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REVIEW ARTICLE

THE ROLE OF HLA TYPING IN RHEUMATIC DISEASES

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Abstract: Association between HLA-DR4 and rheumatoid arthritis (RA) has been known for 4 decades, and amino acid sites within HLA-DRB1 (11/13, 71, 74) are highly associated with RA. HLA is not useful for diagnosis or prognosis, but it may help predict severe and erosive disease. Since 90% of patients with ankylosing spondylitis (AS) and 50-70% of other spondyloarthritis (SpA) patients are HLA-B*27 positive, HLA is a stronghold of diagnostic algorithms. Genetic predisposition to juvenile idiopathic arthritis (JIA) is mainly due to HLA class II, and to a lesser extent to HLA class I. Although HLA plays a role in rheumatic disorders, its clinical relevance is not homogeneous. When classical biomarkers are lacking or in complex cases, HLA typing may provide support for the management of patients.

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INTRODUCTION

Over 100 diseases are associated with classical HLA class I and II genes.[1] When addressing the relevance of HLA and disease association studies, we should not forget that ethnic differences, the definition of the patient group, the advancements of typing techniques, statistical methods and, finally, linkage disequilibrium might all influence the application of research results to clinical practice.

HLA and disease association studies can be divided in two distinct eras, the first pioneering phase spanning from the late '70s to the beginning of the 21st century, and the second period of the past 20 years, which we might call the genomic era.

The main findings and knowledge obtained in the pioneering phase of HLA and disease association studies are described in Thorsby and Lie's review published in 2005.[2] The authors discuss the contribution of HLA genes to specific autoimmune diseases and, for some of these, an indication of Relative Risk (RR) is provided. The RR is of significance for clinical application since it indicates the likelihood that the disease will occur among individuals positive for the predisposing allele, compared with individuals negative for the same allele. Thorsby and Lie clarify some mechanisms involved in autoimmune disease pathogenesis and suggest that, for those diseases which develop gradually over a long period of time, for example type 1 diabetes, knowing the susceptibility genes may allow early diagnosis and thus improve the management of the affected patients.

The Human Genome Project[3] paved the way for the newer studies published in the first twenty years of the new century. In 2007, the Wellcome Trust Case Control Consortium published a genome-wide association study (GWAS) of 14,000 cases of seven common diseases, three of which have variable autoimmune pathogenetic components (Crohn's disease, rheumatoid arthritis (RA) and type 1 diabetes).[4] It is interesting to note that, for RA and type 1 diabetes, the vast majority of the independent association signals could be mapped to chromosome 6

and, more specifically, to the Major Histocompatibility Complex (MHC).

Information obtained through genomic studies shed more light on HLA association and autoimmune diseases than the previous approaches. Trowsdale and Knight[5] describe the impact of the new technologies on these studies and, in particular, the authors comment on the importance of GWAS in revealing new HLA specificities and underline the multifactorial nature of autoimmune conditions. Moreover, they comment on the new imputation approach to HLA typing, a method first described by Leslie and coworkers in 2008.[6] As an alternative to direct HLA sequencing, imputation allows the screening of a large number of samples with fewer costs, guaranteeing robust statistical power; imputation is a means of addressing and partly overcoming the problems related to linkage disequilibrium. One such example is the pivotal study by Raychaudhuri and colleagues[7], which analyzed more than 5,000 RA patients comparing them with 15,000 controls.

More recently, studies aimed at improving the insight into MHC susceptibility for autoimmune diseases have been published.[8] Specifically, fine-mapping approaches have generally confirmed the previous main results and have identified new alleles or single amino acids associated with diseases. To further study the immunogenetics of autoimmune disease, a specific chip, called Immunochip (Illumina, Inc., CA), was created about 10 years ago and widely used to study several conditions, for example Crohn's disease, type 1 diabetes, RA, ankylosing spondylitis (AS).[9] It contains a concentrated panel of SNPs from the MHC and is cheaper and faster than ordinary GWAS.

It is important to point out that these are population studies conducted for research, which help in revealing molecular pathways of disease pathogenesis, but are not intended for clinical use.

Although it is difficult to estimate the number of clinical HLA disease association studies annually performed in Europe, a study carried out by EFI (European Federation for Immunogenetics)[10] indicated that, in 2012, EFI accredited laboratories performed approximately 225,000 HLA and disease association studies.

