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The Rheumatoid Arthritis *HLA-DRB1* Shared Epitope

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Abstract

Purpose of review—To update progress made between December 2008 and November 2009 on the role of the rheumatoid arthritis (RA) shared epitope (SE) in the etiology and pathogenesis of RA.

Recent findings—New evidence has been recently presented to suggest that non-inherited HLA antigens originating through pregnancy or exposure to maternal antigens in utero could contribute to RA development in SE-negative women. An interaction between smoking and SE-coding non-**04 HLA-DRB1* alleles (particularly *HLA-DRB1*01* and *HLA-DRB1*10*) was formally established for the first time. Progress has been made in determining the relative contributions and the interaction of the SE, *PTPN22* and smoking in conferring the risk of anti-citrullinated protein antibodies (ACPA)-positive and –negative RA. The autoantigen which ACPA recognize in a significant number of RA patients has been identified as citrullinated α enolase and the importance of genetic factors in ACPA-negative RA has been highlighted. Additionally, associations of RA risk with several new genetic markers have been reported. Among them: 2 new MHC, non-*DRB1*loci, a polymorphism marker in MHC class I polypeptide-related sequence A (MICA), an allele of the Fcγ receptor; a polymorphism marker in the β2 adrenergic receptor and a low-inducible allele of the cytochrome P450 subtype 1A2.

Summary—While the mechanistic basis of SE-RA association remain an enigma, observations made during the last year shed new light on the conditions in which the SE - alone or in combination with other genes or environmental factors - affects the risk of RA and the phenotype of the disease.

Keywords

HLA-DRB1 alleles; gene-environment interaction; anti-citrullinated protein antibodies

Introduction

HLA-DRB1 alleles that code a “shared epitope” (SE) – a five amino acid sequence motif in residues 70–74 of the HLA-DRβ chain - are associated with severe rheumatoid arthritis (RA, references 1–4). The better-known SE-coding alleles include members of the *HLA-DRB1*04* allele group (for example, **0401, *0404, *0405, *0408*), *HLA-DRB1*0101* or **0102*, *HLA-DRB1*1402* and *HLA-DRB1*1001*. Additional alleles are listed in Table 1. The purpose of this review is to discuss some publications in the past 12 months that could help advance our understanding the role of the SE in RA.

The Shared Epitope Hypothesis – Work in Progress

The mechanism underlying SE-RA association is unclear. The common hypotheses attribute it to presentation of arthritogenic antigens (5), or T cell repertoire selection (6). However, it should be pointed out that data supporting antigen-specific responses as the primary event in

RA are inconclusive. Additionally, several non-RA human diseases (7–12), and experimental animal models of autoimmunity (13–16) have been shown to associate with SE-coding alleles as well. Furthermore, the antigen-presentation hypothesis is difficult to reconcile with SE allele-dose effects on RA penetrance and disease severity (discussed in 17).

Unlike the uncertain mechanism, the association itself has remained largely unchallenged for more than two decades. One notable refinement of the proposed SE motif, however, relates to position 70 of the DRβ chain. In addition to what has been long considered, current evidence suggests that while glutamine or arginine in position 70, are indeed critical for RA risk, aspartic acid in that position confers protection (18). To further define structure-phenotype relationships on the DRβ 70–74 region, Carrier and associates (19*) have recently studied the effect of the DERAA sequence (residues 70–74 encoded by several *HLA-DRB1* alleles, including the RA-protective *HLA-DRB*0402* allele) on disease outcomes in patients with early arthritis. Their data showed that in patients without early erosions, DERAA-coding *DRB1* alleles were strongly protective against severe disease at 30 months. Thus, in addition to prior indications that aspartic acid in position 70 may reduce RA risk, it now appears that it also may reduce disease severity. How this effect is consistent with the antigen presentation theory remains a mystery.

It is estimated that up to 20% of RA patients are SE-negative. The possibility that non-inherited maternal HLA antigens (NIMA) could explain RA development in these individuals has been examined previously but yielded inconclusive results. In 2009, however, two studies provided new evidence that could support a role for non-inherited HLA antigens. Examining a large cohort of families from the North American Rheumatoid Arthritis Consortium, Guthrie *et al.* (20**) have found a significant association between RA risk and HLA-DR4-coding NIMA in women less than 45 years of age, but not in older women, or men. Along the same lines, a European group has recently demonstrated that compared with healthy women, RA female patients had a higher frequency and higher levels of *HLA-DRB1*04* microchimerism (21**). The prevalence of non-inherited genes in larger patient populations and their pathogenic mechanism are unknown.

