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The role of ERAP1 in autoinflammation and autoimmunity

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ABSTRACT

Autoimmune and autoinflammatory diseases affect millions worldwide. These classes of disease involve abnormal immune activation of both the innate and adaptive immune systems. While both classes of disease represent a spectrum of aberrant immune activation, excessive activation of the innate immune system has been considered causal for the inflammation and tissue damage found in autoinflammatory diseases, while excessive activation of the adaptive immune system has been thought to primarily contribute to end-organ symptoms noted in autoimmune diseases. Interestingly, the endoplasmic reticulum aminopeptidase 1 (ERAP1) protein, well known for its aminopeptidase function as a "molecular ruler", trimming peptides prior to their loading onto MHC-I molecules for antigen presentation in the ER, has also been shown to be genetically associated with both autoinflammatory and autoimmune diseases. Indeed, this multifaceted protein has been found to have many functions that affect both the innate and adaptive immune responses. In this review, we summarize these findings, with an attempt to identify the possible ERAP1 dependent mechanisms responsible for the pathogenesis of multiple, ERAP1 associated diseases.

1. Introduction

Historically, immune-mediated diseases could be sub-classified as disorders of primarily the innate or adaptive immune systems, resulting in autoinflammatory and autoimmune diseases, respectively [1]. Interestingly, it is now thought that the autoinflammatory and autoimmune diseases lie on a spectrum of immune activation, where the contribution from the innate and adaptive immune systems varies from one disease to another, rather than being purely due to excessive activation of only the innate or adaptive immune systems *per se* [2]. In support of this, there is increasing evidence that in both autoinflammatory and autoimmune diseases there is an inappropriate activation of the innate immune system. In autoinflammatory diseases, the overactive innate immune system directly causes inflammation and damage, while in autoimmune diseases, the abnormal activation of the innate immune system is the initial step that leads to pathogenic adaptive immune responses ultimately responsible for end-organ tissue damage.

It is thought that during the earliest phases of onset of the autoimmune diseases, innate immune responses are initiated via pathogen recognition receptors (PRRs) such as toll-like receptors (TLRs) and inflammasomes, that then activate both innate immune cells such as dendritic cells (DCs) and macrophages, as well as adaptive immune cells such as B and T cells [3]. The later phases of autoimmune diseases involve adaptive immune activation where autoantibodies and autoreactive T cell-mediated responses prevail [1]. Increasingly, it is recognized that inflammasome activation may be the connecting link between autoinflammation and autoimmunity given their importance for activation of not only the innate immune cells but also activation of autoreactive B cells and T cell polarization [3]. Dysregulation of Th17

Abbreviations: AIM2, absent in melanoma 2; AS, ankylosing Spondylitis; BD, Behcet's disease; CARD, caspase activation and recruitment domains; CARD8, caspase recruitment domain-containing protein 8; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DSS, dextran sodium sulfate; EAE, experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; ERAP1, endoplasmic reticulum aminopeptidase 1; GWAS, genome-wide association studies; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; IDDM, insulin dependent diabetes mellitus; IL-1RII, type II IL-1 decoy receptor; KIR, killer cell Ig-like receptor; LIR, leukocyte Ig-like receptor; MHC-I, major histocompability complex class I; MS, Multiple sclerosis; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa B; NK, natural killer; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; NOD2, nucleotide-binding oligomerization domain containing 2; PBMC, peripheral blood mononuclear cell; PRR, pathogen recognition receptor; SI, sacroiliac; SNP, single nuclear polymorphism; TAP, transporter associated with antigen processing; tDCs, tolerogenic dendritic cells; TLR, toll-like receptor; TNBS, trinitrobenzene sulfonic acid; Tregs, regulatory T cells; UPR, unfolded protein response

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cells is also important in the pathogenesis of autoinflammation and autoimmunity, for example, TGF-β, IL-6, and IL-23 promote Th17 differentiation, and IL-27 downregulates Th17 cells via IL-10 production.

There is increasing evidence for inflammasome involvement in autoinflammatory and autoimmune diseases, where inflammasomes are thought to play initial key roles in innate immune system mediated tissue damage as well as the activation of adaptive immune responses. In autoinflammatory diseases, particular attention has been given to the inflammasome-mediated pathways required for caspase-1 activation and IL-1β production. For example, several single nuclear polymorphisms (SNPs) in nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) have been identified to increase susceptibility to Crohn's disease [4]. Indeed, enhanced inflammasome activation and IL-1 β production have been shown in patients with either Crohn's or long-standing ulcerative colitis [5]. Additionally, polymorphisms in NLRP3, the P2X7 receptor, and NLRP1 have also been shown to be associated with rheumatoid arthritis, multiple sclerosis (MS), systemic lupus erythematosus, and other autoimmune diseases [6].

