On the connection between HLA and rheumatoid arthritis

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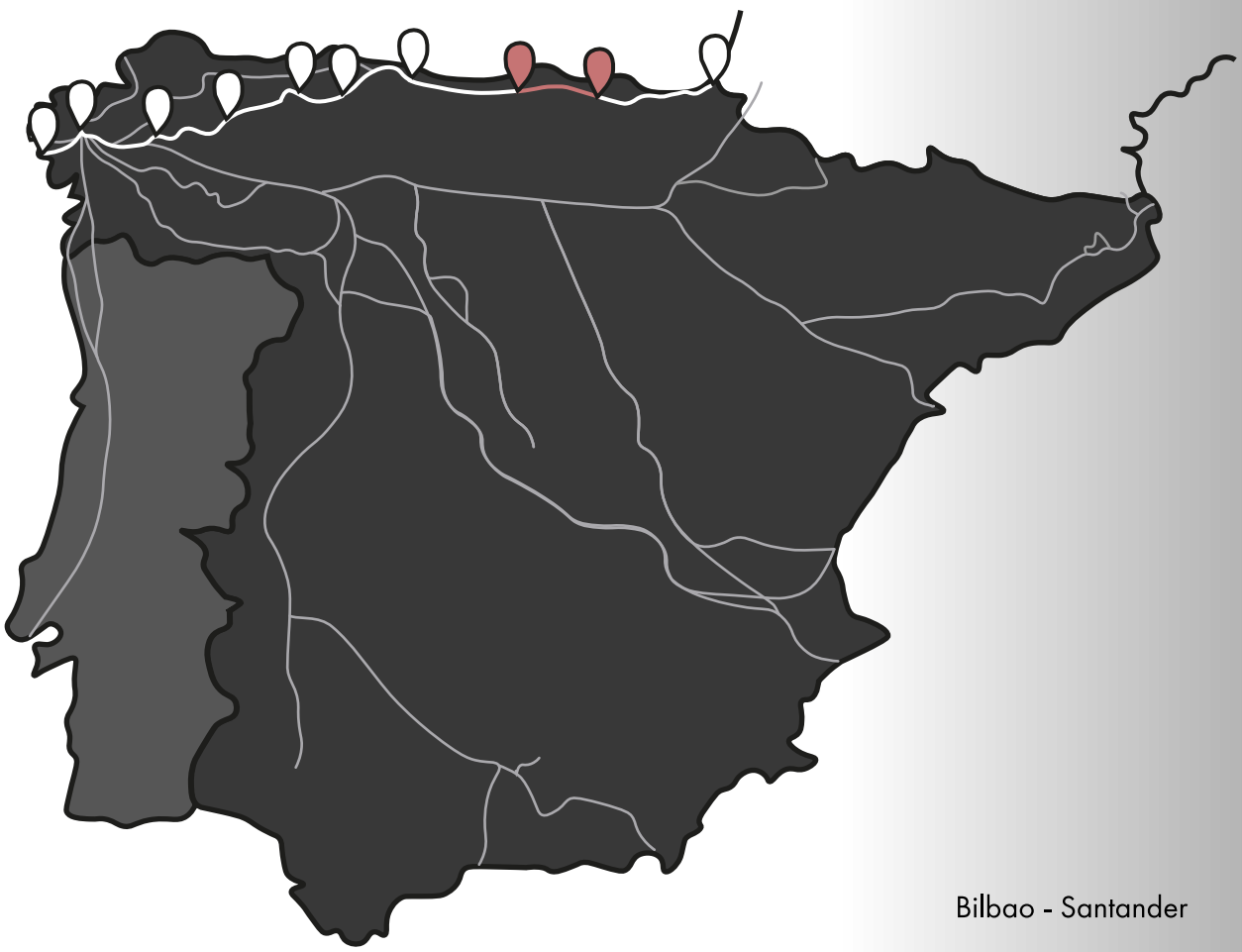

