Human Immunology 80 (2019) 277–280

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Editorial

Endoplasmic reticulum aminopeptidases as a double-faced tool to increase or decrease efficiency of antigen presentation in health and disease

'Well, in OUR country,' said Alice, still panting a little, 'you'd generally get to somewhere else — if you ran very fast for a long time, as we've been doing.'
'A slow sort of country!' said the Queen. 'Now, HERE, you see, it takes all the running YOU can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!'
[Lewis Carol: "Through the Looking-Glass, and What Alice Found There" (1871)]

The vertebrate organisms (as well as all others) coexist not only with other members of their own species, but also with a great multiplicity of other species. Some of these are symbiotic or commensal microorganisms, others are infectious and nosogenic microbes and parasites, still others are providing food ingredients. All of them differ from us biochemically, and these differences are recognized by our immune system, both innate and adaptive. Infectious microorganisms and parasites try to escape this recognition by variation and molecular mimicry, and this drives the evolution of components of the immune system according to Red Queen Paradigm (RQP), i.e., an evolutionary race between infectious agents and molecules of the immune system [1–3]. As Jack [3] points out, "Host–pathogen interactions are classical RQPs—arms races in which the pathogens are forever evolving new virulence strategies while the host responds with ever more effective resistance mechanisms."

During their evolution, vertebrates "elaborated" a sophisticated immune system able to specifically recognize foreign (potentially harmful) molecules and distinguish them from self-components of their organism [4,5]. Infectious agents, particularly microbes, evolve very fast in response to the immune defenses of their hosts. The vertebrates, which have much lower speed of reproduction and cannot therefore compete with them by evolving equally fast, developed an extremely high variability of molecules of their immune system. The highest diversity exists in receptors of T and B lymphocytes, generated by somatic recombination of multiple gene segments; a single individual possesses millions of different lymphocyte clones with specific receptors for different antigens. The enormous diversity of receptors ensures successful recognition of all potential antigens [6,7]. For other elements of immune system, evolution found a different solution: very high polymorphism of major histocompatibility antigens, called human leukocyte antigens (HLA) in humans [8]. Numbers of HLA alleles now reach thousands (http://hla.alleles.org/nomenclature/stats.html). Thus, each HLA allele occurs with a low frequency in the population, usually less than 10% each [www.allelefrequencies.net], hence the difficulty in finding an unrelated histocompatible donor for transplantation. HLA molecules serve as antigen-presenting devices on the cell surface, with class II molecules (HLA-II) presenting extracellular antigens to CD4+ T cells, whereas class I molecules (HLA-I) present intracellular antigens to CD8+ T cells which may eradicate cells infected with intracellular pathogens as well as tumour cells [12,13]. It should be kept in mind, however, that HLA-I may also present peptides derived from extracellular proteins due to a process called "cross-presentation" [11]. The biological meaning of the high diversity of HLA genes and molecules lies in the fact that each HLA allotype binds and presents a different repertoire of antigenic peptides ("immunopeptidome"), derived from the extracellular sources in the case of HLA-II, and from the intracellular proteins in the case of HLA-I. Thus, the very high variety of antigen-presenting devices (HLA molecules) provides human (and other vertebrate) populations with a good chance to present highly immunogenic peptides, even from novel pathogens, allowing a sufficient proportion of individuals to be resistant to a given infection, cancer or autoimmune or autoinflammatory disease [8,12].

For HLA-I, peptides are produced in the cytosol by protein degradation in the proteasome or by other cytosolic proteases, and transported by the transporter associated with antigen processing (TAP) to the endoplasmic reticulum. There, they may be bound by nascent HLA-I molecules if they are of proper length and amino acid composition. HLA-I molecules preferentially bind peptides 8–10 amino acid (aa) long [14], whereas the proteasome produces peptides of a wider length distribution (2–25 aa [15] and TAP transports mostly longer peptides (preferentially 8–12 aa, but also, albeit less efficiently, up to 40 aa [16]), which then, if too long, must be trimmed in the endoplasmic reticulum to be bound by HLA-I. This trimming is executed by endoplasmic reticulum aminopeptidases (ERAPs) which are the topic of this special issue.

