Immunogenetic and immunological aspects of rheumatoid arthritis : DERAA and anti-citrulline reactivity can make the difference

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Chapter 3

Protection against Rheumatoid Arthritis by HLA: Nature and Nurture

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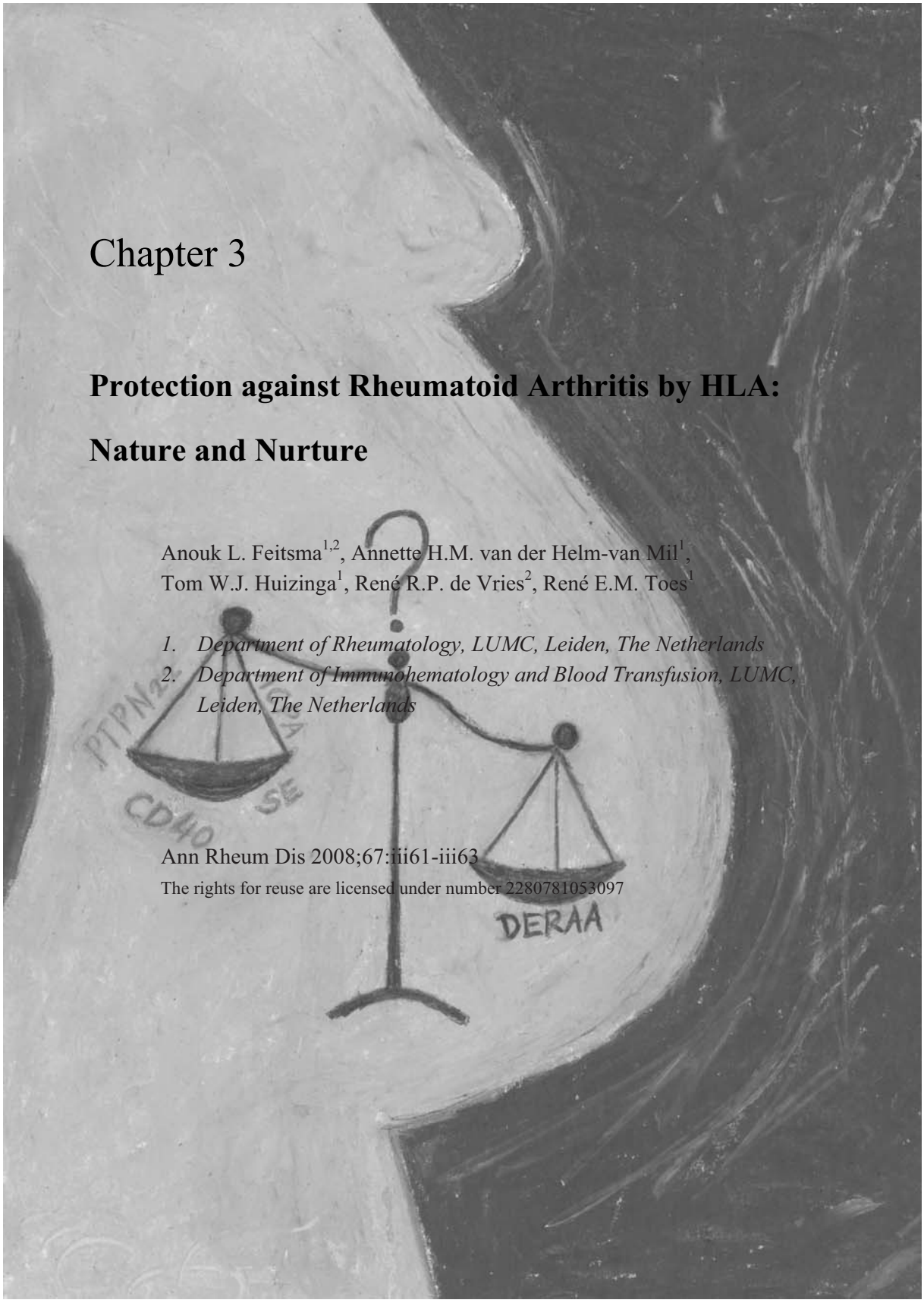

Ann Rheum Dis 2008;67:iii61-iii63

Abstract

The human HLA-region contributes most to the genetic risk for Rheumatoid arthritis (RA), either in a predisposing- or a protective fashion. HLA-DRB1-molecules containing the amino-acid sequence “DERAA” at position 70-74 of the DRbeta chain are less often present in RA-patients compared to controls. It has been proposed that antigens transmitted from the mother, but not genetically inherited (called NIMA), can influence RA-susceptibility. Confrontation of the fetal/newborn immune system with the NIMA is supposed to have a lifelong modulating impact on the immune response of the child and thus on the chance to develop RA. Up to now, no protective NIMAs were described in autoimmunity. Recently, we studied whether “DERAA”-containing HLA-DRB1-alleles as NIMA are associated with a protective effect. We showed a protective NIMA-effect in RA as “DERAA”-positive mothers -in contrast to “DERAA”-positive fathers- could transfer protection to their “DERAA”-negative child. The implications of this finding as well as possible explanations are discussed.