The SE and Citrullinated Proteins

Genetic factors, with the SE being the strongest among them, play a significant role in RA susceptibility. Intriguingly, despite the major role of genetic factors in RA susceptibility, the concordance rate of this disease in monozygotic twins is only 15% (22). It has been therefore hypothesized that while RA susceptibility is determined genetically, disease onset may depend on non-genetic (*i.e.* environmental), epigenetic or posttranslational events.

Deimination of arginine to citrulline by peptidyl arginine deiminase (PADI) is one of the better-characterized posttranslational protein modifications in RA. Several citrullinated proteins have been identified in the disease (23–25). However, the significance of citrullination remains unclear, because deiminated proteins have also been found in target tissues of non-RA inflammatory diseases as well as non-inflammatory conditions (Reviewed in 26).

Over the past few years, anti-citrullinated protein antibodies (ACPA) have been convincingly shown to be specific serological markers for RA, particularly in SE-positive patients. These observations have lead many to propose that ACPA may be playing a direct pathogenic role in RA. Experimental data to support this hypothesis have been reported in some arthritis models (*e.g.* 27,28). However, other animal studies have failed to demonstrate direct involvement of anti-citrullinated proteins immunity in experimental arthritis (29–31).

In human RA, likewise, there is presently no conclusive evidence to directly implicate ACPA as an effector mechanism in disease pathogenesis.

Relevant to the focus of this review, ACPA are found in SE-positive, but rarely in SE-negative RA patients (32). Furthermore, a SE gene-dose effect on RA risk has been documented in ACPA-positive RA patients (33). Based on these findings and a reported high-affinity interaction between citrullinated peptides and SE-positive HLA-DR molecules (34), it has been hypothesized that the association between SE and ACPA represents SE-restricted presentation of citrullinated antigen(s). It should be noted however that: 1. The identities of such putative antigens remain unknown; 2. There are no RA-relevant functional data to substantiate this hypothesis to date; 3. A recent study has shown that antibodies against PADI4 are also highly-specific for RA (35), raising the possibility that antigenically diverse targets within the PADI pathway are peculiarly immunogenic and arguing against antigen-specific effector role of ACPA; 4. Recognition of a sequence-independent post-translational modification, rather than a *bona fide* antigenic epitope, is inconsistent with what is widely considered MHC-restricted antigen specific recognition; 5. As discussed above, the antigen presentation paradigm is inconsistent with the promiscuous SE-disease association, and the observed SE gene-dose effects on disease susceptibility, penetrance and severity (reviewed in 17).

The co-segregation of ACPA-positivity and SE-coding alleles among RA patients has continued to attract research attention in 2009. Several studies focusing on the genetic basis of ACPA-positivity or -negativity have been published. For example, one study (36**) quantified the contribution of genetic risk factors, particularly the SE, to ACPA-positive versus – negative RA by computing their heritability in 148 RA twin pairs in which at least 1 twin had the disease. Comparable heritability rates were found in the ACPA-positive and – negative RA subsets. However, the presence of SE explained 18% of the genetic variance in ACPA-positive individuals, but it contributed only 2.4% of the variance in the ACPA-negative group. The authors concluded that while genetic predisposition plays a significant role in susceptibility to both types of RA, the development of an ACPA-negative disease depends on as yet unidentified non-SE genetic factors.

The identity of putative genetic modulators of ACPA positivity in RA has been reported by Lundström *et al.* (37**). In that study, a case-control analysis of 1,352 RA patients and 922 controls revealed that *DRB1*13* has a dual role: It protects against ACPA-positive RA, but in combination with *DRB1*03* it increase the risk of ACPA-negative disease. Partial corroboration of the putative role of *DRB1*03* was obtained in a recent study that carried out a genome-wide association analysis of ACPA titers in RA (38). That study confirmed that *HLA* is the most important gene region for ACPA in RA. Further, the two top single nucleotide polymorphism (SNP) markers, which associated inversely with ACPA titer, were found to be in moderate linkage disequilibrium with *DRB1*03* alleles, which have been previously found to associate with low ACPA titers (39).