ERAPs in humans are encoded by two genes, *ERAP1* and *ERAP2*, located on chromosome 5q15 in opposite orientation [17,18]. Both these genes, particularly *ERAP1*, are polymorphic, and many single nucleotide polymorphisms (SNPs), silent or changing encoded amino acids, have been described [9,10]. SNPs within one gene, *ERAP1* or *ERAP2*, are in strong linkage disequilibrium (LD) resulting in ten most frequent *ERAP1* haplotypes comprising over 99% of population [19], but much weaker LD is observed between these two genes [20]. Some of these SNPs change enzymatic activity, substrate sequence and/or length specificity, or protein expression [12,18]. The most drastic effect is of the SNP rs2248374 in *ERAP2*: the A allele gives protein expression, whereas the G allele does not, because it causes nonsense-mediated decay of mRNA and the resulting lack of protein [21]. Both alleles are distributed with similar frequencies in most human populations, therefore about 25% (AA) are high ERAP2 expressors, 50% (AG) have lower ERAP2 levels, and about 25% are deprived of this enzyme [21].

https://doi.org/10.1016/j.humimm.2019.03.011

Available online 27 March 2019

Recently, another SNP (rs75862629) located between *ERAP1* and *ERAP2* genes, which inversely regulates expression of both, was described [22].

As stated above, several SNPs regulating activity and/or expression of ERAPs have been described. It is not surprising that they were also found associated with many immune-mediated diseases [9]. Indeed, effects of ERAP1 and ERAP2 gene polymorphisms on immunopeptidome were observed. These effects are most clear in the case of HLA-I alleles most strongly associated with autoimmune/autoinflammatory diseases (ankylosing spondylitis, Behcet disease, birdshot chorioretinopathy and psoriasis) [23].

CD8+ cytotoxic T lymphocytes, expressing clonally different T cell receptors (TCR) are selected and mature in the thymus (hence their name: Thymus-derived lymphocytes). This selection (positive [24] and negative [25]) is necessary to ensure recognition of foreign antigens presented by self MHC molecules, but simultaneously to avoid production of self-reactive, potentially auto-aggressive T cells [13]. Not surprisingly, ERAPs seem to play a role also in thymic selection by affecting the immunopeptidome of antigen-presenting cells in the thymus: *ERAP2* haplotype associated with the highest ERAP2 expression contained all risk alleles for autoimmune diseases and malignancies; however, no such phenomenon was observed for *ERAP1* [26,27]. Unfortunately, these studies have not been continued. Of note in this context, however, is the observation that *ERAP2* was found among other immune response-related genes which were significantly hypoexpressed in the thymuses of children with Down syndrome [28].

Here, in this special issue on “Biology of Endoplasmic Reticulum Aminopeptidases”, we have seven reviews written by top researchers in the field. First, **Tony Kenna and colleagues** [29] describe studies on crystal structures of ERAP1, ERAP2 and the related molecule, IRAP (insulin-regulated aminopeptidase). This latter molecule is active not in classical peptide processing for MHC-I binding in the endoplasmic reticulum, but is present in another intracellular compartment and involved in cross-presentation of internalized extracellular antigens by MHC-I molecules [11]. The crystal structures of ERAP1 revealed two conformations, open (substrate-receptive) and closed (catalytic). So far, only a closed conformation of ERAP2 and a semi-closed conformation of IRAP have been detected. Further on, the authors present functional effects of key *ERAP1* and *ERAP2* single nucleotide polymorphisms (SNPs) and diseases associated with these SNPs and strongly associated with specific *HLA-I* alleles, i.e., ankylosing spondylitis, psoriasis, birdshot chorioretinopathy and Behcet disease. Finally, they discuss recent experiments on inhibitors of ERAP1, ERAP2 and IRAP, potentially useful in treatment of *HLA-I*-associated immune-mediated diseases.

In the second review, **Irini Evnouchidou and Peter van Endert** [30], after a short review of literature on ERAP functions and autoimmune disease associations, present two models of the ERAP1 trimming mechanism, the “molecular ruler” model and the “MHC-I-template model”, and consider the pros and cons of each of them. ERAP1 preferentially trims peptides (9)10–14 amino acids long [9]. The “molecular ruler” model is based on detection of (i) the active site which binds the N-terminus of the peptide and trims the N-terminal amino acid (mostly hydrophobic [9]), and (ii) the regulatory site which binds a hydrophobic C-terminal amino acid and thereby stimulates the active site. On the other hand, in the “MHC-I-template model”, the C-terminus of the MHC-I-bound peptide remains buried in the MHC-I peptide-binding groove, while a partial dissociation from MHC-I exposes peptide N-terminus to trimming by ERAP1 [31–34]. The end result is an 8–9 amino acid-long peptide bound to MHC-I. Then, Evnouchidou and van Endert [30] present data on concerted trimming by an ERAP1-ERAP2 heterodimer, and its effect, stronger than that of each ERAP separately, on antigenic epitope presentation.

