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CHAPTER 11

General discussion
ANTIGEN PRESENTATION

Expression of HLA class II molecules

HLA class II molecules are well-known for their role in the adaptive immune system. Particularly, these molecules are involved in presenting peptide-ligands that can be epitopes for CD4+ T cells. The expression of HLA class II molecules is restricted to professional antigen-presenting cells (e.g. macrophages, B cells and dendritic cells) and to specialized cells in the thymus involved in T cell education. In inflammation, these cells can increase their HLA-class II expression under the influence of IFN-gamma and IFN-gamma can also induce the expression of HLA-class II molecules by non-professional cells[1]. In Chapter 2, we now describe for the first time HLA class II expression by mast cells, which is sufficient to activate CD4+ memory T cells. The expression of HLA-class II molecules is largely influenced by the presence of IFN-gamma. Interestingly, we also show that mast cells can internalize antigen and process antigen to T cell epitopes. This is particular interesting because mast cells are located at strategic locations throughout the body (e.g. the skin and the gut) and can therefore be one of the first cells that present antigens to nearby memory CD4+ T cells.

Presentation of ligands by HLA class II molecules

Since the early 90s ligands have been eluted from different HLA class II molecules[2-7]. These studies have provided numerous information on the nature of HLA class II ligands and have clearly demonstrated that these ligands are very different from HLA class I ligands, both in their origin, the pathway of antigen-processing, as well as in length and in restriction. Also, these studies have provided insights in how structural difference between different HLA class II alleles are reflected in distinct peptide-binding repertoires. In Chapter 3 and 4, we describe two distinct HLA-class II molecules: HLA-DQB1*06:03 and HLA-DRB1*03:01 and correlate identified ligands to functional characteristics of the peptide-binding pockets.

In the last 25 years, there has been an enormous development in the instruments capable of measuring HLA-ligands. Whereas in the 90s, the number of ligands isolated from a single HLA class II molecules were generally below a hundred, in Chapter 3, we isolated over 13.000 ligands from a single HLA class II molecule, HLA-DRB1*03:01. This is the largest set of identified ligands that have ever been described and allowed us to perform analyses that were previously not possible. These analyses have provided novel insights into the nature of HLA class II ligands. For instance, we have shown that HLA class II ligands are not only conserved in amino acid positions involved in interacting with the HLA molecules peptide binding pockets, but also in N- and C-terminal residues projecting outside the HLA class II molecule.
TOWARDS A MODEL FOR RA RISK.

Rheumatoid arthritis patients are a highly heterogeneous population[8]. Interestingly, ACPA autoantibodies can subdivide RA patients in two distinct disease subsets, ACPA-negative and ACPA-positive RA patients. These patient subsets strongly differ with regard to important disease outcome measures (disease progression, chance for remission and radiographic damage), and risk factors (both genetic and environmental) (discussed in Chapter 5)[9-14].

The HLA class II locus is the most important risk factor for the development of RA, but the associated haplotypes differ between ACPA-positive and ACPA-negative RA (discussed in Chapter 6)[9, 11, 15, 16]. In Caucasians, HLA-DRB1*01/10:DQ5- and HLA-DRB1*04:DQ7/DQ8-encoding haplotypes are strongly overrepresented in ACPA-positive RA patients compared to healthy controls or ACPA-negative RA patients[17-19]. In addition to predisposing haplotypes, the HLA-DRB1*13:DQ6 haplotypes associates with protection from ACPA-positive RA[14, 20, 21].

Only a small percentage of donors carrying risk haplotypes will develop ACPA-positive RA. This shows that their presence is not sufficient for disease development and that other factors are required and these findings suggest that the development of ACPA+ RA involves multiple hits. This is further supported by reports demonstrating that ACPA-positivity can precede the onset of RA by up to ten years and that different environmental/genetic risk factors underlie the development of ACPA-positivity and the transition to ACPA-positive RA (Chapter 7)[22, 23].

Positivity for risk haplotypes is not required for the development of ACPA-positive RA as about 15% of patients are negative for these haplotypes. These patients are homozygous for a wide variety of other HLA-DR subtypes. The difference in the relative distribution of HLA-DR genotypes between ACPA+ RA patients and healthy controls (generally presented as odds ratios) could therefore best be explained by the sum of the risk to undergo the multiple hits required for disease development. This sum could be influenced by different factors (Figure 1):

1. Hit number. Etiological pathway in individuals positive for high-risk haplotypes possibly require less hits (or less rare hits) (Figure 1A).
2. Hit risk: Etiological pathways in those positive for high-risk haplotypes might require more prevalent hits (Figure 1B).
3. Multiple pathways: Individuals positive for high-risk haplotypes might develop ACPA-positive RA via more different routes (Figure 1C).

The distribution of HLA-DR haplotypes in ACPA-positive RA patients is most likely explained by a combination of the three factors (Figure 1D).

