Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology REVIEW ARTICLE doi:10.1111/cei.13020

The interplay between HLA-B27 and ERAP1/ERAP2 aminopeptidases: from anti-viral protection to spondyloarthritis

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Accepted for publication 26 July 2017
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Summary

The human leukocyte antigen class I gene HLA-B27 is the strongest risk factor for ankylosing spondylitis (AS), a chronic inflammatory arthritic disorder. More recently, the Endoplasmic Reticulum Aminopeptidase (ERAP) 1 and 2 genes have been identified by genome wide association studies (GWAS) as additional susceptibility factors. In the ER, these aminopeptidases trim the peptides to a length suitable to fit into the groove of the major histocompatibility complex (MHC) class I molecules. It is noteworthy that an epistatic interaction between HLA-B27 and ERAP1, but not between HLA-B27 and ERAP2, has been highlighted. However, these observations suggest a paramount centrality for the HLA-B27 peptide repertoire that determines the natural B27 immunological function, i.e. the T cell antigen presentation and, as a by-product, elicits HLA-B27 aberrant behaviours: (i) the misfolding leading to ER stress responses and autophagy and (ii) the surface expression of homodimers acting as ligands for innate immune receptors. In this context, it has been observed that the HLA-B27 carriers, besides being prone to autoimmunity, display a far better surveillance to some viral infections. This review focuses on the ambivalent role of HLA-B27 in autoimmunity and viral protection correlating its functions to the quantitative and qualitative effects of ERAP1 and ERAP2 polymorphisms on their enzymatic activity.

Keywords: ankylosing spondylitis, autoimmunity, endoplasmic reticulum aminopeptidases, HLA-B27

Introduction

A group of human disorders classified as autoimmune and/or autoinflammatory share a similar immunopathogenic ground in which the principal risk factor is a 'tagging' human leukocyte antigen (HLA) class I gene. In addition, a prominent role has been attributed to Endoplasmic Reticulum Aminopeptidase (ERAP)1 and, in some cases, ERAP2 genes. We are referring to immunological disorders such as Behçet's disease (BD), psoriasis (Ps), birdshot chorioretinopathy (BSCR) and ankylosing spondylitis (AS), each associated by a variable relative risk (odds ratio) with the HLA-B51, HLA-C*0602, HLA-A29 or HLA-B27 genes, respectively [1,2]. Besides peculiar clinical hallmarks due to distinct polygenic backgrounds, such diseases usually affect body sites that undergo physical stress and are located at either external barriers (oral mucosa, skin, gut, eye) or at interior sites (joints, enthesis, cardiac valves, blood vessel walls) [1]. The endogenous and/or exogenous aetiological triggers are usually unknown. However, the co-occurring association with both single HLA class I genes and endoplasmic reticulum (ER) aminopeptidases involved in the final cut of HLA peptides points unequivocally at antigen processing and imbalanced peptide repertoire as putative unifying pathogenic key events. This concept is strengthened further by the fact that the association of ERAP1 in BD, Ps and AS reveals an epistatic gene–gene interaction with HLA-B51, HLA-C*0602 and HLA-B27, respectively [3–5]. This review will discuss how the classical and non-classical HLA-B27 functions could be influenced by the peptide repertoire that, in turn, is finely shaped by ERAP1 and 2 allelic variants and how the different settings can affect AS and the superior anti-viral immunity (Fig. 1).

HLA-B27, a molecule with two faces: the involvement in spondyloarthritis

HLA-B27 went into the spotlight during early 1970s, when a strong association with AS and other related immune-

