

Review

## Association of MHC and rheumatoid arthritis

### Association of RA with HLA-DR4: the role of repertoire selection

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#### Abstract

Most patients with rheumatoid arthritis (RA) express HLA-DR4, HLA-DR1 or HLA-DR10. These alleles share a common amino acid motif in their third hypervariable regions: the shared epitope. In normals and patients with RA, HLA-DR genes exert a major influence on the CD4  $\alpha\beta$  T-cell repertoire, as shown by studies of AV and BV gene usage and BV BJ gene usage by peripheral blood CD4  $\alpha\beta$  T cells. However, the rheumatoid T-cell repertoire is not entirely under HLA-DR influence, as demonstrated by discrepancies in VB JB gene usage between identical twins discordant for RA and by contraction of the CD4  $\alpha\beta$  T-cell repertoire in RA patients. Shared epitope positive HLA-DR alleles may shape the T-cell repertoire by presenting self peptides to CD4 T cells in the thymus. Peptides processed from HLA-DR molecules and encompassing the shared epitope may also be presented by HLA-DQ and select CD4  $\alpha\beta$  T cells in the thymus. Thus, shared epitope-positive alleles impose a frame on the T-cell repertoire. This predisposing frame is further modified (by unknown factors) to obtain the contracted rheumatoid repertoire.

**Keywords:** HLA-DR, rheumatoid arthritis, T cell repertoire

#### Introduction

Most patients with RA express particular HLA-DR alleles. These include HLA-DRB1\*0401, \*0404, \*0405 and \*0408 (identified by serology as HLA-DR4), HLA-DRB1\*0101 and \*0102 (HLA-DR1), and HLA-DRB1\*1001 (HLA-DR10). These alleles share a common structural motif in the third hypervariable region of their  $\beta_1$  chains. This motif is called the shared epitope [1]. Its amino acid sequence, using the one letter amino acid code, is QKRAA (glutamine, lysine, arginine, alanine, alanine) on HLA-DRB1\*0401, QRRAA on HLA-DRB1\*0404, \*0405, \*0408, \*0101 and \*0102, and RRRRAA on HLA-DRB1\*1001. The shared epitope is believed to contribute to the development of rheumatoid

arthritis. However, it is not known how. Two opposite hypotheses propose either that the shared epitope allows the presentation of target antigenic peptides to T lymphocytes or that the shared epitope helps select the T cell repertoire. In this review, we will focus on the influence of shared epitope positive HLA-DR alleles on CD4 $\alpha\beta$  T cell repertoire selection.

#### Preliminary: how to study the T cell repertoire ?

It is known that  $\alpha\beta$  T cells use a highly polymorphic  $\alpha\beta$  surface receptor associated with a signal transducing unit to recognize antigenic peptides in association with class II major histocompatibility complex (MHC) molecules. Thus,

the  $\alpha\beta$  T cell repertoire could be characterized by analyzing  $\alpha\beta$  heterodimers on T cells or identifying recognized peptide–MHC complexes on antigen presenting cells. Unfortunately, most studies on the human repertoire have lacked this kind of accuracy, analyzing either T cell responses to proteins or peptides, or the expression of  $\alpha$  or  $\beta$  T cell receptor (TCR) genes/chains by CD4 T cells. In the future, the use of soluble peptide–MHC tetramers should allow a better view of  $\alpha\beta$  T cells and their targets. Still, these studies are purely descriptive. There is little information on the functional efficiency of the T cell repertoire, which is whether or not a given repertoire is efficient at controlling a particular bacteria or virus. This efficiency of the  $\alpha\beta$  T cell repertoire may also be influenced by HLA-DR.

### HLA-DR influence on the T cell repertoire in normals and RA patients

The variable regions of TCR  $\alpha$  and  $\beta$  chains are encoded by rearranged variable (V), diversity (D) (only in  $\beta$  chains), and joining (J) segments, with additional N diversity sequences [2\*\*]. Whereas identical BV, D or BJ genes can be used by many different T cell receptor  $\beta$  chains, the sequence of the VDJ junction that constitutes the third complementarity determining region is unique to a clone.