Summary

Rheumatoid arthritis (RA) is a complex genetic disorder in which the HLA-region contributes most to the genetic risk. HLA-DRB1-molecules containing the amino-acid sequence QKRAA/QRRAA/RRRAA (i.e. HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001 and *1402) at position 70 to 74 in the third hypervariable region of the DRB1 chain are associated with susceptibility to RA. HLA-DRB1 molecules containing the amino acids “DERAA” (i.e. HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302 and *1304) at the same position are associated with protection from RA.

Interestingly, not only inherited but also non-inherited HLA-antigens from the mother can influence RA-susceptibility. We have recently described a protective effect of “DERAA”-containing HLA-DRB1 alleles as non-inherited maternal antigen (NIMA).

The underlying mechanism of this protective effect is currently unknown, although a possible explanation is covered below. In this review, an overview of the current knowledge on protection against RA is given and the inherited and NIMA effect of “DERAA”-containing HLA-DRB1 alleles are compared.

HLA-DRB1 "DERAA"-positive alleles protect against RA

Rheumatoid arthritis (RA) is a complex genetic disorder in which the HLA-region contributes most to the genetic risk. Especially HLA-DRB1 molecules sharing a common epitope, R(Q)K(R)RAA, (i.e. the amino acids Arginine, (Glutamine), Lysine, (Arginine), Arginine, Alanine, Alanine) at position 70-74 in the third hypervariable region of the DRB1 chain, the so-called shared epitope (SE), are associated with both susceptibility to and severity of RA (1-4). The shared epitope is present in the HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001 and *1402 molecules. At the same position as the SE, the amino acids “DERAA” (i.e. the amino acids Aspartic acid, Glutamic acid, Arginine, Alanine, Alanine) can be present in other HLA-DRB1 molecules (i.e. HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302 and *1304). Individuals carrying HLA-DRB1 alleles that express this “DERAA”-sequence display a lower susceptibility to develop RA and suffer from less severe disease as compared to individuals with ‘neutral’ (SE- and “DERAA”-negative) HLA-DRB1 alleles. The odds ratio of individuals carrying HLA-DRB1 alleles that express the “DERAA”-sequence compared to individuals with “neutral” (SE- and “DERAA”-negative) HLA-DRB1 alleles to develop RA is 0.5-0.7, indicating that “DERAA”-

positive individuals have a lower susceptibility to develop RA (5-8). The protective effect associated with “DERAA” is also found after stratification for the presence or absence of HLA-SE alleles. This indicates that the protective effect associated with “DERAA”-expression cannot be explained by an overrepresenation of SE alleles in patients, resulting automatically in a lower frequency of other HLA alleles in RA patients. Thus, the “DERAA”-containing HLA-DRB1 alleles are independently associated with a reduced risk to develop RA (5).

It is unclear whether the entire “DERAA” motif is essential for the protection or that only certain amino acids of this motif confer the same effect. In contrast to several reports showing the protective effects by “DERAA”-containing HLA-DRB1 alleles to the development and severity of RA (5,9,10), other reports hypothesize that the amino acids “RAA” at position 72-74 in the third hypervariable region influence the susceptibility to RA development whereas the amino acids at position 70 and 71 modulate this effect (11,12). In these articles it is indicated that HLA alleles expressing the ^{70}ERAA^{74} sequence or the Aspartic acid (D) at position 70 both have a lower frequency in RA patients as compared to healthy controls. Further, it has also been described that protection is mainly associated with the Aspartic acid (D) at position 70 (8,13).

Thus, despite these differences in nomenclature and stratification, it is getting increasingly clear that some HLA alleles confer susceptibility, whereas others are associated with protection.

The mechanism of protection is unknown, but it has been proposed that it is mediated by T cells recognizing peptides containing the “DERAA”-sequence presented by HLA-DQ molecules (14). Whether these T cells have a regulatory phenotype or are deleted in the thymus by negative selection is still a subject of research.