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Chapter 2

HLA class II and RA: the bumpy road of revelation

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease primarily targeting the joints. Approximately 1% of the population is affected by RA and, despite the improvements in therapeutic interventions, elucidation of the disease pathogenesis is still in its infancy. RA patients can be subdivided on basis of the presence of autoantibodies, especially Anti-Citrullinated Protein Antibodies (ACPA). ACPA$^+$ and ACPA$^-$ disease most likely differ in etiology, as different genetic and environmental risk factors are associated with these two disease entities. For ACPA$^+$ RA disease, the genetic factors associating with disease mainly comprised of Human Leukocyte Antigen (HLA) class II molecules. The predisposing HLA-DR alleles have been depicted as the "HLA Shared Epitope (SE) alleles", as these alleles encode a similar sequence, the Shared Epitope-sequence, within the beta chain of the HLA-DR molecule. In addition to the involvement of the HLA-SE alleles in the development of ACPA$^+$ RA disease, other HLA-DR molecules have been shown to confer protection against this disease entity. The protective HLA molecules have, instead of the SE motif, a different but shared sequence at the same location in the beta chain of HLA-DR molecules, consisting of the amino acid residues DERAA. The possible contributions of the predisposing and protective HLA molecules in association with ACPA-positive RA are discussed in this review.

Introduction

Rheumatoid Arthritis (RA) is a chronic autoinflammatory disease that primarily affects the joints. Worldwide the prevalence ranges between 0.5%-1% with a few populational exceptions and a higher occurrence in females than in males(1). One of the first humoral markers to be identified within the RA patient population has been the presence of rheumatoid factor (RF), an antibody that targets the Fc region of immunoglobulin G (IgG) molecules(2). However, this specific antibody is also present in a variety of other diseases. Another antibody response with higher specificity for RA was shown to target citrullinated proteins. Citrullination is a post-translational modification converting peptidylarginine residues into peptidylcitrulline involving the peptidylarginine deiminase (PAD) enzyme. A consequence of this modification is the reduction of the net-positive charge of the protein. Not all RA patients produce these antibodies, making a distinction between the RA populations that do or do not harbour Anti-Citrullinated Protein Antibodies (ACPA). ACPA positivity has been shown to be a predictive marker for the development of RA in patients with joint complaints(3) and patients harbouring ACPAs experience a more aggressive disease progression than patients without ACPA(4). This distinction between these two disease entities was further emphasised by differences in underlying genetic risk factors and environmental risk factors, pointing towards a different etiology and pathophysiology between $ACPA^+$ and $ACPA^-$ RA disease.

HLA associations with RA

In several twin studies, the heritability of RA was estimated to be approximately 60%, pointing towards a substantial influence of genetic risk factors on the development of RA disease(5). Recent Genome Wide Association Studies (GWAS) have identified 101 Single Nucleotide Polymorphism (SNPs) in total, showing the highest contribution of the *hla-drb1* gene to the development of RA(6-9). *Hla-drb1*-encoded proteins are components of Human Leukocyte Antigen-DR (HLA-DR) molecules and together with HLA-DQ and HLA-DP, they represent the major determinants in the induction of adaptive immune responses. They are expressed, amongst others, by antigen-presenting cells (APCs) and are able to present peptides to $CD4^+$ T cells. In the 1970s, *HLA-Dw4* was shown to be present in the majority of the RA patients(10) which was confirmed by serological HLA-typing identifying HLA-DR4 and HLA-DR1 in association with RA. Nowadays, the list of HLA alleles conferring increased risk for RA development are largely known (listed in table 1) albeit with altered nomenclature. The predisposing HLA-DR alleles were found to have a particular sequence in common, located in the beta chain (HLA-DRB1) at positions 70-74(11). This has later become known as the Shared Epitope sequence and as such, the HLA-DR alleles carrying this particular sequence were designated as "Shared Epitope alleles" (SE alleles). In 2005, it was discovered that the genetic contribution of the HLA locus did not apply to RA as such, but rather to ACPA-positive RA only(12). These data are important as they indicate that ACPA-positive and ACPA-negative RA represent different disease entities with a different underlying pathophysiology. More recently, positions 11 and 13 (table 1), which are also part of the