### HLA haplotypes determine AV and BV gene usage by peripheral blood T cells

AV and BV gene usage on peripheral blood  $\alpha\beta$  T lymphocytes was first analyzed by labeling T cells with monoclonal antibodies specific for particular V $\alpha$  or V $\beta$  families. This approach showed that HLA haplotypes exert a major influence on expression of AV and BV genes by peripheral blood T cells. In this analysis, HLA-identical siblings and twins had almost identical percentages of peripheral blood T cells expressing the analyzed V $\alpha$  and V $\beta$  chains [3]. Similar results were obtained using polymerase chain reaction technology: BV gene expression was identical in twins, even if they were discordant for RA [4]. These analyses contradict earlier work that suggested that the peripheral T cell repertoire in RA was characterized by expansion of T cells expressing a particular BV [5].

### BJ gene usage by T cells expressing particular BV genes: BV BJ gene usage

More precise TCR B gene expression analysis can be performed by identifying and quantifying, for each of the 24 BV gene families, the BJ genes to which they rearrange. This is performed by first amplifying cDNA from T lymphocytes with a BV family specific and a BC primer, then hybridizing the amplified material with a labeled probe specific for each of the 13 BJ families. This technique allows one to evaluate the percentage of genes from a given BV family, which is rearranged to each of the 13 BJ genes. This analysis confirmed the very strong influence of HLA-DR on the CD4-positive T cell JB gene repertoire in

the BV3, BV14, BV17, BV8 and BV5 families [6,7]. According to this analysis, the peripheral CD4 T cell repertoire from healthy shared epitope positive subjects was different from that of healthy shared epitope negative subjects. Finally, when identical twins discordant for RA were studied, their BV BJ repertoires appeared almost identical [8]. However, statistical analysis demonstrated that they were slightly different [9]. Similar results were obtained by Walser-Kuntz *et al* when comparing the repertoires of HLA-DR matched RA patients and controls [7]. Thus, HLA-DR molecules exert a very strong influence on BV BJ gene usage and may allow the emergence of a repertoire that will help development of RA. However, discrete differences exist between the CD4 T cell repertoires of RA patients and HLA matched controls. These differences cannot be attributed to genetic factors.

### Individual sequences

A further step in the analysis of the BPCR repertoire was taken by Wagner *et al* who studied the representation of individual, arbitrarily picked TCR  $\beta$  chains in the peripheral repertoire of RA patients and normals [10\*\*]. This was achieved by amplifying particular BV BJ combinations in cDNA prepared from different numbers of T cells and looking for a particular third complementarity determining region among the amplified rearranged genes by hybridization with a third complementarity determining region specific probe.

This analysis suggested that, in healthy individuals, the median frequency of individual TCR  $\beta$  chains was 1 in 24 million. In RA patients, however, this frequency was 10 times higher, which indicated a 'contraction' of the T cell repertoire, with less diversity and emergence of dominant clones. These findings were valid for both memory (CD45RO+) and naive (CD45RO-) CD4 T cells.

Thus, individual sequence analysis suggests that RA patients have a contracted peripheral  $\alpha\beta$  T cell repertoire and that this pattern is associated with rheumatoid arthritis [11]. This contraction is not observed in HLA matched controls, which indicates that at least part of this process is independent of HLA-DR.