Interestingly, genome-wide association studies (GWAS) have also uncovered associations between endoplasmic reticulum aminopeptidase 1 (ERAP1) gene polymorphisms and increased susceptibility to both autoinflammatory and autoimmune diseases [7–10]. Therefore, investigating ERAP1 dependent mechanisms in these diseases may further enlighten the complex interactions between the innate and adaptive immune systems specifically, as well as foster understanding to the development of autoinflammatory and autoimmune diseases generally. In this review, an overview of known ERAP1 functions is provided with a particular focus on its functions in innate and adaptive immunity and how these functions might relate to autoinflammation and autoimmunity.

2. ERAP1 biological functions

2.1. ERAP1 background

The ERAP1 protein belongs to the oxytocinase subfamily of M1 zinc metallopeptidases, and functions as a molecular ruler of the peptides destined for antigen display by major histocompatibility complex class I (MHC-I) molecules [11]. Specifically, upon degradation of cytosolic proteins by the proteasome, peptides are transported into the endoplasmic reticulum (ER) via transporter associated with antigen processing (TAP). ERAP1 then trims the N-terminus of peptides that are 9–16 amino acids long down to the optimal size of 8–10 amino acids for loading onto awaiting MHC-I; antigen-loaded MHC-I complexes are then shuttled to the cell surface for antigen presentation [12].

Multiple polymorphisms in the *ERAP1* gene have been found to be associated with autoimmune and autoinflammatory diseases through genome-wide association studies (GWAS). In 2007, the Welcome Trust Case Control Consortium and Australo-Anglo-American Spondylitis Consortium identified five SNPs (rs27044, rs17482078, rs10050860, rs30187, rs2287987) in the *ERAP1* gene to be associated with Ankylosing Spondylitis (AS) [8]. Interestingly rs30187 SNP was also found to be associated with insulin dependent diabetes mellitus IDDM [7], Crohn's disease and MS [13]. Meanwhile, rs17482078 and rs10050860 have been shown to be associated with Behcet's disease [9].

2.2. ERAP1 and adaptive immunity

It is well-accepted that ERAP1 affects antigen presentation, via its peptide trimming activity [14–17]. The rs2287987 polymorphism is located at the active site, rs30187 and rs10050860 are located at the domain junctions, while rs27044 and rs17482078 are located on the inner surface of the peptide-binding cavity of ERAP1 [18]. These polymorphisms affect substrate specificity and catalytic activity of ERAP1 in a substrate-dependent manner. Our groups have demonstrated that depending on the ERAP1 allele, the trimming efficiency of ERAP1 changes, thereby affecting not only the availability of specific peptides for MHC-I loading, but also affecting the surface levels of MHC-I globally. Specifically, when *ERAP1* alleles harboring SNPs associated with a high risk for developing AS were co-expressed with an AS-associated MHC-I gene [19], human leukocyte antigen *(HLA)-B*27*, there was a reduction in the surface levels of HLA-B*27, as compared to ERAP1 alleles containing AS protective SNPs [20]. In our survey of ERAP1 allele activity, we showed that the two high AS risk associated variants of ERAP1 (rs30187 and rs27044) had differing effects on enzymatic activity and substrate specificity [21], where one reduced and the other increased ERAP1 catalytic activity, but ultimately they both resulted in reduced HLA-B*27 surface expression levels [18].

Reeves et al. also showed that ERAP1 allele combinations seen in AS patients have a reduced ability to generate peptides that ultimately results in reduced overall cell surface levels of HLA-B*27 and MHC-I in general [22]. All AS combinations resulted in reduced peptide availability, regardless of whether the ERAP1 SNPs yielded reduced or increased peptidase activity of ERAP1, as both under-trimming and over-trimming activities ultimately lowered the availability of correct sized peptides for MHC-I display. This suggests that in diseases associated with ERAP1, its aminopeptidase activity plays at least a partial role in their pathogenesis, where ERAP1 activity needs to be "just right" to avoid a disease process as the presence of specific ERAP1 SNPs influences its activity and peptide specificity. We have also shown that a complete absence of ERAP1 results in the reduction of MHC-I surface levels [23], as well as completely shifts peptide immunodominance by dictating which peptides are ultimately presented by MHC-I to cytotoxic T lymphocytes (CTLs) [24]. Mice expressing a human *ERAP1* gene containing high AS risk SNPs were also found to generate unique antigen-specific T cell clones upon vaccination with foreign antigens, as compared to mice expressing an AS protective human *ERAP1* allele [25]. We also confirmed that human ERAP1 alleles also ultimately determined the overall surface MHC-I levels in these mice.