In 2010, a study group established by the Italian Association of Transplantation Biology and Immunogenetics (AIBT) wrote practical recommendations for HLA association studies of relevance for clinical practice.[11] On a more local basis, of the 2700 clinical requests for HLA and disease association studies processed by our laboratory between 2009 and 2014, 50% regarded rheumatological diseases[12], and it is of note that this proportion is increasing.

The aim of this paper is to review the role of HLA in some rheumatic diseases, namely rheumatoid arthritis (RA), spondyloarthritis (SpA), both axial (axSpA) and peripheral, including psoriatic arthritis (PsA), and

Juvenile Idiopathic Arthritis (JIA), and to give some practical indications on the role of HLA typing in the management of these conditions.

DISCUSSION

Rheumatoid arthritis (RA)

RA is the most common inflammatory arthritis which primarily targets synovial joints resulting in significant pain, functional limitations and mortality.[13] Early recognition of this disease, along with its extra-articular manifestations, can lead to faster time to treatment and better health outcomes, in addition to preserved joint functionality. The most relevant biomarkers used in clinical management of RA are Rheumatoid Factor (RF) and antibodies to citrullinated peptides (ACPA)[14]; however, neither of the tests is sufficiently specific to establish the diagnosis of RA, and prognosis varies widely with seropositive and seronegative patients. HLA-DR4 has been known to be associated with European RA patients for more than 4 decades[15], and it is widely accepted that the HLA-DRB1 gene is the major genetic susceptibility locus for RA. The HLA alleles DRB1*04:01,*04:04,*04:05 and *04:08 mostly explain the originally observed serological association. Moreover, some HLA-DRB1*01 and DRB1*10 alleles have also been found to be associated with RA in patients who are negative for DRB1*04. In the late '80s, it was found that the associated alleles shared a region of highly similar amino acid (a.a.) sequence, leading to speculate that this portion of the HLA-DRB1 molecule (a.a. 70 to 74), also called shared epitope (SE), controls susceptibility to disease.[16]

In non-European populations with a low frequency of HLA-DRB1*04, the associated alleles still contain the SE. Table 1 shows the relevance of ethnicity on HLA association in RA.

As reported in the introductory section, a fine-mapping study on approximately 5,000 RA seropositive patients and 15,000 controls, conducted by Raychaudhuri and co-workers[7], showed that a small number of amino acid (a.a.) sites within the HLA-DRB1 protein (positions 11/13 which are tightly linked, 71 and 74) and single a.a. polymorphisms in HLA-B (position 9) and HLA-DPB1 (position 9), all located in the peptide binding grove, almost completely explain the MHC association to RA risk. Individuals of European origin can be classified into 16 different risk categories solely based on their two field HLA-DRB1 type or on the a.a. carried at positions 11/13, 71, and 74 of HLA-DRB1.[17] The highest risk categories correspond to SE-positive individuals, while the lower risk categories are defined by protective HLA alleles (for example, HLA-DRB1*13:01). The importance of HLA-DRB1*13:01 as a protective allele in the European population was demonstrated by van der Woude and colleagues in 2010.[18]

Table 1. HLA and Rheumatoid Arthritis (RA) in different ethnic populations

	SEROPOSITIVE RA	SERONEGATIVE RA
Associated HLA alleles	**Caucasians:** HLA-DRB1*04:01 in 50-60% of pts RR of 5 – 11 HLA-DRB1*04:04 in 27-37% of pts RR of 5 – 14 HLA-DRB1*10:01 in 1.5% of pts RR of 2.3 HLA-DRB1*01:01 in 13-27% of pts RR of 1 to 2 HLA-DRB1*04:08 **Japanese:** HLA-DRB1*04:05 **Chinese:** HLA-DRB1*04:04, *04:05 **Yakima, Tlingit, Pima Native-American:** HLA-DRB1*14:02 **Spanish, Basque, Israeli Jews:** HLA-DRB1*01, *10	HLA-DRB1*03 HLA-B*08
Protective HLA alleles	HLA-DRB1*13:01	

Studies which have addressed the role of HLA in seronegative RA[19] demonstrate associations with HLA-DRB1*03 and HLA-B*08, suggesting that seronegative RA is genetically distinct from the seropositive disease, and probably has diverse autoantigens contributing to pathogenesis.