### How could shared epitope positive HLA-DR molecules select the T cell repertoire?

$\alpha\beta$  T cells rearrange their TCR genes before undergoing positive and negative selection in the thymus. In positive selection, CD4 T cells are selected for affinity for self class II MHC and self peptides. In negative selection, CD4 T cells with high affinity for self class II MHC and self peptides are eliminated. HLA-DR molecules may control the binding of self peptides in the thymus, thus influencing the positive and negative selection processes. However, there is little information on the identity of the self peptides involved in  $\alpha\beta$  T cell selection, with exception for self peptides derived from self MHC molecules. Indeed, MHC molecules are

highly polymorphic, hence their processing can generate highly polymorphic peptides. MHC derived self peptides are well represented among peptides presented by MHC molecules and may be important in shaping the T cell repertoire in a MHC specific way. In early studies, development of a mouse model of autoimmunity was attempted by immunizing normal mice with peptides derived from the third hypervariable region of their E $\beta$  chains. To our surprise, it was observed that this polymorphic part of E $\beta$  was strongly tolerated [12]. We later made the same observation in normal humans expressing RA associated HLA-DRB1\*0401. In these subjects, peptides encompassing the shared epitope in the third hypervariable region of HLA-DRB1\*0401 did not trigger proliferation of peripheral blood T lymphocytes, even though they were presented by HLA-DQ. This suggested that peptides from the third hypervariable region of HLA-DRB1\*0401, presented by HLA-DQ, could be involved in negatively selecting the T cell repertoire [13\*,14\*]. The fact that this property was not observed for peptides from the same area of other HLA-DRB1 alleles is surprising. It may indicate that HLA-DRB1\*0401 has an original processing, perhaps related to an original intracellular route [15]. Of interest, the third hypervariable region of HLA-DRB1\*0401 has significant sequence homology with proteins from many infectious agents, like Epstein-Barr virus gp110 or *Escherichia coli* DnaJ [16,17]. In 1995, Albani *et al* demonstrated that synovial fluid T cells from patients with rheumatoid arthritis proliferate to peptides from DnaJ containing the QKRAA motif, and it was proposed that the third hypervariable region of HLA-DRB1\*0401 could be involved in positive selection of the  $\alpha\beta$  T cell repertoire [18]. Thus, peptides derived from the third hypervariable region of HLA-DRB1\*0401 may be involved both in positive and negative selection of the T cell repertoire. A direct demonstration of this hypothesis will be difficult to provide. However, a recent study by Bonnin *et al* in the mouse demonstrated that a peptide from the third hypervariable region of the E $\alpha$  chain could positively select T cells specific for a bacterial homologue of this peptide [19\*]. In short, HLA-DR molecules may shape the T cell repertoire by binding unknown peptides involved in thymic selection. Whereas the identity of these peptides is not known, it is likely that HLA-DR4 derived peptides are involved in this process.

### Is an HLA-DR4 shaped repertoire efficient at controlling pathogens?

HLA-DR genes associated with RA shape the T cell repertoire, as assessed by analysis of T cell receptors. Does this influence infection control? Some information in the field of herpes viruses suggests it does. The replication of herpes viruses is controlled in part by T cells specific for the highly conserved family of glycoproteins B. T cell responses to Epstein-Barr virus gp110 (the glycoprotein B of Epstein-Barr virus) and human cytomegalovirus glycoprotein B are lowest in normal

subjects expressing HLA-DR4 [20,21]. This may provide a clue to the origin of the herpes virus overload observed in patients with RA.

### Conclusion: HLA-DR is not enough to make a rheumatoid repertoire

HLA-DR molecules exert a tight control over the peripheral blood  $\alpha\beta$  T cell repertoire. This control may involve the intervention of HLA-DR derived peptides in the processes of positive and/or negative selection in the thymus. Still, the rheumatoid T cell repertoire is not entirely under HLA-DR influence, as demonstrated by discrepancies in VB JB usage between identical twins discordant for RA and by the contraction of the repertoire in RA patients. The shape imposed on the repertoire by HLA-DR probably constitutes a predisposing frame, which is further modified to obtain the contracted rheumatoid repertoire. The nature of the factor(s) that determine the final modeling of the HLA-DR preshaped repertoire is a mystery.

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