SE-environment Interaction

As discussed above, it is widely believed that cooperation between genetic and environmental factors is required for RA disease onset. Consistent with this model, a gene-environment interaction between the SE and smoking has been reported by several groups prior to 2009 (40,41). For example, a highly significant interaction between the SE and smoking in RA risk has been reported by a Swedish group, particularly in SE homozygous individuals (40). Similar conclusions have been reached by a more recent Danish study (41). Different from the North European studies, early reports on SE-smoking interaction in US populations had been inconclusive. However, in early 2009, a very large US study (42**)

suggested that similar interactions exist in North Americans as well. Studying over 60,000 subjects, the authors have demonstrated strong gene-environment interaction between the SE and smoking when stratifying by pack-years of smoking rather than by ever smoking. This US study shows that the extent of the exposure is important and corroborates the role of SE-smoking interactions previously observed only in North European patient populations. Thus, there is compelling evidence for interaction between the SE and tobacco exposure in the etiology of RA in different geographic locations across the globe.

Previously reported interactions between the SE and smoking have largely focused on the *DRB1*04* alleles. In a paper published in mid-2009 (43*), the spectrum of SE-coding alleles was expanded to include *DRB1*01* and *DRB1*10*. The study concluded that a strong interaction between smoking and SE in the development of ACPA-positive RA exists in all the SE-coding alleles tested (*DRB1*04*, *DRB1*01* and *DRB1*10*). Whether other SE-positive alleles (*e.g. DRB1*1402*) show the same interaction remains to be determined.

Previous studies have suggested that the interaction between the SE and smoking results primarily in anti-citrulline immunity. In an attempt to formally address this question, Morgan *et al.* (44*) examined the interactions between the SE, *PTPN22* and smoking in the development of ACPA-positive and – negative RA, in a cohort of approximately 5,000 patients and 3,700 controls. Their results suggested that while *PTPN22* primarily associated with ACPA-positivity, the SE was found to be independently associated with rheumatoid factor.

The antigenic specificity of ACPA in gene-environment interactions has been examined in a recent study (45**). Here, the authors determined to what extent autoimmunity to citrullinated α-enolase accounts for the observed associations among smoking, SE, *PTPN22* and ACPA. Antibodies to this autoantigen were found in 43–63% of ACPA-positive RA patients. Citrullinated α-enolase reactivity marked a subpopulation with a very strong odds ratio (OR) for RA development, when the combined effect of SE, smoking and *PTPN22* was considered. The ACPA-positive, citrullinated α-enolase-negative subpopulation showed a much weaker OR. The authors concluded that citrullinated α-enolase is a specific autoantigen that links smoking and genetic risk factors in RA.

The Ongoing Quest for Other Genes

Although the SE is the strongest known genetic factor in RA, it is clearly not the only one. Over the past two decades there has been continuous effort to identify additional genetic risk factors for RA, both within and without the HLA region. Notable studies published during the past year are discussed below.

In a case-control study involving 855 RA patients and 977 controls, Vignal *et al.* (46*) have identified 18 SNPs in 14 non-*DRB1* HLA-region genes that showed strong association with RA. After multivariate logistic regression analysis the model was narrowed down to *DRB1* plus 3 markers, two of which were independent of *DRB1* and one (*DQA2*) was found to be in linkage disequilibrium with a SE-coding allele (*HLA-DRB1*0101*). Thus, in addition to identifying new RA-associated SNPs, this study argues against a direct effect of the *HLA-DQ* locus, a question which has long been a subject of controversy.

A recent study implicated another HLA-region gene, *MICA* (MHC class I polypeptide-related sequence A), as an RA susceptibility locus (47*). Using both family-based and case-control cohorts, a significant association between RA and *MICA*-250, independent from SE-coding alleles was identified. Interestingly, *MICA*-250 was found to be in complete linkage disequilibrium with a *MICA* functional variant previously shown to confer differential allelic affinity between MICA and its receptor (NKG2D).

Associations with *FCGR* (the locus that codes Fcγ receptors) have been investigated in 2049 RA patients and 1156 controls by Robinson *et al.* (48) with particular attention to the *FCGR3A*-158F/V genotype. A modest association (OR 1.2) with homozygosity for *FCGR3A*-158F/V was found when all RA patients were analyzed. Somewhat stronger association was found when men only were considered (OR 1.7). No evidence for interaction with the *HLA-DRB1* SE was found.