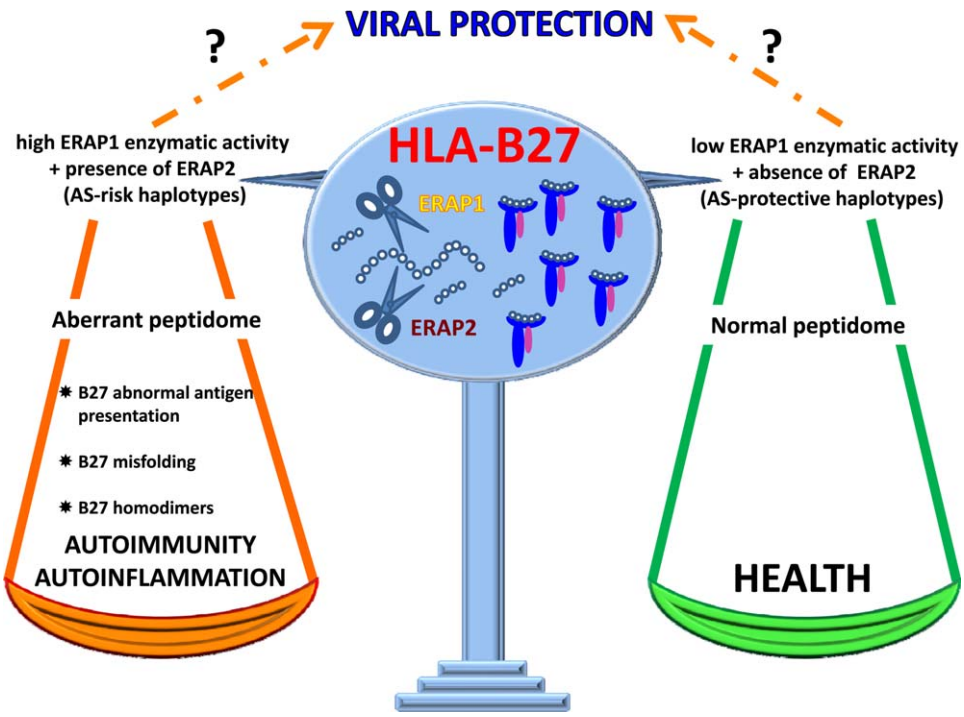

Fig. 1. Cartoon illustrating the interaction between human leukocyte antigen (HLA)-B27 and Endoplasmic Reticulum Aminopeptidase 1 and 2 in ankylosing spondylitis and antiviral defence. HLA-B27 carriage, in association with high peptide-trimming activity ERAP1 haplotypes and the concomitant expression of ERAP2 predisposes to AS. This genetic ground would affect the B27 peptidome impairing HLA-B27 functionality. It is currently unknown whether the so-called 'aberrant' peptidome contains additional pathogenic epitopes and/or loses peptides relevant for the stability of B27 complex or other functions. Indeed, an aberrant peptidome could impact on antigen presentation as well as on the rate of B27 misfolding thus activating ER stress, unfolded protein response or autophagy. In addition, the formation of B27 homodimers can activate cells expressing killer-cell immunoglobulin receptor (KIR)3DL2 and leukocyte immunoglobulin-like receptors (LILRB2) innate immune receptors. Conversely, the HLA-B27 co-inherited with ERAP1 haplotypes of low enzymatic activity, and in the absence of ERAP2, would display a proper and non-disease prone B27 peptidome. With regard to viral infections, it is still unknown whether the protective behaviour of HLA-B27 correlates with a high or low activity of ERAP1, and/or with the presence or absence of ERAP2.

mediated conditions [reactive arthritis (ReA), psoriatic arthritis, enteropathic arthritis and acute anterior uveitis] was established [6,7]. During the next 40 years, although rapid progress in many research fields enabled acquisition of a deep knowledge of the biochemical and functional properties of HLA-B27, its exact role in AS onset has remained elusive.

