proteins in the liver[195]. Elevated CRP levels is also a classification criteria for RA[196]. Extra-articular manifestations, such as splenomegaly, which have been observed in the mouse model, have not been reported in the rat, although ankylosis in the tail is frequently seen in DA but not in LEW rats[27]. Depletion of CD4 T cells prior to immunization effectively prevents development of PIA[183]. T cell dependency has also been shown in other adjuvant models[197] and in collagen-induced arthritis (CIA)[198]. However, while T cells do not contribute to the disease progression in CIA, they appear to do so in adjuvant arthritis (induced by complete Freund's adjuvant)[198] and, possibly, in PIA[183]. Neither depletion of CD8 T cells nor γδT cells influence the development of adjuvant arthritis[199,200], and at least CD8 depletion does not prevent arthritis development in DA rats immunized with pristane (Tuncel et al., *unpublished data*).

An MHCII restriction has been shown in PIA transfer for both RT1-D and RT1-B molecules[201]. However, these experiments were based on administration of whole antibodies and the effect on arthritis may not be limited to the blocking capacity of the antibodies. At least the RT1-D antibody has opsonizing properties and injection into rats leads to a substantial cell death of MHCII positive cells, and possibly also T cells[202]

(Tuncel et al. *unpublished data*). Hence, the MHCII restriction cannot easily be interpreted from these studies. However, we have now determined the MHCII dependency in the induced model using $F(ab')^2$ fragments of RT1-D and RT1-B specific antibodies (*Study II*).

It is possible that intradermal or subcutaneous injections of oil cause cell death in the draining lymph nodes where the oil accumulates[194,203]. Necrotic cells may certainly provide a rich source of antigens for T cell activation but it seems unlikely that those antigens would drive a tissue-specific immune response. However, release of DNA into the pericellular space may promote activation of APCs by nucleic acid-sensing toll-like receptors (TLRs). Nucleic acids may indeed promote activation of T cells[204]. Pristane may also be incorporated into the plasma membrane of APCs and other cells, leading to an unspecific immune activation and subsequent release of pro-inflammatory cytokines such as IL-6 and IL-1. Adjuvanticity has been shown to be strictly associated with the length of the hydrocarbon chain and appears to be strongest in hydrocarbons of intermediate length (C15-C20)[205]. Lipophilic hydrocarbons can disturb the integrity of membranes in microorganisms and increase the permeability for ions[206]. Indeed, an influx of ions, in particular Ca^{2+}, would have profound impact on cellular activation[207].

Innate responses appear to be essential for the induction of autoreactive T cells in PIA. Acute phase responses, which are induced by innate cells, are raised prior to disease onset and the levels of AGP in undifferentiated PIA correlate with disease progression and neutrophilia in immunized rats[208] (Tuncel et al. *unpublished data*). This suggests that IL-6 and possibly also IL-1β, which both increase upon pristane immunization, may be essential for the induction of T cells response, and at least IL-6 is also found in the murine PIA model. Pristane may also facilitate uptake of cartilage-derived antigens from the lymph; however, since pristane lacks the emulsifying capacity of Freund's adjutant, pristane cannot encapsulate antigens into micelles.

Whether exposure to mineral oils and other adjuvants is a risk factor in RA is still debated[209]. Squalene, which is a cholesterol precursor that is arthritogenic in DA rats, is a common constituent in cosmetics and in vaccine adjuvants and several cosmetic products induce arthritis when injected intradermally in DA rats[209-211]. Moreover, percutaneous (on the skin) applications of incomplete Freund's adjuvant has been shown to induce arthritis in DA rats, although only on abraded skin[212]. A Swedish case-control study showed that men suffer 30% increased relative risk of developing RA if

exposed to mineral oils[213], while mineral oils in skin products does not appear to confer risk to RA[209]. Moreover, a long-term follow-up study of Army recruits who received influenza virus vaccines emulsified in incomplete Freund's adjuvant (which is no longer FDA approved for use in humans) found no evidence for increased incidence of autoimmune disease[214].