Then, **Arie Admon** [35] discusses the substrate preference of ERAP1 and its effect on the immunopeptidome, reviewing on this occasion the methods used for immunopeptidome analysis. He argues against the necessity of the ERAP-mediated trimming for a sufficient supply of HLA-I binding peptides. He notes that although many peptides are indeed trimmed by ERAPs, the majority do not need this to bind HLA-I. Nevertheless, he gives examples of disease associations of *ERAP1* and *ERAP2* alleles. These facts are not necessary contradictory, as an antigenic peptide contributing to a disease may require trimming, although the majority of peptides bound by a given HLA-I allotype may not.

In the next article, **Yuliya Pepelyayeva and Andrea Amalfitano** [36] discuss the differences and similarities between autoinflammatory and autoimmune human diseases which were originally thought to be associated with innate or adaptive immunity, respectively. However, the distinction between these two types of disease is not always so clear-cut: the contribution of innate and adaptive immune reactions in different diseases intermingle to different extent, and adaptive immunity is initiated by elements of the innate immune system. Then, the authors give a detailed review of the role of ERAP1 in these two types of diseases. Therefore, they focus on ERAP1 functions in both innate and adaptive immunity and how they may relate to autoinflammation and autoimmunity. This is followed by a detailed review of HLA-associated diseases (ankylosing spondylitis, Behcet disease, inflammatory bowel disease, insulin-dependent diabetes mellitus, multiplex sclerosis, and psoriasis). Although some of these diseases are generally considered as *HLA* class II-associated, a contribution of *HLA-I* genes was also reported recently, which explains associations of ERAP1 SNPs with them. In conclusion, the authors describe their own recent result showing that ERAP1 helps the immune system to maintain normal numbers of Tr1 regulatory cells, although the exact mechanism is still unknown.

ERAP2, sharing 49% homology with ERAP1 [37] and similar crystal structure [38], is nevertheless different from it in several aspects: (i) no open structure has been detected so far; (ii) in contrast to ERAP1, it does not possess a peptide C-terminal amino acid-binding site and therefore trims shorter peptides such as octamers, and its activity quickly decreases with longer substrates; and (iii) again in contrast to ERAP1, it trims not hydrophobic, but basic, especially Arg, residues [9]. In this issue, **Lopez de Castro and Stratikos** [39] review the current knowledge on ERAP2 structure and its differences from that of ERAP1. They explain the specificity of ERAP2 for the peptide N-terminal positively charged amino acid such as arginine by its interaction with a negatively charged glutamic acid residue in position 181 of the enzyme. Further on, the authors discuss the variation of ERAP2 single nucleotide and amino acid sequence (much less extensive than that of ERAP1) and its consequences for the enzyme expression and function. Next, they present current knowledge of ERAP2-disease associations and explain them by the effect of ERAP2 on the immunopeptidome in disease-associated HLA-I allotypes. They also give a hypothetical alternative explanation for the concerted action of ERAP1 and ERAP2: other than heterodimer augmenting activity of its components, they propose that non-productive substrates or products of one enzyme may be trimmed by the other one and vice versa, thus reciprocally modulating the activity of each other. Finally, Lopez de Castro and Stratikos deliberate about a possible use of ERAP2 inhibitors in the clinic – a topic not much exploited, as this enzyme was long considered to be only auxiliary for ERAP1 function.