The normal immunological function of HLA class I molecules is generally to present endogenous peptides of microbial or self-origin to $CD8^+$ T lymphocytes. These are generally peptides of 8–10 aa in length, which in the case of HLA-B27 are stabilized through two principal anchors: the first is almost exclusively an Arg at position 2 (P2), whereas the second, at the peptide carboxy-terminus (pΩ), can admit several different amino acids [8]. Accordingly, one of the first and more popular theories to explain the B27 involvement in AS was the so-called arthritogenic peptide hypothesis. This implies the activation of an autoreactive HLA-B27-restricted cytotoxic $CD8^+$ T cell response primed by cross-reactive microbial antigen(s), thus breaking self-tolerance and perpetuating an autoimmune process leading to tissue damage [9]. In accordance with this hypothesis, HLA-B27-restricted $CD8^+$ T cells reactive against both self-peptides and enterobacterial antigens were found in the synovial fluid of patients with AS and ReA [10]. Moreover, in HLA-B27-positive AS patients, our group has described autoreactive B27-mediated cytotoxic T lymphocyte (CTL) responses triggered by a self-peptide from the vasoactive intestinal peptide receptor type I (VIPR) and cross-reacting with a viral epitope from latent membrane protein 2 (LMP2) of Epstein–Barr virus (EBV) [11]. However, this theory has not received further support, for several reasons. Unlike another related disease, namely ReA [12], AS is not usually preceded by microbial infections, even though the intestinal microbiota has been evoked recently to have an inciting role on B27-restricted T cell responses [13]. Furthermore, the HLA-B27 transgenic rat model seems to disprove the possibility that HLA-B27 is uniquely responsible for disease because of its classical antigen-presenting functions, given that the lack of $CD8^+$ T cells does not prevent spondyloarthritis in this context [14].

The HLA-B27 family consists of more than 160 alleles (https://www.ebi.ac.uk/cgi-bin/ipd/imgt/hla/allele.cgi), whose ancestral subtype is the HLA-B*2705 that is distributed

ubiquitously worldwide and is strongly AS-associated [15]. Most B27 alleles are rare, so that their relation to AS is unknown. Nevertheless, at least two alleles, the HLA-B*2706 and HLA-B*2709, are not risk factors for AS [15–17]. These alleles have limited polymorphic positions compared to the AS-associated HLA-B*2705 allele. In particular, the B*2709 differs from the B*2705 only for a His instead of an Asp at position 116, whereas the B*2706, an Asian allele, displays two substitutions (Asp114His, Tyr116Asp) compared to the B*2704 allele, which is strongly AS-associated in the same geographic area [16]. These polymorphisms are clustered mainly around the pockets of the peptide-binding cleft, and influence the peptide repertoire. Therefore, based on the arthritogenic peptide theory, the disease-inducing peptides should be exclusive ligands of the AS-associated alleles and not of B*2706 and B*2709 alleles. In contrast with this assumption, recent biochemical studies from Purcell's group, analysing a large peptide data set from the AS-associated and non-AS-associated B27 alleles expressed by transfected C1R cells, failed to identify qualitative changes in their peptide repertoire [18]. Rather, quantitative differences have been found which justify the interest towards a panel of 26 peptides eluted in lower abundance from the non-AS-associated alleles [19]. In addition, another study in which structure, peptide specificity, folding and stability of either AS-associated or non-AS-associated subtypes on C1R cells were correlated with the constitutive peptidomes, reached similar results. Indeed, very few peptides emerged as connected strictly with the disease [20]. Unfortunately, these studies are limited by experimental requirements that allow preferential analysis of certain cell types. Therefore, little is known so far about the peptide repertoire of either AS-associated or non-AS-associated subtypes in the target tissues, where the differences could be more informative.

Alternative areas of intense investigation have considered aberrant and potentially pathogenic biochemical features of HLA-B27 that set this allotype apart from other HLA class I molecules. First, the HLA-B27 displays an altered folding rate during the assembly into the ER [9,21,22]. This misfolding is a consequence of the particularly slow HLA-B27 maturation rate that triggers the endoplasmic reticulum (ER)-associated degradation (ERAD) of the heavy chains [22]. However, the accumulation of misfolded heavy chains, aggregates or even dimeric structures, which do not transit further along the secretory pathway, generates ER stress and the unfolded protein response (UPR) [22–24]. In turn, the UPR promotes cytokine dysregulation and activates the interleukin (IL)-23/IL-17 axis [25]. This mechanism has been well documented in transgenic rat model for spondyloarthritis but not in humans, and its pathological implication in AS remains controversial [25,26]. In this regard, the occurrence of B27 misfolding in gut of AS patients does not seem to activate UPR but rather autophagy, that would be the leading mechanism modulating the intestinal production of IL-23 in the disease [27]. Another peculiarity of the HLA-B27 is related to its expression on the cell surface as a non-canonical form made by β2m-free homodimers [9,21,28]. The formation of homodimers arises from endosomal recycling compartments and is caused by the impaired and highly reactive cysteine 67 (Cys67) located into the B pocket of the peptide groove [29]. The pathogenic effects of these structures would depend upon their engagement by both leukocyte immunoglobulin-like receptors (LILR)B2 and killer-cell immunoglobulin receptor (KIR)3DL2 innate immune receptors expressed by natural killer (NK) and T cells, thus triggering an inflammatory cascade [30]. In patients with spondyloarthropathies, more $CD4^+$ T cells expressing KIR3DL2 have been found and the binding with B27 dimers would licence pathogenic T helper type 17 (Th17) cell polarization [31,32].