In our survey of ERAP1$^{-/-}$ mice, we recently detected reduced levels of type 1 regulatory (Tr1) T cells in their spleens [26]. Tr1 cells are Foxp3- CD4 + T cells and are a less well studied immunosuppressive class of regulatory T cells, as compared to, for example, Foxp3 + regulatory T cells (Tregs). Tr1 cells are characterized by high levels of IL-10 and TGF-β cytokine secretion [27]. The presence of Tr1 cells and their normal function are essential for attenuation of tissue inflammation and prevention of autoimmunity, indeed Tr1s have also been implicated in the pathogenesis of a variety of inflammatory diseases including IDDM, inflammatory bowel disease (IBD) and MS, diseases also associated with ERAP1 (see below) [28].

2.3. ERAP1 and innate immunity

Interestingly, ERAP1 has also been shown to have a direct role in innate immunity and may contribute to autoinflammatory and autoimmune diseases via additional functions [29,30]. For example, ERAP1 is involved in proteolytic cleaving of several cytokine receptors, including TNF-R1, IL-6Rα and type II IL-1 decoy receptor (IL-1RII) [31–33]. This shedding function of ERAP1 allows for regulation of receptor availability on cell surfaces, thereby mediating immune responses. However, it is important to note that the shedding function of ERAP1 has not been confirmed in ERAP1 knockout mice and soluble levels of receptors have been shown to be independent of ERAP1 polymorphisms in AS patients [34].

Overexpression of the *ERAP1* gene in COS-7 fibroblast-like cells has been shown to induce its secretion [35], suggesting that in certain conditions, ERAP1 may function as a soluble protein in the extracellular milieu or circulation. One group confirmed that ERAP1 can be secreted from cells after LPS and IFN-γ stimulation [29]. Interestingly, while ERAP1 does not have an ER-retention sequence, it has been shown that

exon 10 contains an amino acid sequence important for its retention in the ER [36]. The authors hypothesized that certain proteins might bind to the exon 10 sequence allowing for its retention in the ER, suggesting that saturation of the binding sites may cause secretion of ERAP1 outside the cells. It is also possible that there are other ER proteins, which compete for the same binding sites as ERAP1, allowing for ERAP1 secretion to take place; however, no such proteins have been identified to date.

ERAP1 secretion from macrophages can be mediated after activation of various TLRs, including TLR1, TLR2, TLR4, TLR6 and TLR9 in myeloid differentiation primary response 88 (MyD88)- and nuclear factor kappa B (NF-kB)- dependent manners, via the calmodulin pathway [37]. Furthermore, TLR-induced secretion of ERAP1 is mediated via IFN-γ, IFN-β, and TNF-α, where all three cytokines are needed for maximal ERAP1 secretion. Once ERAP1 is secreted by the macrophages, it stimulates their phagocytic activity [29].

Based on these results our lab further investigated the function of secreted ERAP1 on immune cells. We have shown that extracellular functions of ERAP1 are dependent on its aminopeptidase activity and do not require re-uptake by the cells to elicit ERAP1's extracellular effects [30]. Exposure of human peripheral blood mononuclear cells (PBMCs) to extracellular ERAP1 protein variants resulted in activation of natural killer (NK) cells, DCs, and T cells, as well as enhanced production of cytokines and chemokines, through mechanisms involving the NLRP3 inflammasome and cathepsin B pathways. Interestingly, the disease associated ERAP1 variants had differing effects upon PBMCs, where exposure to AS predisposing variants (rs30187 and rs27044) enhanced production of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 and enhanced T cell and NK cell activation of treated human PBMCs. This suggests that polymorphisms in *ERAP1* may predispose individuals to autoinflammatory and autoimmune diseases at least in part due to their effects on ERAP1's extracellular functions.

ERAP1 functions are also important in NK cell biology. Our group has extensively studied ERAP1$^{-/-}$ mice, and we have shown that NK cells from the ERAP1$^{-/-}$ mice exhibit increased activation in response to innate immune stimuli [38]. Evaluation of the splenic NK cells from ERAP1$^{-/-}$ mice revealed that ERAP1$^{-/-}$ mice have increased numbers of licensed, and terminally mature NK cells. Additionally, compared to identical innate immune stimulations of splenocytes derived from WT mice, stimulation of splenocytes from ERAP1$^{-/--}$ mice resulted in increased levels of NK activation markers and proinflammatory cytokine secretion including TNF-α, IL-6, and IL-1β, all of which are also implicated in autoinflammation and autoimmunity [38]. Moreover, it was shown that loss of ERAP1 in human cell lines enhanced their killing by NK cells via interaction between MHC-I and inhibitory NK cell receptors, killer cell Ig-like receptors (KIR) and CD94/NKG2A [39].