The role of genetic and environmental factors on the pathogenesis of RA is illustrated in McInnes and Schett's excellent review published in 2011.[13] Although the association between HLA and RA is widely accepted, according to some authors, HLA would only contribute approximately 50% of the global genetic risk. Okada and co-authors recently published a paper[20] which lists the known genes associated to RA, which amount to about 100. Some of the most relevant non-HLA genes associated with the disease are *PTPN22*, *CTLA4*, *TRAF1* and *STAT4*.

In the last 10 years, approximately 900 studies have been published on HLA and RA (data obtained from PubMed, accessed on 29 January 2020). This testifies to the interest in the role of immunogenetics in this specific rheumatologic condition. However, from a practical point of view, most clinicians do not use HLA typing as a genetic marker for the clinical management of RA.

Diagnosis

Contrary to other autoimmune diseases, such as AS, the alleles strongly associated to RA are common in the normal population. A study, published in 1992, calculated that individuals carrying HLA-DRB1*04:01, *04:04 or *01:01 had a risk of developing RA of 1:46.[21]

In 2013, Balandraud and co-workers[22] published a paper on a patient population of 857 individuals affected by ACPA-positive RA and 2178 controls from South Eastern France. The authors demonstrated that the risk of developing the disease was correlated with both HLA-DRB1 alleles carried by patients. Two tables are presented in the study: the first reports the Odds Ratio (OR) of single alleles or allele groups, whereas the second illustrates the risk associated with the genotypes. The single allele bearing the greatest risk is HLA-DRB1*04:08, with an OR of 10.22. On the other hand, the genotype with the highest OR (28.2) is HLA-DRB1*04:01,*10.

Although the role of HLA-DRB1 in RA is well-established, the availability of non-expensive serological biomarkers, together with clinical and radiological evaluation, does not recommend HLA typing for diagnosis at present.

Prognosis, severity and response to treatment

The presence of ACPA at disease onset allows for the identification of patients at greater risk of severe erosive disease and radiological progression[23, 24], independently of RF positivity.[24] Patients with high titre ACPA should be carefully monitored. Generally, these antibodies are present in patients bearing the RA-associated amino acids at positions 11/13, 71 and 74 of HLA-DRB1[7], therefore HLA typing for prognosis cannot be recommended.

As far as severity is concerned, the most significant genetic marker of disease severity is valine at position 11 of HLA-DRB1, as illustrated by Viatte and Barton.[17] Valine at position 11 is independent from SE and, according to the authors, it is associated with radiological severity, mortality and treatment response. However, the authors believe more data is necessary to confirm these findings before recommending HLA typing for severity and treatment response in RA.[25]

Spondyloarthritis (SpA)

SpA are a family of rheumatic disorders which share some clinical features: inflammation of axial joints (especially the sacroiliac joints), asymmetric

oligoarthritis (especially of the lower extremities), dactylitis (sausage digits), enthesitis (inflammation at sites of ligamentous or tendon attachment to bone). SpA may also display some additional clinical signs such as skin and genital lesions, eye and bowel inflammation, an association with preceding or ongoing infectious disorders, a positive family history, elevated acute phase reactants and, most importantly, a strong association with HLA-B*27.[26]

The SpA family may be classified according to the distribution of joint involvement as being predominantly axial (axSpA) or peripheral (peripheral SpA):

1) Axial SpA
   - Ankylosing spondylitis (AS)
   - Nonradiographic axial SpA (nr-axSpA)
2) Peripheral SpA
   - SpA associated with psoriasis or psoriatic arthritis (PsA)
   - SpA associated with Crohn disease and ulcerative colitis
   - Reactive arthritis
   - Juvenile onset SpA

Categories do not necessarily represent discrete entities, and the clinical, laboratory and imaging findings can overlap. Table 2 illustrates the main HLA associations with the different subsets of SpA. The present review will mainly address AS, as a prototypical form of axSpA, and PsA, as an example of peripheral SpA.