As trimmers of precursors of antigenic peptides, ERAPs also play a role in the immune response against malignancies. Loss, retention or acquisition as well as imbalances of ERAP1 and ERAP2 expression in different solid tumor tissues as compared to normal counterparts has been observed [41]. Here, **Doriana Fruci and colleagues** [40] present the state of the art of ERAP expression in normal tissues and in cancer, put on the background of the ERAP functions. ERAP2 was less studied in this respect because of a lack of a homologous gene in mice, but *ERAP1*-deficient mouse models were extensively examined. These experiments revealed strong anti-tumor immune response when the host did not possess *ERAP1* but the tumor was positive and vice versa, proving that ERAP1 activity or its absence contributes to production of the repertoire of the tumor-specific immunopeptidome. Both *ERAP1* and *ERAP2* genes produce several transcript variants, resulting in two

different ERAP1 proteins and a lack of ERAP2 protein expression in about 25% of people. The effect of ERAP1 and ERAP2 activity on tumor biology is difficult to generalize, given the heterogeneous expression of both ERAP1 and ERAP2 in different human tumors. Somatic mutations of *ERAP* genes are rare, usually in less than 1% of tumors, but some non-silent mutations were found in more than one tumor. However, expression quantitative trait loci (eQTL) in humans that affect expression of ERAP1 and ERAP2 have been recently found to associate with immune infiltration of tumours and efficacy of immune checkpoint inhibitor therapy, marking these two genes as predictors of productive immune responses against cancer [27]. Finally, the effect of ERAP expression changes on natural killer cell activity against cancer is discussed.

The last review in this issue concentrates on the role of *ERAP1* and *ERAP2* gene polymorphisms in susceptibility to autoimmune, infectious and malignant diseases. Here, **Yao and colleagues** [42] give a wide spectrum of single nucleotide polymorphisms in *ERAP1* and *ERAP2* and frequencies of their alleles, as well as haplotypes composed of these SNPs, in all human populations examined so far. Then, they review in detail the diseases associated with these polymorphisms in different human populations. This is the most detailed and updated description of *ERAP* polymorphisms in different ethnicities in health and disease hitherto published.

In this issue, we have also three original reports on associations of *ERAP* polymorphisms with diseases.

The etiology of Behcet disease (BD), an inflammatory disorder which affects multiple parts of the body including recurrent relapsing oral and genital ulcers, ocular involvement, skin lesions, arthritis and vasculitis, is still unknown. Its association with *HLA-B*51* was described more than half a century ago and reproducibly confirmed since that time [43]. Efforts to discover other genes in the vicinity of *HLA* failed, indicating that their apparent associations with BD result from linkage disequilibrium with *HLA-B*51* [44]. This, however, does not exclude contribution of genes from other chromosomes. Indeed, several genes from other regions have been implicated, one of them being *ERAP1* [9,23,36,39,45]. *ERAP1* SNPs and their low-activity haplotype were confirmed as a strong risk factor for BD in *HLA-B*51*-positive individuals in a large (1900 patients and 1779 controls) Turkish cohort [46]. ERAP1 SNPs and haplotypes in the Spanish population displayed a similar trend but did not reach statistical significance (patient and control groups were much smaller than in the Turkish study [47]). Two *ERAP1* SNPs, rs1065407 and rs10050860, were found to protect against BD in Chinese, and this was associated with higher *ERAP1* mRNA expression [48]. Here, **Maria Carmela Padula** [49] and colleagues report on two new *ERAP1* polymorphisms, Glu183Val and Phe199Cys, detected only in a small group of 50 BD Southern Italian patients but not in 50 controls. Therefore, if they are present in healthy population at all, their frequency will be below 2%. All Phe199Cys-positive patients had also the Glu183Val polymorphism (both were in heterozygous combinations). Interestingly, the same authors previously described another novel ERAP1 variant, Arg53Pro, also only in BD patients [50]; however, it was present in other individuals not possessing the variants found here [49]. Minor alleles of previously known *ERAP1* SNPs rs30187, rs17482078 and rs27044 were significantly more frequent in patients than in controls. The authors also provided a 3-dimensional protein structure prediction and calculated that Val183 was associated with a more stable protein chain, while Cys199 destabilized the structure. It would be desirable to test much higher numbers of patients and healthy controls to assess the frequencies of these variations in BD and check whether they are present, although rare, in the normal population of Southern Italy.

Ankylosing spondylitis (AS) is another autoimmune/autoinflammatory disease strongly associated with *HLA-I*, *HLA-B*27* in this case. *ERAP1* and *ERAP2* polymorphisms are the second risk factor for this disease, as has already been documented beyond doubt [9,23,29,36,39]. Here, **Wiśniewski and colleagues** [51] present their results on *ERAP1* and *ERAP2* SNPs and their haplotypes in a relatively small (180 individuals) sample of Polish AS patients and almost three times higher number of controls. They confirm both the associations of *ERAP1* SNPs (rs2287987, rs30187, rs27044) and of high ERAP1 activity and presence of ERAP2 with AS, and protection by low active ERAP1 and lack of ERAP2. Similar effects of *ERAP1/ERAP2* haplotypes were observed in familial studies in Canada [52], but the present work is the first case-control study on such haplotypes in AS.