Table 1: HLA class II alleles and their associated risk to ACPA+ RA disease development

HLA-DRB1	Amino acid position HLA-DRB1								SE	Risk Association	
Allele	11	13	67	70	71	72	73	74	sequence	Susceptible*	Protective*
*01:01	L	F	L	Q	R	R	A	A	✓	✓	
*01:02	L	F	L	Q	R	R	A	A	✓	✓	
*01:03	L	F	I	D	E	R	A	A			✓
*03:01	S	S	L	Q	K	R	G	R			
*04:01	V	H	L	Q	K	R	A	A	✓	✓	
*04:02	V	H	I	D	E	R	A	A			
*04:03	V	H	L	Q	R	R	A	E			
*04:04	V	H	L	Q	R	R	A	A	✓	✓	
*04:05	V	H	L	Q	R	R	A	A	✓	✓	
*04:07	V	H	L	Q	R	R	A	E			
*04:08	V	H	L	Q	R	R	A	A	✓	✓	
*07	G	Y	I	D	R	R	G	Q			✓
*08	S	G	F	D	E	R	A	L			
*09:01	D	F	F	R	R	R	A	E		✓	
*10:01	V	F	L	R	R	R	A	A	✓	✓	
*11:01	S	S	F	D	R	R	A	A			
*11:02	S	S	I	D	E	R	A	A			
*11:03	S	S	F	D	E	R	A	A			
*12	S	G	I	D	R	R	A	A			
*13:01	S	S	I	D	E	R	A	A			✓
*13:02	S	S	I	D	E	R	A	A			✓
*13:03	S	S	I	D	K	R	A	A			
*14:01	S	S	L	Q	R	R	A	E			
*14:02	S	S	L	Q	R	R	A	A	✓	✓	
*15	P	R	I	Q	A	R	A	A			

* Risk association is based on the meta-analyses from de Vries et al. 2002; van der Woude et al. 2010a; Willkens et al. 1991

‡ The amino acids located at the given positions are based on Bondinas et al. 2007

Table 1: Association of HLA-DRB1 alleles with ACPA⁺ RA disease

Depicted are the residues located at various positions for a diversity of HLA-DRB1 alleles. Not all known HLA alleles are shown here. The presence of the SE sequence is indicated with a tick, as well as the association with predisposition or protection against ACPA⁺ RA disease for each allele.

peptide-binding groove, have been implicated in the association between HLA and RA(13). However, as these positions are the most polymorphic in the HLA region, these 2 positions most likely represent the best proxy for the predisposing HLA molecules explaining their association with RA in statistical terms.

Shared epitope alleles and RA

Epidemiology

The contribution of HLA alleles to RA development has been extensively studied by means of meta-analyses in different populations, including the Asians, Caucasians and native Americans. These studies show that especially the HLA-SE alleles increase the risk of developing RA in every population, although there are discrepancies in the degree of contribution of each allele when comparing populations(14-16). The presence of combined SE alleles within individuals increases the risk of