HLA and Ankylosing Spondylitis (AS)

AS is a potentially disabling SpA with a predominant involvement of the spine and/or sacroiliac joints.[27]

Prevalence of AS ranges from 0.12 to 1.8% in Europeans and is a function of the distribution of HLA-B27 in the population.[28] In Asia, prevalence is 0.17% whereas, in Africa, it is 0.07%.[29]

The association between HLA-B27 and AS is very strong and has been known since 1973.[30] HLA-B27 is present in 90-95% of AS patients in the USA, Europe and China. However, in the general population, only 5% of individuals carrying HLA-B27 will develop AS or another form of SpA. The contribution of HLA to AS heritability is about 23%. Therefore, even though HLA-B27 is necessary for the development of AS, apparently it is not sufficient. The RR is greater than 150, the highest for all HLA associations with autoimmune diseases.[2]

There are more than 300 HLA-B27 alleles (IMGT/HLA database, Release 3.39.0, access date: 25 February 2020), not all of which are similarly associated with SpA and AS. The differences between alleles may determine the peptide repertoire, the molecular structure and the biogenesis, all factors which can influence the antigen presentation and the immune response. HLA-B*27:05, the ancestral HLA-B27 allele from which B*27:02, *27:04 and *27:07 derive, is strongly associated with AS in Europeans. Conversely, HLA-B*27:04 is more frequently associated with AS (and more generally with SpA) in Chinese and Japanese individuals. It is interesting to note that HLA-B*27:06 and *27:09 are not associated with either axial or peripheral SpA.[31]

In addition to HLA-B27, other HLA-B alleles such as *13:02, *40:01, *40:02, *47:01 and *51:01 seem to show some degree of association with AS, as demonstrated by the study published in 2015 by Cortes and colleagues.[32]

The role of HLA-B27 in the pathogenesis of AS is still not clearly understood. As discussed by Bowness in his 2015 review[33], at the moment there are two hypotheses. The first is the classical binding of antigenic peptides to HLA-B27 and their presentation to cytotoxic T cells,

Table 2. HLA and Spondyloarthritis (SpA)

HLA	Axial SpA		Peripheral SpA			
	AS	**nr-axSpA**	**Psoriatic arthritis**	**Reactive arthritis**	**SpA associated with IBD**	**Juvenile SpA**
HLA-B27 HLA-B27 is present in approximately 8% of Caucasians Only 5% of HLA-B27+ subjects develops AS	90-95% of patients are + Associated HLA-B27 alleles: **B*27:05** (Caucasians) **B*27:04** (Asians) HLA-B27 alleles **NOT** associated: **B*27:06** **B*27:09**	75-85% of patients +	< 50% of patients +	30-50% of patients + Considerable variability Among in-patients with more severe cases, 60-80% of patients +	< 50% of patients +	< 50%
Other HLA specificities			HLA-C*06:02 HLA-B*08 HLA-B*38 HLA-B*39			

Legend: IBD - Inflammatory Bowel Disease

arthritogenic or self-antigens might be responsible for the disease. The second theory regards the misfolding of the HLA-B27 molecule, which leads to its accumulation in the endoplasmic reticulum, stress and autophagy which can induce the production of inflammatory cytokines such as Il-23.

More recently, the role of the gut microbiome and the damage to intestinal mucosal barriers in the pathogenesis of SpA has been addressed.[34-36]

For a more detailed description of the pathogenesis of AS, readers are referred to Ranganathan[37] and Brown.[38]

HLA and Psoriatic Arthritis (PsA)

Approximately 30% of patients affected by psoriasis will develop psoriatic arthritis at some stage of their disease; in the USA, the prevalence is estimated as ranging from 0.06 to 0.25%, although figures could be higher due to under-reporting.[39] Even though HLA-C*06:02 is strongly associated with cutaneous psoriasis[40] and appears to be relevant as a marker of response to treatment[41], its correlation with psoriatic arthritis is less significant.[42] Indeed, HLA-B alleles B*08:01, B*39:01 and B*38:01, in addition to B*27:05, are more commonly associated with PsA.[43] On the other hand, it has been shown that the HLA-B alleles B*44:02, B*44:03 and B*40:01 are protective for the development of PsA.[43] Pathogenesis of PsA, including the role of non-HLA genes, is thoroughly discussed by Veale and Fearon in a recent review.[44]

Practical aspects

Among the most relevant authors who have studied the clinical utility of HLA-B27, Lim, Sengupta and Gaffrey, in their review published in 2017[4], give a detailed account of the possible role of HLA-B27 typing on risk assessment, screening, diagnosis, treatment and prognosis.

The first aspect which must be highlighted is that an early diagnosis of axial and peripheral SpA is of paramount importance for the clinical outcome.

HLA is now accepted as part of the diagnostic algorithm of SpA, together with the clinical evaluation of the rheumatologist or the general practitioner. It is important to bear in mind that the presence of HLA-B27 alone is not diagnostic for axSpA, and the absence of HLA-B27 cannot exclude axSpA in European individuals since:

- 85-95% of AS patients and 75-85% of nr-axSpA are HLA-B27-positive;
- HLA-B27 is present in about 8% of the general population;
- only 5% of HLA-B27 subjects will develop AS.