If misfolding as well as homodimer formation can be the cause of AS pathogenesis, then the disease-associated B27 subtypes should differ from the non-disease associated HLA-B*2706 and 09 alleles in this aspect. Several investigations have addressed this issue, and the results were not always consistent [8,33–35]. One study failed to establish a total correspondence between being a disease-associated B27 subtype and a lower folding efficiency, as the AS-associated B*2707 allele behaved as the non-AS-associated B*2706 and B*2709 alleles [36]. Moreover, the two structurally close but differently AS-predisposing B*2705 and B*2709 alleles have been shown to have similar intracellular trafficking and propensity to form oligomers [33]. However, more recently, it has been described a greater tendency of AS-associated B*2702, B*2705, B*2707 alleles to accumulate in 'dynamic' intracellular vesicles as misfolded/aggregate proteins in comparison with the non-associated B*2706 allele [37]. This behaviour has been correlated with the B27 protein level.

One important question is whether a differential amount of cell surface free heavy chain (FHC) and/or homodimers, recognized by KIR3DL2 on NK and $CD4^+$ T cells, discriminates some B27 alleles from others, justifying their distinct AS-association. So far, this matter has not been explored in an *in vivo* setting in which cells from AS patients are compared with the controls expressing the different B27 alleles. However, no significant difference in cell surface expression of FHC among several B27 subtypes expressed on C1R cells has been reported [38]. Moreover, one report highlighted that B*2706, unlike B*2709, expressed the highest surface level of FHC, probably reflecting the poor tapasin dependence during the assembly process which produces more dissociation-prone heterotrimeric complexes [39]. More recently, Cauli and co-workers, using transfected cells, have shown that a higher cell surface FHC expression of B*2705 *versus* B*2709 could contribute to the differential disease-association [40].

The formation of B27 dimers and oligomers is a complex process in which a series of unpaired Cys residues

(positions 67, 308 and 325) appear to play important roles, but the conserved Cys residues at 101 and 164 could also be relevant [22,30,41,42]. These Cys residues are shared by all B27 alleles; therefore, other residues must influence their reactivity and accessibility to the oxidizing environment of the ER for a time sufficient to allow the formation of aberrant disulphide bonds. A pivotal role has been ascribed to residues 114 and 116 located in the F pocket of the binding groove, which notoriously influence the repertoire of bound peptides but also chaperone association, assembly process, maturation rate and, lastly, heavy chain dimerization [43,44]. Interestingly, these residues distinguish the AS-associated from the non-AS-associated alleles [9,16]. Of note, Tyr116 in B*2706, much more than His116 in B*2709, appears to impact positively on the assembly kinetics, thus reducing dimer formation [44]. These data can also be interpreted on the basis of recent biophysical and computational analyses. Indeed, several studies have described an enhanced degree of flexibility and disorder of B*2705 and B*2704 peptide-binding cleft in comparison to that of B*2706 and B*2709 alleles [45–47]. This would influence the tapasin dependence, the folding dynamics and the stability of HLA–peptide complexes overall [46].

