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How microchimerism can impart HLA susceptibility to rheumatoid arthritis

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Rheumatoid arthritis, a chronic inflammatory joint disease, is strongly associated with HLA-DRB1*01 and *04 alleles that have in common similar 5-amino acid motifs in the third hypervariable region of DRβ1 (QKRAA, QRRAA, RRRAA), the so called shared epitope (SE). Most patients with RA carry 1 or 2 doses of the SE, with particular genetic combinations at higher risk. In recent work we provided evidence that patients who lack HLA-DRB1*01 and/or *04 alleles can acquire RA susceptibility through fetal, maternal or iatrogenic microchimerism. We also discuss how Mc carrying HLA-DRB1*04 alleles is more likely to be present in the peripheral blood of RA patients compared to Mc carrying HLA-DRB1*01 alleles. We further analyze our results in light of the hierarchy for RA risk with different combinations of the SE. How Mc could contribute to RA susceptibility and whether it also contributes to the hierarchy of risk observed with particular combinations of SE-containing alleles is certainly the beginning of an intriguing story and may offer hope for future therapeutic and/or preventative interventions.

Key words: microchimerism, HLA, shared epitope, rheumatoid arthritis

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From early genetic studies to recent Genome Wide Association Studies, Human Leukocyte Antigen (HLA) genes are unequivocally the strongest genetic factor risk for Rheumatoid Arthritis (RA) and most autoimmune diseases. In RA, a similar five amino acid motif, located in the middle of one of the two alpha helices that delineate the peptide binding groove of HLA-DR molecules, referred to as the shared epitope (SE) is a strong risk factor for the disease.[1] This motif of the third hypervariable region of HLA DRβ1 chains is present on some HLA-DR1 and DR4 molecules. The RA-associated allele DRB1*0401 encodes the SE sequence $^{70}QKRAA^{74}$ and the DRB1*0101, *0404, *0405 and *0408 alleles encode the SE sequence $^{70}QRRAA^{74}$. Additionally in some populations, HLA-DRB1*1001 is overrepresented in RA patients and encodes the SE motif $^{70}RRRAA^{74}$. All the SE motifs above have in common a positive charge which might allow conditions to bind negatively charged peptides.

Although HLA genes contribute to RA risk in the majority of patients, there are still a non-negligible percentage of patients who are negative for the SE. This percentage varies from one cohort to another, but in general about 20% of patients do not carry the SE.

Interestingly a number of studies describe a gender disparity in genetic predisposition to autoimmune diseases.[2,3] Women with RA are less "genetically predisposed" than men, as men with RA have more often 2 doses of SE compared to women. Moreover McGregor et al. showed that men with RA have increased heterozygosity.[3] The HLA-DRB1*0401/*0404 genotype is associated with a 90-fold increased risk in men and this is more than doubled when age at disease onset is below 30.[3] Eberhardt et al. reported that RA patients who did not express disease associated HLA-DRB1 alleles developed RA later in life and postulated that without certain genetic factors more time is needed for clinical disease to develop.[4]

Therefore the question arises whether women could acquire genetic predisposition later in life. In the light of recent knowledge, that fetal and maternal cells commonly persist for decades after delivery in the mother and the child, creating respectively fetal and maternal microchimerism (Mc), women could acquire genetic susceptibility through Mc.[5,6]

In a recent publication in *Arthritis & Rheumatism*, we hypothesized that RA patients without the SE would have Mc with greater frequency and possibly at higher quantities than patients who already possess 1 or 2 doses of the SE.[7] We then designed HLA-specific quantitative PCR for HLA-DRB1*04 and HLA-DRB1*01 and tested women with RA and healthy women who lacked DRB1*04 and/or DRB1*01 alleles with the appropriate assay. To our surprise, SE-negative patients were not more microchimeric than SE positive patients (1 or 2 doses). However, there was a significant difference in frequency ($p = 0.00006$) as well as in quantities ($p = 0.0095$) of HLA-DRB1*01 and/or DRB1*04 Mc between SE-negative women with RA and SE-negative women without RA indicating a link between Mc and disease.[8]

But the most intriguing observation was that the quantity of DRB1*04 microchimerism reached higher levels than that of DRB1*01 microchimerism. Moreover, patients with HLA-DRB1*04 in their own genetic background were less prone to HLA-DRB1*01 Mc (3/17) than were HLA-DRB1*01 women for HLA-DRB1*04 Mc (11/33). Although these results were of borderline significance they merit further attention.

Several studies report the importance of the gene dosage of the SE in RA and a hierarchy of risk for RA with various SE positive HLA-DRB1 genotype combinations. Indeed having 2 doses of SE is at higher increase for RA risk and more severe articular damage, but all alleles encoding the SE are not similar in their strength of association with disease.[9] Interestingly, the strongest association with RA is not conferred by homozygosity such as HLA-DRB1*0401/*0401 (QKRAA/QKRAA) or HLA-DRB1*0101/*101 (QRRAA/QRRAA), but rather by heterozygosity, as HLA-DRB1*0401/*0101 or HLA-DRB1*0401/*0404 (QKRAA/QRRAA), known as compound heterozygosity.[10,11] Compound heterozygosity may be particularly associated with more severe disease.[12]

Our results illustrate differences in SE strength, as it is widely recognized that DRB1*04 is a stronger risk factor for RA than is DRB1*01.[9] They also illustrate that compound heterozygosity can be acquired through Mc. Individuals already genetically predisposed with HLA-DRB1*0101 alleles, could for example acquire later in life, through fetal Mc, HLA-DRB1*0401 susceptibility. This could indicate the importance of Mc as a mini-transfer of susceptibility in RA but could also refer to patient or donor HLA genotype influence. As our group previously described, women who had a child positive for HLA-DQA1*0501 had more often fetal Mc within their T lymphocytes.[13] Similarly, maternal Mc was more frequent in children with juvenile dermatomyositis (JDM), but also in their siblings when they were carrying HLA-DQA1*0501, the disease-susceptibility allele.[14] We then suggested, in 2000, that the greater magnitude of association when the child had HLA-DQA1*0501 suggests that patients with autoimmune diseases who lack disease specific HLA molecules could be investigated for persistent microchimerism as an alternative source of HLA disease-associated molecules or peptides.

This hypothesis is now confirmed in RA, but we still do not know whether this is because microchimeric cells with a specific genotype are better retained by individuals with particular genotypes. The limit in our Quantitative PCR assays is that we could not test the highest risk compared to other genotype combinations, which is DR4-positive compound heterozygotes.[10] In other words, whether Mc could contribute to the genetic combination at highest risk for RA: HLA-DRB1*0401^{host}/*0404^{Mc} or HLA-DRB1*0401^{Mc}/*0404^{host}. Further analyses are needed to understand in detail how Mc can impart HLA susceptibility to RA.

We also don't yet know what factors influence persistence of detectable Mc. We recently found that maternal Mc present among mononuclear cells from two patients with systemic sclerosis (SSc) disappeared under cyclophosphamide treatment (unpublished). It is, however, difficult to correctly analyze drug effects on Mc levels while also recognizing levels fluctuate over time and patients have different treatments and different environmental exposures.

Thus our data points to the importance of further investigation of Mc in healthy individuals in order to understand what factors lead to Mc becoming detrimental in a particular genetic background, remaining neutral or in other instances even beneficial, with the hope that this knowledge might lead to future therapies capable of reversing Mc destiny.

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