There are different approaches to the role of HLA-B27 typing in the diagnostic algorithm of SpA. Among the most accepted algorithms, is the one published in 2013 by van den Berg and co-workers[46], and validated by the Assessment of Spondyloarthiritis International Society cohort. This protocol recommends HLA-B27 for individuals with chronic back pain (> 3 months, < 45 years of age onset) and negative radiographic studies with less than 4 SpA clinical features, as illustrated by Figure 1. Some authors suggest using HLA-B27 for screening of patients with chronic low back pain or inflammatory back pain (IBP), in whom axSpA is suspected:

- in chronic back pain patients, the probability of axSpA is 5% in HLA-B27-negative individuals and 30% in HLA-B27-positive subjects;
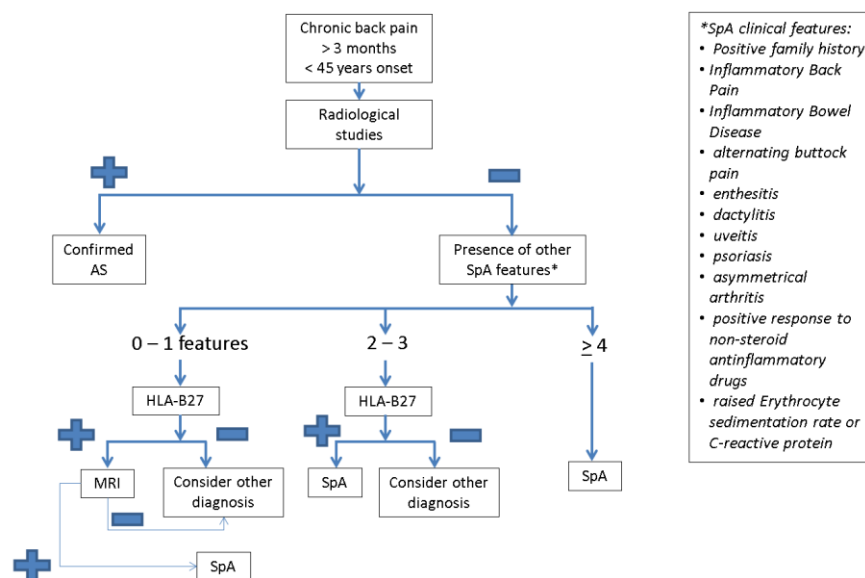- in IBP patients, probability of axSpA is 14% in HLA-B27-negative and 60% in HLA-B27-positive individuals.[47, 48]

Figure 1. Diagnostic algorithm of SpA (modified from van den Berg et al[37])

As far as the peripheral (non-axial) SpAs are concerned, there are no pathognomonic laboratory tests. As shown in Table II, HLA-B27 is present in approximately 50% of reactive arthritis and peripheral SpA without comorbidities. The frequency of HLA-B27 decreases in psoriatic arthritis and SpA associated with inflammatory bowel disease (IBD). Similarly to axial SpAs, HLA-B27 alone is not diagnostic of SpAs, and, conversely, most features of peripheral SpA can occur in HLA-B27-negative patients.

Juvenile Idiopathic Arthritis (JIA)

The present accepted definition of juvenile idiopathic arthritis (JIA) is chronic arthritis persisting for more than 6 weeks in children below 16 years of age, without any apparent etiology. It is the most frequent joint disease of childhood, with an annual incidence of 2-20 cases per 100,000 individuals in high-income countries.[49]
JIA is characterized by a variable pattern of articular involvement and systemic symptoms, and thus it has been classified in several subtypes.[49] JIA, as most autoimmune diseases, is a multifactorial condition caused by the interaction between environment and a genetic background. There is wide evidence that HLA plays an important role on the predisposition to different JIA subgroups.[50]

In an attempt to better define HLA associations with the different subsets of JIA, together with colleagues from the University Hospital in Pavia, we carried out a meta-analysis[51] according to a protocol published in the PROSPERO International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO) with registration number: CRD42017073065. We started out by evaluating 565 papers, nine of which met our eligibility criteria. The main findings of our study can be summarized as follows:
1) HLA-DRB1*08 is a strong factor predisposing to JIA, both for oligo-articular and poly-articular forms (Odds Ratio 6.0);
2) HLA-DRB1*01 and HLA-DRB1*04 may be involved in the genetic predisposition of RF+ forms of JIA;
3) HLA-DRB1*11 was confirmed to be predisposing to oligo-articular JIA;
4) HLA-DRB1*04 was confirmed to have a role in systemic JIA. Importantly, RF positivity seems to select the JIA clinical subset with the strongest immunogenetic similarities with adult RA.