HLA-B27, a molecule with two faces: protection from viral infections

Being a carrier of HLA-B27 certainly represents a risk condition for the development of autoimmune rheumatic diseases, but there are also benefits concerning a superior protection against a variety of viruses [48,49]. Together with few other HLA class I molecules of the B locus, the HLA-B27 is associated with long-term non-progression to AIDS in patients, called 'elite controllers', which maintain a low viral load and remain asymptomatic for longer [50]. Furthermore, recent studies have revealed in HLA-B27 subjects a high rate of spontaneous clearance of hepatitis C virus [48,51]. The reasons for this are not completely understood, although virological and immunological explanations have been anticipated. First, during HIV and hepatitis C virus (HCV) infections, viral escape from HLA-B27-restricted cytotoxic T cells targeting immunodominant epitopes is undoubtedly a difficult process. It usually requires multiple compensatory mutations to counterbalance structural and functional constraints having a high cost for the viral fitness [48,52,53]. Secondly, a number of immunological benefits of the virus-specific, HLA-B27-restricted $CD8^+$ T cells have been described pertaining to broader polyfunctionality and higher functional avidity [54]. Furthermore, special thymic selection inducing a larger B27-driven $CD8^+$ T cell precursor repertoire, preferential usage of certain T cell receptor (TCR) clonotypes associated with higher cross-reactive and, finally, better capacity of evasion from regulatory T cell (T_{reg})-mediated suppression have also been documented [55–58]. Moreover, rapid and efficient processing of the proper immunodominant epitopes would contribute to these successful B27-restricted T cell responses [59].

Recently, in HLA-B*2705 subjects, mainly patients with AS, our group has described the capacity to elicit a vigorous HLA-B27-restricted $CD8^+$ T cell response against an EBV epitope from EBNA3A (RPPIFIRRL) which was already known as immunodominant in another restriction context, namely the HLA-B7 molecules [60,61]. This presentation is somewhat intriguing, as the peptide is a suboptimal B27 ligand and is expected to shift into the peptide-binding cleft to permit the fitting of N-terminal Arg into the B pocket, while leaving the A pocket empty [60]. Of note, almost 70% of B*2705 individuals possess such 'unexpected' $CD8^+$ T lymphocytes that share a common TCR β-chain repertoire. Interestingly, no reactivity has been found in B*2709 healthy donors and, accordingly, the non-AS-associated B*2709 allele appears unable to present such suboptimal epitope. This is a further evidence supporting the different plasticity of B*2705 v*ersus* the B*2709 antigen-presenting groove [49]. Overall, this finding allows us to speculate that for some HLA-B27 alleles, possibly those associated with AS, the real pool of bound ligands is larger than anticipated on the basis of biochemical data. This would enhance the ability to mediate anti-viral protection while increasing the risk of autoimmunity.

The ERAP1 and ERAP2 aminopeptidases: functional role and allelic variants

ERAP1 and ERAP2 are endoplasmic reticulum-resident aminopeptidases trimming peptides to an optimal length for binding with major histocompatibility complex (MHC) class I molecules [62,63]. They belong to the M1 family of zinc-metallopeptidases and share 49% of sequence identity. In humans, ERAP1 and ERAP2 genes are located on chromosome 5q15 in opposite orientation and, conceivably, share regulatory elements [62].

ERAP1, besides its role in antigen processing, exerts several other biological functions promoting innate immune responses, or even regulating angiogenesis and hypertension/blood pressure [64]. ERAP1 is a highly dynamic molecule switching from a lower-activity open conformation to a higher-activity closed conformation [65]. This conformational transition is induced by the substrate upon binding to a regulatory site neighbouring the catalytic domain [63]. Through a mechanism named 'molecular ruler', whereby the enzyme itself acts as a peptide-length template, ERAP1 trims peptides of 9–16 residues very efficiently while sparing shorter peptides [66–68]. The substrate specificity of ERAP1 is dictated by the N- and C-terminal residues of the peptide as well as by the internal sequence [67,69–71]. ERAP1 shows preferences for hydrophobic residues, while basic and acidic amino acids are poor substrates; finally, Pro is never hydrolyzed [69,70]. The trimming activity of

Table 1. Single nucleotide polymorphisms (SNPs) in Endoplasmic Reticulum Aminopeptidase (ERAP)1 and ERAP2 associated with ankylosing spondylitis (AS)