Non-inherited "DERAA" from the mother also gives protection to the child for RA development

In 1954 Owen *et al.* described that Rhesus D (RhD) negative children were tolerant to the RhD antigen when they had a RhD positive mother, probably due to exposure to the RhD antigens during pregnancy (15). This was the first time that a biological effect of non-inherited maternal antigen (NIMA) was described. This terminology is exemplified in Figure 1. Confrontation of the fetal/newborn immune system with the NIMA may have a lifelong influence on the immune response of the child. This phenomenon has considerable implications for transplantation and most studies on

NIMA are coming from the transplantation field. Claas *et al.* described that renal transplant patients often do not generate antibodies against the mismatched HLA antigens of their mother in comparison to those of their father and are therefore tolerant for this HLA mismatch when they are transplanted. This is associated with a longer transplant survival (16-18). This is exemplified best in a study by Burlingham showing that haplo-identical NIMA-mismatched sibling transplants have a graft survival similar to that of HLA-identical siblings, whereas NIPA-mismatched sibling transplants did as poorly as did recipients of maternal and paternal grafts (19).

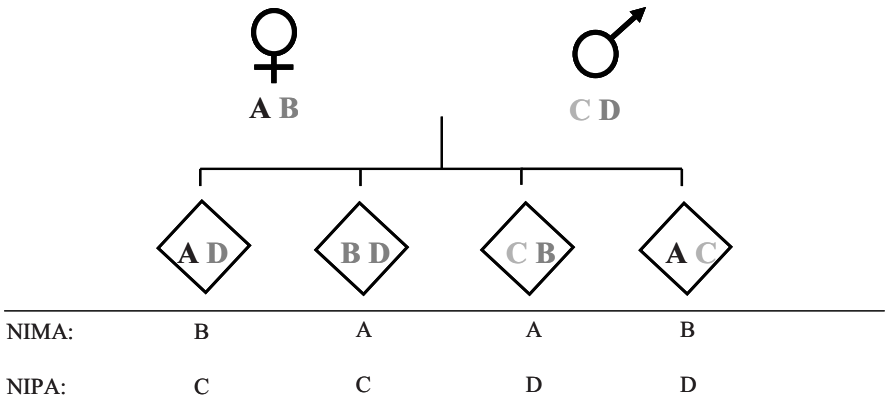

Figure 1. Terminology of non-inherited maternal antigen (NIMA) and non-inherited paternal antigen (NIPA). The terminology is orientated from the point of view of the child. ◊ gender can be male or female.

We have recently shown that there is also a protective effect on the development of RA of HLA-DRB1 molecules that contain the amino acid sequence "DERAA" when presented as NIMA on the development of RA (20). We anticipated that if non-inherited "DERAA"-containing HLA-DRB1 alleles of the mother protect the child to RA development, it is expected that the frequency of mothers of RA patients bearing a "DERAA"-containing HLA-DRB1 allele is lower compared to the general population. Indeed, using a cohort of Dutch RA patients together with their parents, we were able to show that the mothers of RA patients showed a significantly lower frequency (16.1%) of "DERAA"-containing HLA-DRB1 alleles compared to the Dutch control population (29.3%; $p = 0.02$). In contrast, the frequencies of "DERAA"-containing HLA-DRB1 alleles in the fathers of the RA patients (26.2%) and the individuals of the healthy control group were comparable. These findings were replicated in the English

multi-case families from Manchester. To further ascertain that the observed difference in frequency of "DERAA"-containing HLA-DRB1 alleles between mothers and fathers of RA patients could indeed be attributed to an effect of non-inherited HLA-antigens, the "DERAA"-positive families with a "DERAA"-negative child (the RA patient) were selected for further analysis. For this analysis, the patients from the UK and the Netherlands were pooled. The odds ratio (OR) for "DERAA"-negative RA patients of having a "DERAA"-positive mother compared to a "DERAA"-positive father was 0.25 (95% CI 0.09-0.65; p=0.003). These results together show that there is a protective effect of "DERAA"-containing HLA-DRB1 alleles as NIMA on development of RA of the child.

Table 1. Comparison of the inherited and NIMA effect of "DERAA"

	Mother	**Child**	**RA patients (n = 89)**	**Controls (n = 206)**
A	pos	pos	7	39
B	neg	pos	6	23
C	pos	neg	8	26
D	neg	neg	68	118

The Dutch families were used for this analysis [20]. Group B vs D is the inherited effect. OR = 0.45 (0.16-1.25); Group C vs D reflects the NIMA effect. OR = 0.53 (0.21-1.31). Pos = positive (hetero-zygote) for "DERAA"-containing HLA-DRB1 alleles. Neg = negative for "DERAA"-containing HLA-DRB1 alleles.