Human reproduction failures are growing problems in developed countries. Spontaneous abortion is the most common complication of human pregnancy, because women frequently decide to conceive later in life and delay motherhood. Approximately 10–15% of pregnancies end in miscarriage during the first trimester. Although most are sporadic and non-recurrent, there is a subset comprising 0.5–5% of couples who suffer from recurrent spontaneous abortion (RSA). As the foetus is semi-allogeneic for the mother, it must be tolerated by her immune system, as perceived 66 year ago [53] by 1960 Nobel prize winner Peter Medawar. On the other hand, natural killer cells, which constitute a majority of lymphocytes in decidua, should be stimulated to invade spiral arteries and remodel them in order to provide the foetus with sufficient amount of blood. This stimulation is achieved, among others, by recognition of paternal HLA-C by natural killer cell immunoglobulin-like receptors, KIRs [54]. It has been established in recent years that interactions with HLA-I of not only T lymphocytes via TCRs, but also NK cells through their KIRs, depends on the activity of ERAPs [55–57]. **Izabela Nowak's group** has already established *KIR* and *HLA-C* gene associations with RSA [58]. Here they exploit this topic further, adding *ERAP1* and *ERAP2* polymorphisms to their analysis [59]. What they found is very interesting: although *ERAP1* rs30187 alone had only a weak effect on susceptibility to RSA, it nevertheless was a risk factor together with the *HLA-C* C2 epitope or with the *KIR* Bx genotype, and an even stronger risk factor when rs30187, *HLA-C* and *KIR* risk alleles were all present. This result is particularly meaningful, because subdivision according to these markers drastically decreased the numbers of individuals, yet the p value was lowest and odds ratio highest when all three markers were present.

In the end, we should mention the synonyms of ERAPs which appear in the literature, particularly in early publications, and which may cause confusion for non-prepared readers. Thus, ERAP1 has been called also: adipose-derived leucine aminopeptidase (ALAP or A-LAP), aminopeptidase regulator of TNFR1 shedding (ARTS1), puromycin-insensitive leucyl-specific aminopeptidase (PILSAP), endoplasmic reticulum aminopeptidase associated with antigen processing (ERAAP; this abbreviation is most frequently used for murine ERAP1 equivalent), and KIAA0525 (https://www.omim.org/entry/606832?search=ERAP1&highlight=erap1). For ERAP2, less intensively studied, only one synonym was used: leukocyte-derived arginine aminopeptidase (LRAP) (https://www.omim.org/entry/609497?search=ERAP1&highlight=erap1).

Acknowledgments

We are very grateful to all the Authors who agreed to write the articles for our Special Issue. P.K. is supported by a grant OPUS 8 2014/15/B/NZ5/03517 from the Polish National Science Centre and 14/2019 from the Hirszfeld Institute of Immunology and Experimental Therapy.

References

[1] L. Van Valen, A new evolutionary law, Evol. Theory 1 (1973) 1.
[2] M.D. Daugherty, H.S. Malik, Rules of engagement: molecular insights from host-virus arms races, Annu. Rev. Genet. 46 (2012) 677.
[3] R.S. Jack, Evolution of immunity and pathogens, in: E. Hsu, L. Du Pasquier (Eds.), Pathogen-Host Interactions: Antigenic Variation v. Somatic Adaptations. Results and Problems in Cell Differentiation, vol. 57, in: D. Richter, H. Tiedge (Series Ed.), Springer, Cham Heidelberg New York Dordrecht London, 2015, p. 1.
[4] M.D. Cooper, M.N. Alder, The evolution of adaptive immune systems, Cell 124 (2006) 815.