	Amino acid position	Major/minor allele amino acid (nucleotide)	AS-risk allele amino acid (nucleotide)	Effects
ERAP1 SNPs				
rs2287987	349	Met (A)/Val (G)	Met (A)	Trimming activity (substrate-dependent)
rs30187	528	Arg (G)/Lys (A)	Lys (A)	Expression level, trimming activity, Substrate specificity
rs10050860	575	Asp (G)/Asn (A)	Asp (G)	Trimming activity
rs17482078	725	Arg (G)/Gln (A)	Arg (G)	Trimming activity
rs27044	730	Glu (G)*/Gln (C)	Gln (C)	Substrate length preference, Trimming activity
ERAP2 SNPs				
rs2549782	392	Asn (T)/Lys (G)	Lys (G)	Trimming activity
rs2248374		null-allele (G)/expressing allele (A)	expressing allele (A)	Presence/absence

*This SNP is also present in an AS-predisposing haplotype [78].

ERAP2 is complementary to that of ERAP1 for both N-terminal substrate specificity and peptide length. Indeed, ERAP2 cleaves positively charged residues preferentially and its activity is maximal on octameric substrates and lower on longer peptides [63,72–74]. Therefore, the two aminopeptidases would operate in a concerted manner, ensuring an efficient generation and/or destruction of MHC class I epitopes for a proper functioning and regulation of the adaptive immunity.

ERAP1 and ERAP2 have been shown to form heterodimeric complexes having an allosteric effect on ERAP1 that acquires an enhanced *in vitro* trimming activity due to a higher substrate binding affinity [75,76]. However, less than 30% of each enzyme is engaged in the heterodimers [75]. Recently, it has also been shown that the ERAP1/ERAP2 dimer could work as a peptide editor by trimming 'on MHC I' substrates until the correct length enabling the MHC groove to reach a closed conformation [77].

ERAP1 is a highly polymorphic gene. The most common protein variants, reported as ERAP1 allotypes, are encoded by haplotypes created by missense variant combinations of SNPs harboured in an ancestral haplotype found in humans as well as in primates [78]. The most investigated ERAP1 allotypes (from 10 to 13) [78,79] are distinguished by enzymatic functions with both qualitative (substrate preferences) and quantitative (high, intermediate and low activity variants) effects [5,64,80–82]. Interestingly, some non-synonymous SNPs influence the gene expression level of ERAP1 [83].

ERAP2, instead, displays poor polymorphism. In the worldwide population, evolution under balancing selection has maintained two main ERAP2 haplotypes: one expressing the protein and the other ERAP2-deficient, because the G allelic variant of SNP rs2248374 induces a truncated form that goes through non-sense-mediated decay [84]. The two haplotypes are almost equally frequent in the different ethnic groups, so that 25% of individuals, being homozygous for the second haplotype, do not express ERAP2. Moreover, a non-synonymous SNP (rs2549782) encoding for the amino acid substitution N392K affects both enzymatic activity and substrate specificity [72]. This functional variant is in strong linkage with SNP rs2248374. Apart from specific ethnic peculiarities, the N392 allelic variant is almost absent in the human populations because of its co-inheritance with the rs2248374 null-allele [84,85].

HLA-B27 and ERAP1/2 as players in ankylosing spondylitis

In 2007, a genome wide association study (GWAS) revealed the association between five ERAP1 SNPs and an increased risk to develop AS [86]. In particular, two of these SNPs, rs30187 (Arg528Lys) and rs27044 (Glu730Gln), reached high statistical significance for AS, as the minor alleles were robustly more frequent in AS patients than controls. Afterwards, several studies replicated these genetic associations, imputed ERAP1 haplotypes and found further associations with other SNPs mapping in the coding, UTR or intronic regions [2,78,87]. In 2011, it was proved that the association of ERAP1 with AS occurred exclusively in HLA-B27-positive patients, pointing at a gene–gene epistatic interaction [5]. Hence, the obvious effort has been to understand the impact of AS-risk ERAP1 polymorphisms on the B27 peptidome and, consequently, on the putative B27 pathogenic functions.