Thus, together these data indicate that both "DERAA"-containing HLA-DRB1 alleles inherited from one of the parents and the presence of "DERAA" containing HLA-DRB1 alleles as a NIMA protect against the development of rheumatoid arthritis. The question that arises from these observations is how the strengths of both effects compare to each other. To answer this question, both effects were compared in the same set of patient and control families. Only Dutch families (20) were included in this analysis for a proper comparison to the control families. The data depicted in table 1 indicate that, indeed, the effect of "DERAA"-containing HLA-DRB1 alleles as NIMA is as strong as the effect observed in case the "DERAA"-alleles are inherited directly from one of the parents. Although not significant (over 7000 families would be required to discriminate whether the inherited and non-inherited protection differ significantly or not), these data indicate that both effects are of the same magnitude.

The comparable effect size described here is similar as the observations made in the transplantation setting (19).

This result would be in line with the assumption that only very few cells can exert the protective effect. One of the few cell populations that can give rise to many different cell types and has a life long existence are the stem cells. During pregnancy the immune systems of mother and child are in close contact and trafficking of cells, antibodies and/or antigens can occur. Therefore the most plausible explanation for the observed NIMA effect of "DERAA"-containing HLA-DRB1 molecules is maternal microchimerism.
Moreover, because the NIMA effect is not taken into account in most studies analyzing the contribution of the HLA system to RA susceptibility, these data also indicate that the association would be even more prominent in case an effect of "DERAA" as NIMA would have been considered.

Microchimerism as a possible mechanism of the NIMA-effect

During pregnancy there is a bidirectional maternal-fetal lymphocytic transfer (21). Occurrence of these cells starts after about three months of gestation and persists till delivery (22). It is shown that the levels of fetal DNA in the circulation of the mother increase during these six months and disappear for the largest part after delivery (23). During pregnancy also cells of the mother migrate to the fetus and may induce lifelong microchimerism in the child (21,24,25). Maternal microchimerism has been shown in mice to induce neonatal B cell (26) and probably also T cell (27) tolerance and is therefore one of the possible mechanisms for NIMA effects (28) Although speculative, we postulate therefore that the protective effect of the DERAA-containing HLA-DRB1 alleles as NIMA on the development of RA is most probably mediated by maternal cells entering the bloodstream and tissues of the child which exert their effect through a change in the immune repertoire and most likely the T cell repertoire of the child. These maternal cells might influence thymic selection or act in the peripheral lymphoid organs, for example as a consequence of the sustained presence of cells from the mother in the child. It has been shown that maternal microchimeric cells can be present in many different cell subsets (29) in both healthy and diseased individuals (30,31) in which they may exert different effects (32,33). Likewise, immune regulatory mechanisms might directly be induced in the fetus as it has recently been described that

the fetus can already develop cytotoxic T cells directed at a maternal minor H antigen *in utero* (34) or becomes sensitized against foreign antigens to which the mother is exposed during pregnancy (35). Although the presence of maternal microchimerism is not rare, there are several reports that the amount of microchimerism influences the sensitivity of an individual to certain diseases (30,31,36,37).
The observation that the inherited and NIMA effect of "DERAA" have approximately similar effect size strengthens the idea that the NIMA effect is caused by lifelong circulating microchimeric cells that play a role in the thymic selection and therefore influence the T cell repertoire. Only when the inherited and the NIMA acquired "DERAA" have the same mechanism of induction of protection, the similar strength can be explained.

Overall, we can conclude from the data presented in this review that the presence of "DERAA"-containing HLA-DRB1 molecules can protect an individual against the development of rheumatoid arthritis. The "DERAA"-containing HLA-DRB1 molecules can either be present since the individual has inherited them directly or because the individual had a "DERAA"-positive mother and acquired some of the "DERAA"-containing HLA-DRB1 molecules during fetal and/or neonatal life. The protective effect that is acquired in either way is of similar strength, which suggests that already a low amount of cells can initiate this protective effect. Further research is required to elucidate the mechanism of protection of both the inherited as the NIMA effect of the "DERAA"-containing HLA-DRB1 molecules. Such research might be very rewarding as it could guide the way to the development of novel therapies initiating protection in a similar manner as provided by "DERAA"-positive mothers to their "DERAA"-negative children.

License statement

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