[5] K.M. Yatim, F.G. Lakkis, A brief journey through the immune system, Clin. J. Am. Soc. Nephrol. 10 (2015) 1274.
[6] M. Kasahara. Variable lymphocyte receptors: a current overview, in: E. Hsu, L. Du Pasquier (Ed.), Pathogen-Host Interactions: Antigenic Variation v. Somatic Adaptations. Results and Problems in Cell Differentiation, vol. 57, in: D. Richter, H. Tiedge (Ed.), Springer, Cham Heidelberg New York Dordrecht London, 2015, pp. 175.
[7] J. Nikolich-Žugich, M.K. Slifka, I. Messaoudi, The many important facets of T-cell repertoire diversity, Nature Rev Immunol 4 (2004) 123.
[8] J. Klein, Natural History of the Major Histocompatibility Complex, John Wiley and Sons, New York, 1986.
[9] J.A. López de Castro, C. Alvarez-Navarro, A. Brito, P. Guasp, A. Martín-Esteban, A. Sanz-Bravo, Molecular and pathogenic effects of endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 in MHC-I-associated inflammatory disorders: towards a unifying view, Mol. Immunol. 77 (2016) 193.
[10] E. Stratikos, A. Stamogiannos, E. Zervoudi, D. Fruci, A role for naturally occurring alleles of endoplasmic reticulum aminopeptidases in tumor immunity and cancer pre-disposition, Front. Oncol. 4 (2014) 363.
[11] M. Embgenbroich, S. Burgdorf, Current concepts of antigen cross-presentation, Front. Immunol. 9 (2018) 1643.
[12] S.C. Meuer, S.F. Schlossman, E.L. Reinherz, Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions, Proc. Natl. Acad. Sci. USA 79 (1982) 4395.
[13] T.W. Mak, M.E. Saunders, The Immune Response. Basic and Clinical Principles, Elsevier, Amsterdam etc., 2006, pp. 26–28.
[14] J.S. Blum, P.A. Wearsch, P. Cresswell, Pathways of antigen processing, Annu. Rev. Immunol. 31 (2013) 443, https://doi.org/10.1146/annurev-immunol-032712-095910.
[15] R.E. Toes, A.K. Nussbaum, S. Degermann, et al., Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products, J. Exp. Med. 194 (2001) 1.
[16] J. Koopmann, M. Post, J.J. Neefjes, G.J. Hammerling, F. Momburg, Translocation of long peptides by transporters associated with antigen processing (TAP), Eur. J. Immunol. 26 (1996) 1720.
[17] A. Hattori, K. Kitatani, H. Matsumoto, S. Miyazawa, T. Rogi, N. Tsuruoka, S. Mizutani, Y. Natori, M. Tsujimoto, Characterization of recombinant human adipocyte-derived leucine aminopeptidase expressed in Chinese hamster ovary cells, J. Biochem. 128 (2000) 755.
[18] T. Tanioka, A. Hattori, S. Masuda, Y. Nomura, H. Nakayama, S. Mizutani, M. Tsujimoto, Human leukocyte-derived arginine aminopeptidase: the third member of the oxytocinase subfamily of aminopeptidases, J. Biol. Chem. 278 (2003) 32275.
[19] M.J. Ombrello, D.L. Kastner, E.F. Remmers, Endoplasmic reticulum-associated amino-peptidase 1 and rheumatic disease: genetics, Curr. Opin. Rheumatol. 27 (2015) 349.
[20] R. Cagliani, S. Riva, M. Biasin, M. Fumagalli, U. Pozzoli, S. Lo Caputo, et al., Genetic diversity at endoplasmic reticulum aminopeptidases is maintained by balancing selection and is associated with natural resistance to HIV-1 infection, Hum Mol. Genet 19 (2010) 4705.
[21] A.M. Andres, M.Y. Dennis, W.W. Kretzschmar, J.L. Cannons, S.-Q. Lee-Lin, B. Hurle, et al., Balancing selection maintains a form of ERAP2 that undergoes nonsense-mediated decay and affects antigen presentation, PLoS Genet. 6 (2010) e1001157.
[22] F. Paladini, M.T. Fiorillo, C. Vitulano, V. Tedeschi, M. Piga, A. Cauli, et al., An allelic variant in the intergenic region between ERAP1 and ERAP2 correlates with an inverse expression of the two genes, Sci. Rep. 8 (2018) 10398.
[23] J.A. Lopez de Castro, How ERAP1 and ERAP2 shape the peptidomes of disease-associated MHC-I proteins, Front. Immunol. 9 (2018).