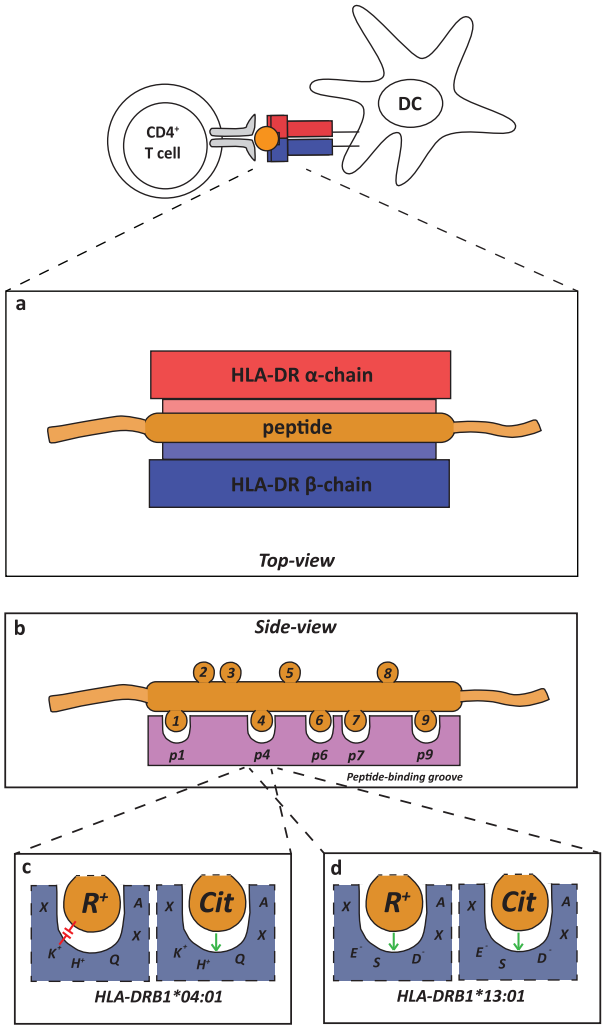

Figure 1: Schematic representation of a peptide binding to a HLA-DR molecule

a) Top view of the peptide binding groove. The peptide-binding groove is formed by the alpha and beta chain, leaving, forming two walls to accommodate the peptide. b) Side-view of the peptide-binding groove without distinction between the two HLA-DR chains. The peptide-binding pockets shown are anchoring points for residues of the peptide. The other residues are available for T-cell receptor recognition. c) Representation of how the Shared Epitope sequence can influence the binding of residues. Due to the presence of positively charged residues within pocket 4, arginine may be repelled and citrulline may be accepted. X represents any amino acid involved in formation of pocket 4. Position of residues within peptide-binding pocket 4 does not represent the actual position. d) Representation of how the DERAA-sequence can influence the binding of residues. Due to the presence of negatively charged residues within pocket 4, both arginine and citrulline may be accepted. X represents any amino acid involved in formation of pocket 4. Position of residues within peptide-binding pocket 4 does not represent the actual position.

developing ACPA+ RA even more(17). Interestingly, even though the SE alleles confer the highest risk, some non-SE alleles have also been identified as predisposing. For example, HLA-DRB1*09:01 is not considered a Shared Epitope allele by a small difference in the 5 residues located at positions 70-74(18), however, in meta-analyses the presence of HLA-DRB1*09:01 has shown an increase in odds ratio(15, 16). This notion indicates that the involvement of HLA class II molecules may be dependent on more than just the Shared Epitope sequence.

Citrulline presentation by Shared Epitope alleles

HLA class II molecules consist of 2 protein chains, the alpha and beta chain (figure 1a), both encoded by different genes. The combination of the two chains together forms a molecule with a peptide-

binding groove for the presentation of peptides (figure 1a). This peptide-binding groove consists of 4-5 peptide-binding pockets, accommodating different residues of the bound peptide ligand (figure 1b). The amino acids involved in the formation of these pockets are key determinants in restricting the peptide repertoire that can be accommodated. Even though peptides of variable lengths can bind to HLA class II molecules, the core sequence of these peptides consists of 9 amino acids. Of these 9 amino acids, 4-5 residues will get enclosed by the HLA molecule, in peptide-binding pockets 1, 4, 6, (7) and 9, whereas the remainder is available for T-Cell Receptor (TCR) recognition (figure 1b).