A negative yet important study on *PADI4*-RA association has been recently reported by Burr *et al*. (49*). Polymorphisms in the *PADI4* gene have been previously shown to correlate with RA susceptibility in Eastern Asians, but their contribution to disease risk in Caucasian populations remained unclear. In the largest study of its kind, this group enrolled over 19,000 UK subjects. No association with RA was demonstrated for the PADI4_94 SNP (as well as 56 other PADI4 SNPs). While significant interaction between SE and *PTPN22* was detected in ACPA-positive RA patients, there was no interaction of the PADI4_94 SNP with either *PTPN22* R620W SNP or SE-coding alleles. Meta-analysis of five published studies confirmed the lack of association between PADI4_94 and RA in European populations.

Another study (50) reported an association between β2 adrenergic receptor polymorphism and RA. Specifically, strong associations were found with receptor variants expressing arginine in position 16. Individuals with Arg16-coding alleles had earlier age of disease onset and Arg16/Arg16 homozygosity was associated with greater incidence of ACPA. Another interesting observation in this study is a possible interaction between Arg16 and the SE in disease risk.

An intriguing study implicating another non-HLA genes in RA has focused on a member of the cytochrome P450 gene family, *CYP1A2* (51**). Cornelis *et al.* studied the association between a common *CYP1A2* polymorphism (−163 A>C) and RA in over 32,000 Korean subjects. The OR of RA among carriers of a low-inducible allele was found to be 1.11 in SE-negative individuals, 0.82 among SE heterozygotes and 0.32 in individuals homozygous for SE-coding *HLA-DRB1* alleles. The significance of these findings is dual: 1. CYP1A2 plays a role in oxidative stress, an aberration which has been long implicated in RA; 2. CYP1A2 is a downstream effector enzyme in the aryl hydrocarbon receptor pathway, which mediates the xenobiotic effect of various polycyclic aromatic hydrocarbons, including those found in tobacco, and plays a role in autoimmunity in mice (6,7). If confirmed in other ethnic populations, the finding reported by Cornelis *et al.*(51**) could open a new investigative path in the search for mechanisms involved in gene-environment interaction in RA.

Conclusion

The SE is the single most significant genetic risk factor for RA. Although the mechanistic basis of SE-RA association is unknown, progress made in the past several years and new observations made during the period reviewed here, help better mapping the main genetic and environmental players which cooperate with the SE or antagonize it. The knowledge recently gained could open new research directions that might help us better understand the enigmatic role of the SE in the etiopathogenesis of RA.

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Abbreviations

ACPA	anti-citrullinated protein antibodies
HLA	human leukocyte antigen
MHC	major histocompatibility complex
MICA	MHC class I polypeptide-related sequence A
NIMA	non-inherited maternal HLA antigen
OR	odds ratio
PADI	peptidyl arginine deiminase
PTPN22	protein tyrosine phosphatase type 22
RA	rheumatoid arthritis
SE	shared epitope
SNP	single nucleotide polymorphism

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with history of ever-smoking were compared. This US study shows that the extent of the exposure is important and corroborates the role of SE-smoking interactions previously observed only in North European patient populations.

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Table 1

Common amino acid sequences in the 70-

Amino acid sequence	SE motif	Coding *HLA-DRB1* alleles
QKRAA	+	**0401;*0409;*0413;*0416; *0421;*1419;*1421*
DERAA	−	**0402;*0414;*0103;*1102;*1116;*1120;*1121;*1301;*1302;*1304;*1308;*1315;*1315;*1317;*1319;*1322;*1416*
QRRAE	−	**0403;*0406;*0407;*0417;*0420*
QRRAA	+	**0101;*0102;*0105;*0404;*0405;*0408;*0410;*0419; *1402;*1406;*1409;*1413;*1417;*1420*
RRRAA	+	**1001*
RRRAE	−	**09;*1401;*1404;*1405;*1407;*1408;*1410;*1411;*1414;*1418*
DRRAA	−	**0415;*0805;*11011;*11012;*11041;*11042;*1105;*1106;*11081;*11082*1109;*1110;*1112;*1115;*1118;*1119;*1122;*1201;*12021;*12022; *12031;*12032;*1305;*1306;*1307;*1311;*1312;*1314;*1321;*1601;*1602;*1605*
QARAA	−	**15;*1309*
QKRGR	−	**03; *0422;*1107*