[24] P. Kisielow, H.S. Teh, H. Bluthmann, H. von Boehmer, Positive selection of antigen-specific T cells in thymus by restricting MHC molecules, Nature 335 (1988) 730.
[25] P. Kisielow, H. Bluthmann, U.D. Staerz, M. Steinmetz, H. von Boehmer, Tolerance in T-cell receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes, Nature 333 (1988) 742.
[26] I.S.M. Gabrielsen, M.K. Viken, S.S. Amundsen, H. Helgeland, K. Holm, S.T. Flåm, B.A. Lie, Autoimmune risk variants in *ERAP2* are associated with gene-expression levels in thymus, Genes Immunity 17 (2016) 406.
[27] Y.W. Lim, H. Chen-Harris, O. Mayba, S. Lianoglu, A. Wuster, T. Bhangale, et al., Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration, PNAS 115 (2018) E11701.
[28] F.A. Lima, C.A. Moreira-Filho, P.L. Ramos, H. Brentani, L.A. Lima, M. Arrais, et al., Decreased AIRE expression and global thymic hypofunction in Down syndrome, J. Immunol. 187 (2011) 3422.
[29] A.L. Hanson, C.J. Morton, M.W. Parker, D. Bessette, T.J. Kenna, The genetics, structure and function of the M1 aminopeptidase oxytocinase subfamily and their therapeutic potential in immune-mediated disease, Hum. Immunol. (2019).
[30] I. Evnouchidou, P. van Endert, Peptide trimming by endoplasmic reticulum aminopeptidases: Role of MHC class I binding and ERAP dimerization, Hum. Immunol. (2019).
[31] S.-C. Chang, F. Momburg, N. Bhutani, A.L. Goldberg, The ER aminopeptidase, ERAP1, trims precursors to lengths of MHC class I peptides by a "molecular ruler" mechanism, PNAS 102 (2005) 17107.
[32] A. Mpakali, Z. Maben, L.J. Stern, E. Stratikos, Molecular pathways for antigenic peptide generation by ER aminopeptidase 1, Mol. Immunol. (2018), https://doi.org/10.1016/j.molimm.2018.03.026 in press.
[33] A. Papakyriakou, E. Stratikos, The role of conformational dynamics in antigen trimming by intracellular aminopeptidases, Front. Immunol. 8 (2017) 946.
[34] A. Papakyriakou, E. Reeves, M. Beton, H. Mikolajek, L. Douglas, Grace Cooper, et al., The partial dissociation of MHC class I-bound peptides exposes their N terminus to trimming by endoplasmic reticulum aminopeptidase 1, J. Biol. Chem. 293 (2018) 7538.
[35] A. Admon, ERAP1 shapes just part of the immunopeptidome, Hum. Immunol. (2019).
[36] Y. Pepelyayeva, A. Amalfitano, The role of ERAP1 in autoinflammation and autoimmunity, Hum. Immunol. (2019).
[37] M. Tsujimoto, A. Hattori, The oxytocinase subfamily of M1 aminopeptidases, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids 1751 (2005) 9.
[38] A. Mpakali, P. Giastas, N. Mathioudakis, I.M. Mavridis, E. Saridakis, E. Stratikos, Structural basis for antigenic peptide recognition and processing by endoplasmic reticulum (ER) aminopeptidase 2, J. Biol. Chem. 290 (2015) 26021.
[39] J.A. Lopez de Castro, E. Stratikos, Intracellular antigen processing by ERAP2: Molecular mechanism and roles in health and disease, Hum. Immunol. (2019).
[40] M. Compagnone, L. Cifaldi, D. Fruci, Regulation of ERAP1 and ERAP2 genes and their disfunction in human cancer, Hum. Immunol. (2019).
[41] D. Fruci, P. Giacomini, M.R. Nicotra, M. Forloni, R. Fraioli, et al., Altered expression of endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 in transformed non-lymphoid human tissues, J. Cell. Physiol. 216 (2008) 742.
[42] Y. Yao, N. Liu, Z. Zhou, L. Shi, Influence of ERAP1 and EARP2 gene polymorphisms on disease susceptibility in different populations, Hum. Immunol. (2019).
[43] M. De Menthon, M.P. LaValley, C. Maldini, L. Guillevin, A. Mahr, HLA–B51/B5 and the risk of Behc¸et's disease: a systematic review and meta-analysis of case–control genetic association studies, Arthritis Rheum. 61 (2009) 1287.