The previously mentioned Shared Epitope sequence either consists of the residues ^{70}QKRAA^{74}, ^{70}QRRAA^{74} or ^{70}RRRAA^{74}. Three (residue 70, 71 and 74) out of the five amino acids are involved in the shaping of peptide-binding pocket 4 of the HLA molecule. The presence of one or two positively charged residues (lysine, K; arginine, R) within the pocket alters the permissiveness of the binding of positively-charged residues at that position, enabling enhanced binding capacity of citrullinated peptides compared to the native peptide (figure 1c). To confirm this enhanced binding capacity, Hill *et al* performed peptide-binding assays showing that the HLA-SE molecules were capable of binding citrullinated peptides with higher affinity than their native counterparts(19). Indeed, subsequent HLA-DRB1*04:01/peptide crystal structures clearly showed that this HLA molecule exhibited an enhanced ability to accommodate citrulline, but not arginine, within peptide-binding pocket 4(20). However, recent evidence indicates that not only the Shared Epitope motif within peptide-binding pocket 4 contributes to citrulline binding, as pockets 7 and 9 are also capable of accommodating citrulline more efficiently. In addition, we have shown that HLA-DQ molecules, that are in tight linkage-disequilibrium (LD) with predisposing HLA-DR molecules (HLA-DQ8 and –DQ7, in LD with HLA-DR4), but also "neutral" HLA-DQ molecules (HLA-DQ2, in LD with HLA-DR3), are able to accommodate citrullinated peptides better as their non-modified counterparts. Moreover, they can do so in multiple pockets(21). Therefore, the association between SE alleles and the development of ACPA$^+$ RA disease may not be explained fully by enhanced binding of citrullinated peptides by these alleles as the interactions between citrullinated peptides and HLA molecules seem to be more complex. In addition, due to the tight LD between HLA-DR and –DQ molecules, it is complicated to define the precise contribution of these molecules to RA pathogenesis.

T-cell recognition of citrullinated peptides

In contrast to the increasing data on the ACPA response, including the ACPA-expressing B-cell response, knowledge on the nature of the T cells involved in RA is limited. As mentioned previously, the HLA class II molecules present peptides to activate and/or modulate the CD4$^+$ T-cell response. T cells can exert a diversity of functions including the promotion of the immune response by helping B cells. Recent studies have shown that the HLA class II locus is associated with established ACPA$^+$ disease, but, surprisingly, only to a limited extend with ACPA positivity in healthy subjects. Therefore,

it is likely that the CD4+ T helper cells restricted by the predisposing HLA molecules play a role in the development of ACPA+ disease rather than in the development of ACPA positivity. The contribution of this T-cell help may lie in the facilitation of the maturation of the antibody response by B cells, as relatively short before the onset of symptoms, the antibody response has been shown to expand in level, isotype usage and fine-specificity recognition profile(22-25). Despite the increasing knowledge on the nature of citrullinated antigens recognised by ACPA, knowledge on T-cell receptor specificity is still scarce. Next-generation sequencing has shown that there might be oligoclonal expansion of T-cell populations, nevertheless common TCR specificities were not identified among different RA patients(26). The difficulty in identifying the T-cell reaction involved in RA pathogenesis might be partially due to the cross-reactive nature of ACPA, being able to recognise many citrullinated proteins. Therefore, the antigens recognised by B cells *in vitro* are not necessarily the same antigens that are recognised by T cells *in vivo*, making it challenging to pinpoint a specific dominant T-cell response in RA patients. Moreover, because of the cross-reactive nature of ACPA, it is conceivable that the autoreactive ACPA-expressing B cells also react to citrullinated proteins from microbes, thereby allowing the recruitment of microbe-directed T cells, instead of autoreactive T cells, in the help provided to ACPA-producing B cells. Therefore, it has yet to be elucidated whether and to what extent T cells are autoreactive in nature. Despite these challenges, studies have shown the existence of T cells specific for citrullinated peptides in mice. For example, HLA-DR4 (HLA-DRB1*04:01) transgenic mice immunised with 2 naturally processed peptides derived from citrullinated vimentin, readily respond to these with the production of interferon-gamma. This response was both restricted to the citrullinated form of the peptide and to the presentation of the peptide in the context of HLA-DR(27, 28). In addition, multiple studies suggest that such citrullinated peptide-specific T cells can also be present in RA patients, although they are found in healthy controls as well(19, 20, 28).