PIA transfer

Pristane as well as incomplete and complete Freund's adjuvants can be used to prime T cells for passive and adoptive transfers. The manifestations of the transferred disease resemble in large the induced model; however, the transfer of pristane-primed cells does not induce a chronic disease, at least not in immunocompetent hosts. In general, passive transfer of cells from complete Freund's adjuvant-primed donors to normal immunocompetent rats is not possible, although at least one study has shown the opposite[215]. An exception is passive transfer of thoracic duct (TD) cells, which consists of highly activated cells and presumably a larger proportion of arthritogenic T cells[216]. In addition, arthritis can be transferred passively from CFA primed rats to thymectomized and irradiated hosts[217], while irradiated, non-thymectomized rats do not develop arthritis when transferred passively with pristane-primed cells (Tuncel and Holmberg, *unpublished data*). A standard protocol has been developed for adoptive PIA transfer, in which pristane-primed T cells are stimulated *ex-vivo* with Concanavalin A (ConA) for 65 hrs before intravenous transfer into naive syngenic hosts (Tuncel et al., *manuscript in preparation*). We and others have previously reported optimal conditions for priming and re-stimulation in PIA and adjuvant transfer models[201,217]; however, a standardized protocol for PIA transfer has so far not been published. At least in our hands, the severity and duration of arthritis are dependent on a number of aspects, of which the age of the recipient, the extension of the priming, the route of the injection and the source of the cells are the most important, whereas the number of transferred cells, at least when injecting intravenously, has little impact on the severity (Tuncel et al., *unpublished data*).

Collagen Induced Arthritis

Collagen-induced arthritis is a classic model of RA and although first reported in rats[218], it is more commonly used in mice[219]. The rat model of CIA is typically induced with autologous rat CII, although other articular collagens, such collagen type XI, can also induce arthritis[220,221]. Mycobacterial supplements in the adjuvant or booster injections are not needed in the rat model but they are typically necessary in the murine model. Mice are in addition fairly resistant to immunization with autologous CII and the T cell response in mouse CIA is therefore directed to a heterologous antigen, while the antibody response is directed to both the inoculated and the endogenous collagen. The manifestations in the joints are similar between CIA and the oil-induced models, with the major infiltrating cells being neutrophils and CD4 T cells and, to a less extent, CD8 T cells[185]. However, while PIA and other adjuvant models preferentially affect larger joints, such as the ankle, CIA targets more the smaller joints in the digits. Mouse CIA lacks several distinctive features of RA, such as RF, ACPAs and a female preponderance[222], and the disease course is typically more monophasic than relapsing-remitting as in PIA. The arthritogenic response is predominantly, if not entirely, directed to collagen type II and even if CII reactive antibodies are present in patients with RA[220], their pathogenic contribution is still elusive. Nevertheless, autoantibodies appear to play an important role in RA pathology, which in particular treatments with rituximab has shown, and CIA therefore fulfils an important role as an antibody driven model.

T cell polarization in experimental arthritis

The discovery of IL-23 by Oppmann and colleges in 2000 [223] challenged the prevailing perception that autoimmune disease was caused by Th1 cells. The new culprit was the Th17 cell and its signature cytokine IL-17. In the end of 1990s, several studies had shown that IFN-γ or IFN-γ receptor knockout mice developed more severe arthritis than their wildtype littermates. The absence of IFN-γ was postulated to promote IL-4 dependent Th2 immunity and, hence, production of autoantibodies[224,225]. However, many of the findings at the time were inconsistent. Boissier, for example, showed that

IFN-γ neutralizing antibodies could prevent the development of CIA in DBA/1 mice when administrated during the priming phase, while the same treatment had the opposite effect when performed on established disease[226]. Yet other studies showed that repeated injections of recombinant IL-12, a cytokine released by macrophages and DCs to stimulate the production of IFN-γ, delayed the onset of CIA in the same strain[227].