TOWARDS A MOLECULAR BASIS

For this thesis we aimed to further elucidate the molecular basis of RA with a specific focus on ACPA-positive RA.
Initial loss of B cell tolerance.

ACPA autoantibodies can be detected in the serum of healthy donors up to ten years before disease onset without any clinical signs of arthritis[23]. Interestingly, the ACPA-response of healthy donors is characterized by low levels, little cross-reactivity and low isotype usage[24]. Recently, it became increasingly clear that the HLA locus is playing only a minor role in the development of ACPA-autoantibodies as it was shown for both predisposing and protective HLA alleles that these do not associate with the presence of ACPA in healthy donors (Chapter 7)[22]. In contrast these HLA alleles associate more with the transition from ACPA-positive to ACPA-positive disease (Chapter 7). This indicates that the initial development of ACPA is triggered by HLA-independent risk factors. These independent risk factors could be genetic factors that shape the threshold for B cell activation (e.g. PTPN22) or perhaps factors that increase “citrulline load”, e.g. polymorphisms in the PADI4 gene, anti-PAD antibodies that enhance PAD function, chronic infection with PAD-containing bacteria or environmental factors like smoking and silica exposure that promote citrullination in the lungs[13, 22, 25-28].

Loss of T cell tolerance

The transition from ACPA-positivity to ACPA-positive RA is characterized by strong changes in the ACPA-response, including a rise in ACPA-levels, isotype-expansion and a more diverse fine-specificity profile[24]. These changes in the ACPA-profile are likely the result of germinal center formation that are induced by T cell dependent B cell activation[29, 30]. A role for T cells during this phase is further
supported by the observation that the HLA class II molecules associated with RA are mainly involved during this particular phase. In this thesis, three different potential pathways were studied (Chapter 8-10) that are summarized in Figure 2. Interestingly, the molecular basis for all these pathways is different. The studied pathways will now be shortly summarized.

**Molecular mimicry**

In Chapter 8, we provide a first molecular basis for the role of both predisposing and protective HLA-alleles in ACPA-positive RA. As discussed, HLA-DRB1*13-positive donors are protected. HLA-DRB1*13 is particularly interesting as it was also shown to protect in the presence of shared epitope alleles indicating that HLA-DRB1*13 could act in the same biological pathway as risk alleles, possibly by preventing a particular hit to occur[14, 31]. In Chapter 7, we studied the protective role of HLA-DRB1*13 during different phases of disease development. We could show that HLA-DRB1*13 does not protect from ACPA-positivity, only from ACPA-positive disease. These data indicate that this allele exerts (part of) its effect in the time-frame between ACPA-positivity and the development of ACPA+ RA. In Chapter 8, we provide an explanation for the protective effect HLA-DRB1*13 alleles in the presence of SE alleles. We could show that predisposing HLA-DQ molecules (those in

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**Figure 2:** Summary of the studied molecular bases for the association between HLA, RA and citrullination
LD with SE-alleles) have an exceptional capacity to present a group of peptides with the core sequence DERAA that are derived from microbes, vinculin and from HLA-DRB1*13. Our data indicate a role for vinculin directed T cells, primed by pathogens in providing help to ACPA-producing B cells and a role for HLA-DRB1*13 in tolerizing this T cell population. Together this pathway explains part of the risk mediated by risk and protective haplotypes. Thus, this elegant pathway can explain a large part of the risk of SE-alleles and the protective effect of HLA-DRB1*13 alleles.

**Enhanced HLA-affinity**

It was previously shown for “Caucasian” SE alleles that these can present citrullinated peptides with an enhanced affinity over their native counterpart[32-38]. In Chapter 10, we show that this capacity is not specific for SE alleles as it can also be found for several predisposing and non-predisposing HLA-DQ molecules, thereby providing a further refinement of the association between HLA-SE alleles, RA and citrullination.

**Enhanced TCR-avidity**

In Chapter 9, we show that not all HLA-SE alleles can present citrullinated epitopes with an enhanced HLA-affinity. SE-allele HLA-DRB1*14:02, frequent in indigenous North Americans, has no enhanced affinity for citrullinated epitopes over their arginine counterpart. However, we could demonstrate that HLA-DRB1*14:02 presents citrulline residues in an orientation different from arginine residues, which could be a target of citrulline-directed T cells, thereby providing an alternative molecular basis for this HLA-SE allele.

**CONCLUSION**

In recent years, novel insights have been provided into the mechanisms underlying the association between the HLA system, RA and citrullination. Further elucidating the molecular basis for these associations will likely contribute to a better understanding of disease pathogenesis. The window between ACPA-positivity and disease development offers a unique opportunity for preventive treatment. As the HLA system plays a key role in disease development, elucidating the molecular basis for the association between HLA, ACPA and RA will be of pivotal importance. Excitingly, the data presented in this thesis together with other recent data, have provided novel insights into this long-known association.

**REFERENCES**

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