[44] N. Mizuki, M. Ota, K. Yabuki, Y. Katsuyama, H. Ando, G.D. Palimeris, et al., Localization of the pathogenic gene of Behcet's disease by microsatellite analysis of three different populations, Invest. Ophthalmol. Vis. Sci. 41 (2000) 3702.
[45] H. Petrushkin, M.S. Hasan, M.R. Stanford, F. Fortune, G.R. Wallace, Behçet's disease: do natural killer cells play a significant role? Front. Immunol. 6 (2015) 134.
[46] M. Takeuchi, M.J. Ombrello, Y. Kirino, B. Erer, I. Tugal-Tutkun, E. Seyahi, et al., A single endoplasmic reticulum aminopeptidase-1 protein allotype is a strong risk factor for Behçet's disease in HLA-B*51 carriers, Ann. Rheum. Dis. 75 (2016) 2208.
[47] M. Conde-Jaldon, M.A. Montes-Cano, J.R. Garcia-Lozano, L. Ortiz-Fernandez, N. Ortego-Centeno, R. Gonzalez-Leon, et al., Epistatic interaction of ERAP1 and HLA-B in Behçet disease: a replication study in the spanish population, PLoS ONE 9 (2014) 1.
[48] L. Zhang, H. Yu, M. Zheng, H. Li, Y. Liu, A. Kijlstra, P. Yang, Association of ERAP1 gene polymorphisms with Behcet's disease in Han Chinese, Invest. Ophthalmol. Vis. Sci. 56 (2015) 6029.
[49] M.C. Padula, P. Leccese, N. Lascaro, T. Carbone, M. Gilio, A.A. Padula, G. Martelli, S. D'Angelo, Genotyping of Italian patients with Behçet syndrome identified two novel ERAP1 polymorphisms using sequencing-based approach, Hum. Immunol. (2019).
[50] M.C. Padula, P. Leccese, A.A. Padula, S. D'Angelo, G. Martelli, ERAP1 molecular characterization: identification of a de novo allelic variant, HLA 92 (2018) 44.
[51] A. Wiśniewski, S. Kasprzyk, E. Majorczyk, I. Nowak, K. Wilczyńska, A. Chlebicki, et al., ERAP1-ERAP2 haplotypes are associated with ankylosing spondylitis in Polish patients, Hum. Immunol. (2019).
[52] F.W. Tsui, N. Haroon, J.D. Reveille, P. Rahman, B. Chiu, H.W. Tsui, R.D. Inman, Association of an ERAP1 ERAP2 haplotype with familial ankylosing spondylitis, Ann. Rheum. Dis. 69 (4) (2010) 733.
[53] P.B. Medawar, Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates, Symp. Soc. Exp. Biol. 7 (1953) 320.
[54] P. Parham, NK cells and trophoblasts: partners in pregnancy, J. Exp. Med. 200 (2004) 951.
[55] H.G. Hilton, P. Parham, Missing or altered self: human NK cell receptors that recognize HLA-C, Immunogenetics 69 (2017) 567.
[56] M.J. Sim, S.A. Malaker, A. Khan, et al., Canonical and cross-reactive binding of NK cell inhibitory receptors to HLA-C allotypes is dictated by peptides bound to HLA-C, Front. Immunol. 8 (2017) 193.
[57] C.A. Stewart, F. Laugier-Anfossi, F. Vely, et al., Recognition of peptide–MHC class I complexes by activating killer immunoglobulin-like receptors, Proc. Natl. Acad. Sci. USA 102 (2005) 13224.
[58] I. Nowak, A. Malinowski, H. Tchórzewski, E. Barcz, J.R. Wilczyński, M. Banasik, et al., HLA-C C1C2 heterozygosity may protect women bearing the killer immunoglobulin-like receptor AA genotype from spontaneous abortion, J. Reprod. Immunol. 88 (1) (2011) 32.
[59] K. Wilczyńska, A. Wiśniewski, A. Malinowski, E. Barcz, J.R. Wilczyński, P. Kuśnierczyk, I. Nowak, ERAP, KIR and HLA-C gene interaction in susceptibility to recurrent spontaneous abortion in the Polish population, Hum. Immunol. (2019).

Piotr Kuśnierczyk*
Laboratory of Immunogenetics and Tissue Immunology, The Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
E-mail address: piotr.kusnierczyk@hirszfeld.pl.

Efstratios Stratikos
National Centre for Scientific Research Demokritos, Agia Paraskevi, Athens, Greece

* Corresponding author.