HLA-DR13 and RA

Epidemiology

Since the identification of the HLA-SE alleles, the majority of studies have focused on the association between the presence of HLA-SE alleles and RA. Another facet of the HLA association with ACPA+ RA is found in the presence of protective HLA alleles. These protective HLA alleles have been categorised according to three classifications: the presence of the sequence "DERAA" at positions 70-74 of the beta chain, the presence of an aspartic acid at position 70 or the presence of an isoleucine at position 67 (Table 1). An extensive meta-analysis on the influence of multiple HLA alleles has shown that despite these categories, HLA-DRB1*13:01, and to a lesser extent HLA-DRB1*13:02, confers protection in ACPA+ RA but not ACPA- RA in four different populations(15, 29). In line with these observations, a different study has shown that HLA-DRB1*13 confers protection against ACPA+ RA rather than ACPA positivity(30). The mechanism how HLA molecules can protect against ACPA+ disease development is yet to be elucidated, nevertheless, several ideas have been postulated.

Citrulline presentation by protective HLA molecules

Despite the in-depth analyses of the association between HLA-DRB1*13 and ACPA+ RA disease, only limited data are available on the ability of HLA-DRB1*13, or other protection-associated HLA molecules, to bind citrullinated or native peptides. Nonetheless, in contrast to the predisposing HLA molecules, the HLA molecules associated with protection seem to be able to accommodate both citrullinated and native peptides with similar efficiency as reported using a model peptide from vimentin(19, 20)(figure 1d). Therefore, it was proposed that due to the increased binding affinity of citrulline for the predisposing HLA molecules, the number of peptide:HLA complexes are increased on the cell surface as opposed to the neutral or protective peptide:HLA complexes(19). As the level of peptides presented by protective HLA molecules is less likely to reach the threshold for T-cell activation, the so called 'biochemical margin of safety'(19, 31), it will not lead to T-cell activation or might even lead to induction of regulatory T cells(31, 32). In addition, peptides that bind with higher affinity to HLA molecules increase the lifespan of the peptide:HLA complex on the cell surface, increasing the opportunity of T cells to recognise the presented peptide(33). However, whether these features are related to the immunogenetic data remains a question that is yet to be elucidated.

HLA-DERAA presentation by thymic APCs

HLA molecules can present an array of peptides derived from endogenous proteins whereby the peptides presented by HLA class II molecules are often derived from proteins from the cell membrane(34). Therefore, it is no surprise that HLA class II molecules also present peptide derived from other HLA proteins(35). Given the protective effects associated with some HLA molecules, it has been proposed that the presentation of a specific epitope derived from HLA molecules protect against development of arthritis through the induction of regulatory T cells recognising such peptides. For example, it was proposed that the murine MHC molecule H-2Ebd is able to confer protection through the presentation of a peptide derived from its high variable domain 3 (HV3) by H-2Aq(36). In this case, it was hypothesised that protection would be mediated through the induction of anergic, regulatory T cells. Because presentation of peptides derived from MHC class II molecules by other MHC class II molecules could potentially lead to the formation of regulatory T-cell responses, it was proposed that T cells specific for the HV3 region are positively selected in the thymus and subsequently could contribute to immune regulation and inhibition of autoimmunity in mice and men(36). Indeed, it has been shown that a peptide derived from the protective HLA-DRB1*04:02, but not from HLA-DRB1*0401, can be presented by HLA-DQ8 and be recognised by HLA-DQ8-restricted T cells, supporting the notion that peptides from the HV3 region of HLA molecules can serve as T-cell targets(37).

HLA-DR13 and RA; how could they connect?

A possible biological explanation of the protective effects associated with HLA-DR13 was found in the presence of the sequence ^{70}DERAA^{74} encoded by HLA-DR13 and other protective HLA molecules(38).