Oppmanns' finding of IL-23 clarified much of this discrepancy. When searching sequence databases for members of the IL-6 family, they identified a novel cytokine they named p19. This cytokine was found to be distantly related to the p35 subunit of IL-12. The p19 cytokine itself had no biological activity but it combined with the p40 subunit of IL-12 to form a composite cytokine. They termed this composite cytokine IL-23 and could show that it activated STAT4. It further had a strong effect on memory T cells in both mice and humans. Hence, since most of the IFN-γ deficient mice were mutated in the p40 subunit, the resulting models were deficient in both IFN-γ and IL-23. Targeting the p40, p35 and p19 subunits independently showed that IL-23 rather than IL-12 was essential for the induction of autoimmunity in several models[228,229]. In arthritis, mice lacking only IL-12 (p35) were shown to develop more CIA while mice lacking only IL-23 (p19) were completely protected[230]. It was further shown that the resistance to CIA in the p19 knock-out mice correlated with an absence of IL-17-producing CD4 T cells, while the induction of collagen-reactive Th1 cells was not affected. Hence, these data provided a link between IL-23 and the expansion of Th17 cells in autoimmunity. Shortly after Oppmanns' study, Lubberts and van den Berg published that blocking of IL-17 suppressed CIA in DBA/1 mice[231]. Th17 driven autoimmunity has since been described for a number of experimental models, such as the K/B×N cell transfer model[232] and the zymozan- or β-glucan-induced SKG model[233].

IL-23 is a key cytokine for a sustained Th17 response but it is not necessary for its induction. A number of independent studies in 2006 showed that two pleiotropic cytokines, IL-6 and TGF-β, were essential for the induction of Th17 cells in mice[234-236] and humans[237]. In the absence of IL-6, polarization of Th17 cells is indeed defective and T cells instead succumb to a regulatory phenotype (manifested by the expression of the FoxP3 transcription factor). The inhibition of autoimmunity in mice deficient for IL-6 appears to be dependent on the expansion of regulatory T cells, and upon depletion of FoxP3+ cells, IL-6 knock out mice regained their susceptibility. Moreover, another cytokine, IL-21, can induce Th17 differentiation in the absence of IL-6[238].

Not all experimental arthritis models are mediated through Th17. The proteoglycan-induced arthritis model (PGIA) has been shown to be independent on IL-17, and Th differentiation in most, if not all, of the rat adjuvant models are still poorly characterized. We have shown that IFN-γ is important for the induction of arthritis in the PIA transfer model, while the role of Th17 cells in this model has still not been characterized[201]. However, we have now addressed the dependency of IFN-γ and IL-17 in the PIA model (*Study II*), and we are currently testing the requirement for IL-17 in the transfer model.

PRESENT INVESTIGATIONS

STUDY I

The major histocompatibility complex (MHC) is a major genetic determinant of thymic selection. However, studies in humans and mice have largely focused on the association to the classical MHC class I and II genes, while the influence of non-MHC genes is still unclear. In this study, we used an outbred cohort derived from 8 inbred rat strains to map variations in CD4 and CD8 T cell numbers and MHC class I and II expression. Blood from ~1400 adult rats was analyzed by flow cytometry. Ten quantitative trait loci (QTLs) reached genome-wide significance (-logP < 4.3); 4 controlled CD4:CD8 ratio, 3 surface expression of MHC class I and 3 surface expression of MHC class II. Three QTLs (one for each phenotype) were identified on chromosome 20 and spanned the MHC region. The confidence intervals of these QTLs were determined to 4.1-9.7 megabases (Mb). To narrow down the QTL regions, we produced a panel of recombinant MHC-congenic strains (RCS). This panel was derived from four inbred MHC-congenic strains that shared a common genetic background (DA); DA.1I, DA.1F, DA.1H and DA.1U. We used a conventional intercross breeding to obtain crossovers in the MHC. Progenies were screened using 67 short-tandem repeat (STR) and SNP markers, which revealed a total of 70 recombinations. These recombinations were located within 5 distinct intervals, which largely overlapped with recombination hotspots in the human MHC (*HLA-DM*, *BTNL2* and *LTA*). By congenic mapping, we could show that two minimal QTLs, a 0.282 Mb interval in the classical MHCIa region (T cell selection QTL 1, *Tcs1*) and a 0.206 Mb interval in the MHCII region (*Tcs2*), were responsible for the QTLs identified in the HS rats. *Tcs1* was associated with variation in T cell numbers and MHC class I expression, whereas *Tcs2* controlled T cell numbers, and both MHC class I and II expression. We showed further that these QTLs controlled the selection of T cells in the thymus rather than the expansion of CD4 and CD8 T cells in the periphery. *Tcs2* contained 12 coding genes that were Sanger sequenced in the five parental strains to identify genetic variants associated with the mapped phenotypes. Polymorphisms in a gene encoding the ER transporter, *Tap2*, were found to be responsible for the variation in CD8 T cell numbers and MHC class I expression. We