Apart from a few exceptions, a higher enzymatic activity marks individual ERAP1 polymorphisms or the entire haplotypes associated with increased risk of AS (Table 1) [2,5,78–80]. Several studies agree to indicate the high trimming Met349/Lys528/Asp575/Arg725/Gln730 haplotype as being associated most strongly with AS risk, while the low trimming Val349/Arg528/Asn575/Gln725/Glu730 haplotype as the most protective [2,78,88]. In contrast, a report has suggested that rare hyperactive or hypoactive allotype

pair combinations found in AS patients could explain the involvement of ERAP1 in the disease [79]. However, such study suffered from low statistical power due to the small cohorts analysed.

Seminal work from Lopez de Castro's group has outlined how the B27 peptidome is influenced by ERAP allotypes [89,90]. The effect impacts mainly the P1 residue and, to a lesser extent, the remaining peptide sequence, the peptide length, the amount of specific ligands and the B27 affinity and thermostability of the overall peptide/B27 complexes. It is noteworthy that another study has shown that ERAP1 silencing, as expected, decreased the amount of B27-bound nonamer peptides and, interestingly, increased the number of longer ligands, especially with extended C-terminus [91].

A key point which is still the subject of debate is the relationship between the enzymatic activity of ERAP1 variants and the amount of cell surface B27 aberrant FHC/dimeric/oligomeric forms. A correlation between AS-protective Arg528 and Glu730 ERAP1 variants and a decreased surface expression of B27 FHC on monocytes from AS patients as well as on B27-expressing cell lines has been reported [92]. In contrast, a previous study documented a higher level of FHC induced by the AS-protective Glu730 ERAP1 variant, while no effect was attributed to Arg528Lys polymorphism [93].

There are also conflicting results on the impact of ERAP1 down-regulation, which was found to correlate with a lower expression of surface B27 FHC and, consequently, with a lower Th17 expansion [92]. Another report showed an opposite effect with a selective increase of FHC for AS-associated B*2705 and B*2704 subtypes but not for non-AS-associated B*2706 and B*2709 alleles on C1R transfectants [93]. Similarly, Tran and co-workers have observed an accumulation of disulphide-linked HLA-B27 dimers on U937 monocytic cell lines following ERAP1 knock-down, while levels of HLA-B18 and HLA-B51 were unaffected [94].

Current data do not allow to establish a link between ERAP1 trimming activity, intracellular B27 aberrant forms, cellular stress and disease. Dendritic cells derived from HLA-B27 patients with AS exhibited ERAP1 over-expression in comparison with healthy controls, but this did not parallel with an altered amount of overall HLA class I dimers [95]. Another study performed in HLA-B27-positive and HLA-B27-negative AS patients, in the presence of risk or protective ERAP1 variants, did not show significant differences in the expression of ER stress markers nor of proinflammatory cytokines, ruling out the ER stress as cause of disease [96].

A very recent work analysed the HLA-B27 peptidome from spleen cells of HLA-B27 transgenic rats in conditions of heterozygous or homozygous deletion of ERAP1 [97]. Interestingly, the knock-out genotype of ERAP1 altered approximately one-third of the B27 peptidome, but was still disease-permissive [97].

Unlike ERAP1, the association of ERAP2 with AS is independent from HLA-B27, occurring in both B27-positive and -negative carriers [98,99]. This finding allows speculation of a role for the two aminopeptidases not necessarily converging on the same mechanism. Notably, the ERAP2 null-variant rs2248374 is strongly protective for AS (Table 1) [99]. Hence, ERAP2 could be involved in the AS acting at two levels; that is, coupled or uncoupled with ERAP1. Accordingly, ERAP2 has been shown to influence directly the B*2705 peptidome, destroying some ligands with N-terminal basic residues and, indirectly, increasing the amount of nonamers through the enhancement of ERAP1 activity [100]. The latest work has demonstrated that the effects of ERAP2 on B27 peptidome could change depending on the amount of ERAP1 trimming [101]. The net consequence of ERAP2 presence/absence on HLA-B27 conformations remains to be determined. In one report, the presence of ERAP2 did not influence significantly the expression of folded and unfolded HLA-B27 molecules, ER stress markers and proinflammatory cytokines in patients *versus* controls [102]. In contrast, another study reported that the loss of ERAP2 induced an increase of FHC B27 level as well as up-regulation of the UPR pathway [103]. Overall, the molecular mechanisms underlying the influence of ERAP2 on AS risk are far from being understood.