Next to several HLA alleles associated with protection against ACPA$^+$ RA, the DERAA-sequence can also be found in a few other human self-proteins including vinculin (VCL). Vinculin is a component of the cytoskeleton, ubiquitously expressed in all cell lineages. It has been shown to be citrullinated within the inflamed joints of RA patients(39) and to be recognised by ACPA in its citrullinated form(38). Moreover, T-cell tolerance against vinculin had previously been reported not to be absolute(40) as under certain pathological conditions the presence of T cells recognising vinculin have been reported. Interestingly, comparing HLA-DRB1*13:01-positive and -negative individuals revealed that T-cell reactivity against "vinculin-DERAA" was readily detectable in many HLA-DR13-negative individuals, but absent in HLA-DR13-positive subjects. Moreover, when binding affinity of the "VCL-DERAA" peptide to different HLA molecules was examined, it was shown that the peptide was able to bind to all HLA-DQ molecules that are associated with ACPA-positive RA (i.e. HLA-DQ7, -DQ8 and -DQ5) as these molecules are in strong linkage disequilibrium with predisposing HLA-DR molecules. In contrast, the HLA molecules analysed that do not associate with arthritis, did not bind this peptide, suggesting a link between the ability to present this peptide and the predisposition to ACPA$^+$ RA. Interestingly, it was subsequently shown that a T-cell clone against the "VCL-DERAA" peptide also crossreacted to several "DERAA"-containing epitopes present in microbes. These peptides bound the predisposing HLA-DQ molecules as well and were recognised by a substantial number of HLA-DR4-DQ8/7.3-positive donors. However, such T cells remained undetectable in donors additionally expressing the protective HLA-DR13 molecule. Therefore, a possible mechanism explaining the presence of "VCL-DERAA"-specific T cells is found in the notion that a breach of tolerance is induced following exposure to a microbe-harboured protein that resembles the VCL-DERAA sequence. This could lead to the induction of cross-reactive T cells that recognise the autoantigen vinculin. Intriguingly, the DERAA-sequence is present in 66% of bacteria and 4% of viruses, indicating that everyone will be exposed to such microbes. Thus, taking both the ability of a VCL-DERAA-directed T cells to respond to "DERAA"-containing epitopes from microbes, and the apparent selective absence of reactivity of such T cells within HLA-DR13$^+$ individuals, a hypothesis describing the connection between HLA and RA has been proposed. In short, upon encounter and peripheral priming by microbial-derived DERAA-containing peptides, "DERAA"-directed T cells will be activated in individuals expressing HLA molecules that can present such peptides (i.e. the HLA-DQ molecules predisposing to RA). Some of these T cells show crossreactivity with VCL-DERAA and thereby allow the provision of help to ACPA-expressing B cells that recognise citrullinated vinculin (a target of ACPA that is also present in citrullinated form in the inflamed joint). In contrast, individuals carrying the protective HLA molecules will not be able to mount such T-cell responses as these T cells are hypothesised to be negatively selected in the thymus by presentation of the DERAAsequence derived from protective HLA molecules. Therefore, by virtue of the absence of such T cells, ACPA-expressing B cells recognising citrullinated vinculin will not receive T-cell help explaining the protective effects of HLA-DR13 to ACPA$^+$ RA(38).

Concluding remarks

The association between the HLA system and RA has been studied intensively in the past 40 years thereby addressing many aspects involved in the pathogenesis of RA. Even though many breakthroughs have been made, there is yet so much more to be elucidated, primarily within the context of which role T cells play during the development of ACPA$^+$ RA. The elucidation of the exact events underlying the development of ACPA$^+$ RA might provide relevant insights into the role of T cells and peptide presentation by HLA class II molecules and thereby the pathogenesis of this frequent autoimmune disease. It is likely that many aspects on the role of T cells in ACPA$^+$ disease will be clarified in the years to come, most likely unveiling several pathways the T cells employ to contribute to the arousal of ACPA$^+$ RA.

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