conclude that surprisingly few regions in the genome (we found only one) control both MHC expression and T cells selection. The MHC region is an exception, in which two distinct sub-regions contribute to a significant overlap in phenotypes. These phenotypes are not solely regulated by classical MHC genes as the regulation by *Tap2* illustrates.

STUDY II

In this study, we followed up *Study I* and analyzed the effect of *Tcs1* and *Tcs2* in pristane-induced arthritis (PIA). We show that *Tcs2*, a 206 kb chromosomal interval in the MHCII region, is associated with disease onset and severity at the acute stage of arthritis. However, neither incidence nor the severity of chronic arthritis was associated with MHCII. In contrast, genetic variation in the MHCI locus (*Tcs1*) did not affect the susceptibility to PIA. The arthritis was particularly severe in rats with $RT1^a$ (DA) and $RT1^f$ (DA.1FR9) haplotypes. These strains encode identical RT1-D genes (orthologues to HLA-DR), which suggested that RT1-D restricted T cells are important in PIA. However, antibodies that block RT1-D did not prevent arthritis when injected at the priming stage in PIA, while antibodies to RT1-B (HLA-DQ) did. The importance of RT1-B was further supported by the expression of this molecule on the cell surface of pristane primed APCs. While RT1-B was highly expressed on the cell surface of DCs, RT1-D instead accumulated intracellularly and was relatively weakly expressed on DCs. To identify which MHC class II-bound antigens are important for priming CD4 T cells in PIA, we analyzed the peptide ligandome of RT1-B and RT1-D molecules. We observed qualitative and quantitative differences in the peptide ligandome between the strains and between the MHC molecules. Peptides derived from ubiquitously expressed auto-antigens described in RA were identified; however, none of the identified peptides were specific to cartilage proteins, nor did they elicit a T cell response when added to pristane primed T cells ex-vivo. The *Tcs2* interval regulates T cell selection as shown previously but neither frequency nor absolute numbers of CD4 and CD8 T cells correlated with arthritis susceptibility. However, the frequency of both CD4 and CD8 recent thymic emigrants (RTEs) was greatest in rats that were protected from severe arthritis. Hence, we could show a correlation between thymic activity and arthritis susceptibility that was determined by MHCII. Surprisingly, T cells expanded more in arthritis-protected strains than in non-protected strains upon pristane injection, which correlated with the frequency of RTEs in these strains. Pristane-primed T cells in the least susceptible strain, DA.1HR61, contained less Th1 cells but more Th17 cells. This shift in Th subsets is likely to explain the arthritis protection in DA.1HR61 since neutralization of IFN-γ suppressed disease when administrated during disease priming, while neutralization of IL-17 had no effect. However, the role of IFN-γ and IL-17 was

the opposite in established disease: while neutralization of IFN-γ was inefficient, neutralization of IL-17 was very effective in reducing inflammation, possibly by inhibiting recruitment of neutrophils to the joints. In conclusion, this study positionsa 206 kb region in the MHCII in an adjuvant model and shows that MHCII genes confer risk to acute but not chronic arthritis.