From the practical point of view, HLA typing is of interest only for research purposes, to the extent that it could shed some light on etiology and pathogenesis of the different JIA forms. However, at present, HLA typing cannot be recommended for diagnosis or prognosis.
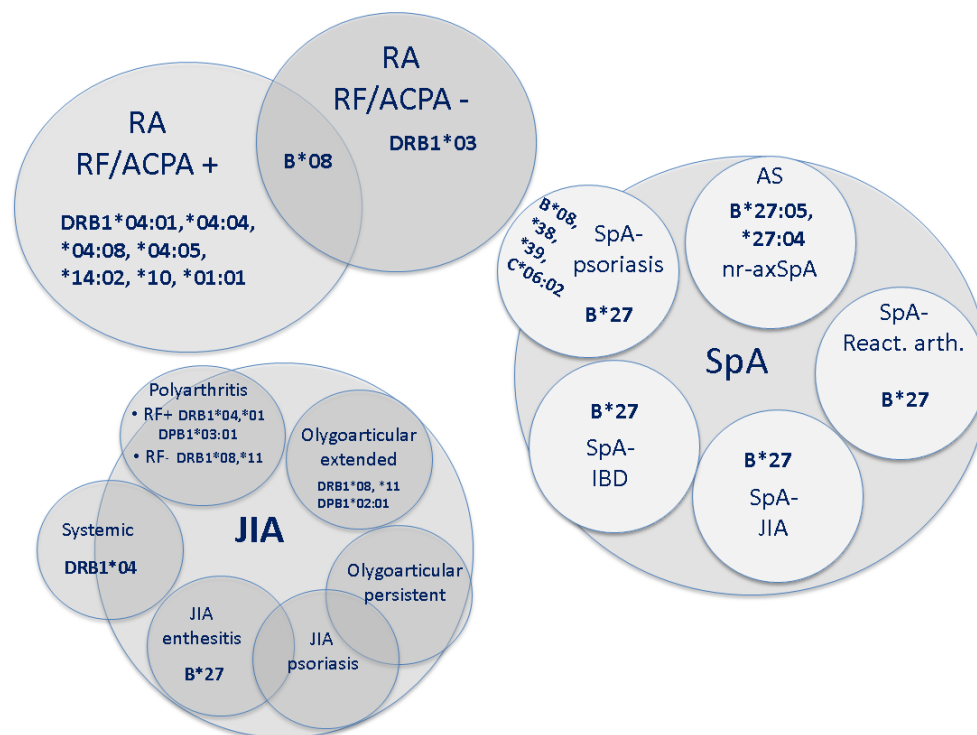

Figure 2. The role of HLA typing in Rheumatic diseases

CONCLUSION

The rheumatic disorders discussed in this review, i.e. RA, SpA and JIA, are, to a variable degree, associated with HLA. Figure 2 aims at summarizing the HLA allelic groups and alleles found to be correlated to the different disease subtypes.

In this paper, we attempted to illustrate the present-day practical use of HLA typing in disease diagnosis and prognosis. It is interesting to note that some authors have a less restrictive view on the practical role of HLA typing; Roudier and colleagues[52] prescribe HLA-A, -B, -C, -DRB1 typing to all patients for whom a rheumatic disease is suspected. According to the authors, this provides a diagnostic orientation in patients with recent onset disease, before the development of typical clinical features, especially in those individuals negative for autoantibodies. This approach is hindered by the elevated cost of HLA typing, and could be more widely adopted should typing costs decrease significantly. In our personal experience, HLA typing has proven useful in supporting diagnosis in some clinically unclear situations and in the early stages of the disease.

Future prospects of HLA studies in rheumatic diseases will benefit from new insights on the non-coding region variation, on the consequences of regulatory variation on HLA expression and its possible impact on disease.[53] Moreover, an emerging role of Natural Killer cells and HLA-C might shed new light on the pathogenesis of rheumatic diseases.[41]

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