HLA-B27 and ERAP1/2 interplay in the anti-viral immunity

One of the first studies investigating the role of aminopeptidases in anti-viral defence reported a genetic association of ERAP2 with natural resistance to HIV [104]. The notion that the relevance of ERAP is within the framework of HLA antigen presentation has been clearly supported by an *in vivo* study performed in a flu-infected murine model based on HLA-B27/ERAP$^{-/-}$ or HLA-B7/ERAP$^{-/-}$ transgenic mice that, unlike humans, express only one ERAP gene (ERAAP) [105]. Interestingly, the analysis of cytotoxic T cell responses directed against two influenza nucleoprotein immunodominant peptides restricted for HLA-B27 (NP) 383–391 and HLA-B7 (NP) 418–426, a protective allele for AS [106], demonstrated that only the HLA-B27-restricted response was ERAP-dependent and its absence led to the reduction of HLA-B27 molecules on the cell surface as well as of NP 383–391-reactive CD8$^+$ T cells [105].

In humans, studies performed *in vitro* on N-terminally extended precursors of naturally processed HLA-B27 antigens from human respiratory syncytial virus (HRSV) have shown that the two aminopeptidases operate in a concerted manner, each using the digestion products of the other as substrate for a further N-terminal cleavage [107].

Seregin and co-workers observed, using an *in vitro* cell-based antigen presentation system, that the high AS-risk

ERAP1 allotype (Met349/Lys528/Asp575/Arg725/Gln730) compared with the low AS-risk allotype (Val349/Arg528/Asn575/Gln725/Glu730) influenced antigen presentation by destroying more rapidly the majority of HLA-B27 peptides, whether from viral, bacterial or self-origin [108]. Hence, the authors speculated that the co-existence of B27 molecules and ERAP1 allotypes with enhanced enzymatic activity alters the normal presentation of microbial and self-peptides to the adaptive immune system setting the conditions to autoimmunity [108].

Another study has reported that the silencing of ERAP1 as well as the AS-protective allelic variant Arg528 reduced the presentation of the HIV-Gag immunodominant HLA-B27 epitope, KK10 [91]. Interestingly, the decreased CTL recognition of the cells expressing the Arg528 ERAP1 variant or the minigenes containing KK10 precursors was reversed by the combination Arg528/730Glu, supporting the concept that the global ERAP1 haplotype modulates the fine enzymatic specificity [91].

It is evident from the above that we still have a fragmented view of the role of ERAP1 and 2 in the HLA-B27-mediated adaptive immunity. It would be most interesting to assess whether the more effective protection against specific viral infections conferred by the HLA-B27 comes from a synergic interplay with particular ERAP1 and 2 haplotypes (Fig. 1).

Closing remarks

It is amazing that after so many decades of intense studies, the role of HLA-B27, the gene associated more strongly with AS pathogenesis, remains uncertain. The new entries, ERAP1 and ERAP2, point strongly at the shaping of the B27 peptidome as a crucial event. Genetic association studies suggest that ERAP1 allotypes with high trimming activity, together with the presence of ERAP2, are strong predisposing factors for AS. However, we still do not know whether or not they contribute directly by destroying the 'normal peptidome' or by generating harmful peptides. Alternatively, their activity can impact upon the B27 complex stability and, consequently, on the chance to form dimers or oligomeric structures. A deeper knowledge of the interplay between HLA-B27 and ERAP1/2 in tuning the antiviral response would certainly help in the understanding of their interconnection in autoimmune diseases.

Acknowledgements

The authors wish to thank Ceschina Foundation and Sapienza University of Rome (Progetti di Ateneo) for their financial support.

Disclosure

The authors declare no disclosures.

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