STUDY III

Collagen type XI (CXI) is a heterotrimeric collagen and a minor constituent of cartilage. The alpha 3 polypeptide (α3) in CXI is encoded by *COL2A1*, which also codes for α1 of collagen type II (CII). Albeit identical amino acid sequences, α3(XI) and α1(II) differ in glycosylation pattern, which could influence T cell recognition. We show in this paper that MHC congenic DA.1F rats immunized with the non-antigenic mineral oil pristane develop T cell and antibody responses to CXI. The reactivity to CXI was confined to the chronic phase of the disease and specific for the $RT1^f$ haplotype. We separated the alpha constituents of CXI by HPLC and could show that the T cell response was specific for the α1 and α2 polypeptides, while no T cell response was detected for α3. Thus, post-translational modifications of α3(XI) do not explain CXI immunity in pristane-induced arthritis. We further used DA.1F recombinant MHC congenic strains from *Study I* to map CXI immunity to an interval of approximately 0.2 Mb in the MHC-II region (*Tcs2*). Sequencing of the coding regions of the MHC class II genes (RT1-B and RT1-D) in DA.1F and DA showed that immunity to CXI is associated with RT1-B. Finally, we show that patients with early stage RA display increased serum reactivity to CXI, which is distinct from the response to CII.

STUDY IV

This is the first paper describing the adoptive transfer of pristane-induced arthritis (PIA transfer). We show in this paper that T cells can be adoptively transferred from pristane-primed donor rats to naive recipient rats after re-stimulation in vitro with Concanavalin A (ConA) or different T cell receptor (TCR) Vα and Vβ specific monoclonal antibodies. We determined the conditions necessary for T cells to become arthritogenic, which included dose of pristane, priming period and re-stimulation protocol. We show that PIA transfer is a mild disease compared to the induced model while the arthritis onset is earlier in the transfer model (day 5 in PIA transfer versus day 10 in PIA). In addition, we show that the transfer model lacks the characteristic relapsing-remitting disease course typical for PIA and that the rats recover completely from arthritis 3-4 weeks post T cell transfer. We characterized the cellular compositions in synovia and blood of PIA rats and compared them to the blood of naive rats. This showed that highly activated T cells (CD54+, CD25+ and MHC-II+) in pristane primed rats migrated to the joints. When stimulated in vitro with ConA, these cells produced high levels of TNF-α and IFN-γ, and anti-TNF-α (etanercept) and anti-IFN-γ (DB-1) pre-treatment of recipient rats suppressed development of PIA transfer. We further determined MHC restriction in the model using MHC-congenic rats. We found that T cells transferred to allogenic, irradiated, recipients were unable to induce arthritis whereas T cells transferred to semi-allogenic or syngenic recipients induced arthritis. We finally showed that pre-treatment of recipient rats with anti-MHC-II monoclonal antibodies could suppress the disease induction, supporting that PIA is dependent on MHC class II restricted CD4+ T cells.

CONCLUDING REMARKS

The work presented in this thesis aims to increase the understanding for the fundamental processes that concern antigen presentation, T cell selection and autoimmunity in the rat. Three of the included papers and manuscripts stem from the generation of a panel consisting of a large number of MHC recombinant inbred strains. Using a model that is essentially unique to the rat, we have shown that MHCII genes influence the onset, severity and progression of autoimmune disease, which has never been shown before. We could also show that T cells in the same model react upon challenge with the minor cartilage component collagen type XI and provided evidence for a similar function of this antigen in human RA. In addition, we showed a number of specific QTLs controlling CD4 and CD8 T cell numbers and provided evidence on gene level for an interaction between MHCI and MHCII genes. Finally, we described a new model for experimental arthritis in which the effector function of autoreactive T cells can be investigated.

In summary, we have provided a physical set of minimal MHC fragments that positions a large number of immune related phenotypes of importance in humans. The panel of congenic strains that was established will remain as an important resource for further studies on the genetic variation in the MHC.

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