Recently we have shown that the absence of ERAP1 also results in reduction of a specific subtype of DCs known as tolerogenic dendritic cells (tDCs), identified as CD11c^{low}CD45RB^{high} cells [40]. These cells are unique in their immature phenotype and in their ability to secrete high amounts of IL-10 and are important for Tr1 cell differentiation and function [26]. It is evident that beyond its function in antigen processing, ERAP1 also functions as a modulator of the innate immune responses, a function which may play a role in the pathogenesis of ERAP1-associated diseases.

3. Role of ERAP1 in human diseases

3.1. Ankylosing Spondylitis

AS is a highly heritable chronic autoinflammatory disease affecting the axial skeleton. It belongs to the family of related immune-mediated arthropathies – spondyloarthritides which also includes reactive arthritis, psoriatic arthritis, enteropathic arthritis, Crohn's disease, undifferentiated spondyloarthritides and juvenile-onset spondyloarthritis [41]. AS affects an estimated 0.9–1.4% of the adult population in the U.S. [42]. AS primarily affects the spine and the sacroiliac (SI) joints, with sacroiliitis being its hallmark feature, being present early on during the disease's progression [43]. The progression of AS is next characterized by excessive osteoproliferation and syndesmophyte formation between adjacent vertebrae, which ultimately results in joint fusion [44]. AS has a wide range of manifestations, including spinal inflammation, chronic back pain, sacroiliitis and loss in the range of motion in the lumbar and thoracic spines [45]. Another common feature of AS is osteoporosis of the trabecular bone of the vertebral bodies, making them susceptible to vertebral fractures and spinal cord injuries [46,47]. Peripheral arthritis, acute anterior uveitis, and inflammatory bowel disease are also prevalent in AS patients, significantly impacting their quality of life [48]. The age of onset of AS symptoms can be in the teen to early twenties [49], although it is estimated that the diagnosis of AS is delayed by 8–10 years due to its gradual progression and ambiguous presentation [50].

In 1973, *HLA-B*27* was the first gene found to be associated with AS; 88% of AS patients were found to be *HLA-B*27* positive [51,52]. Since then multiple other HLA-B alleles have also been found to be associated with AS, including *HLA-B*13:02*, *HLA-B*40:01*, *HLA-B*40:02*, *HLA-B*47:01*, and *HLA-B*51:01*. Meanwhile, *HLA-B*07:02* and *HLA-B*57:01* are protective against AS [19]. *HLA-B*27:05*, *HLA-B*27:02*, *HLA-B*27:04*, and *HLA-B*27:07* are the most common subtypes that have been shown to be associated with increased risk of AS, while two subtypes *HLA-B*27:06* and *HLA-B*27:09* are not associated [53]. *HLA-B*27* accounts for 20–50% of the total genetic susceptibility of AS [48]. Interestingly, while 90% of AS patients are *HLA-B*27* positive, the risk of developing AS in *HLA-B*27* positive individuals is only about 5–10%, suggesting the involvement of other genes in the susceptibility of this disease. In 2007, five SNPs: rs27044, rs17482078, rs10050860, rs30187, rs2287987 in the *ERAP1* gene were found to be associated with AS [8]. This study estimated that 26% of the risk in the development of AS is attributable to ERAP1. There is an epistatic gene-gene interaction between specific *ERAP1* variants and *HLA-B*27* gene, where they together increase the risk of developing AS [19,54]. This finding makes sense molecularly, as ERAP1 and HLA-B*27 functionally interact due to their involvement in the same molecular pathway of antigen presentation, and suppression of ERAP1 has been shown to increase the levels of free heavy chains and surface HLA-B*27 bound to extended peptides [55].

Despite the knowledge of *HLA-B*27*'s association with AS for over 40 years, we still do not fully understand the molecular mechanism through which it contributes to the pathogenesis of AS. Several hypotheses explaining the role of HLA-B*27 in AS have been proposed. The arthritogenic peptide hypothesis proposes that arthritis-causing peptides derived from a pathogen are preferentially presented by HLA-B*27 and are recognized by autoreactive CD8+ T cells resulting in chronic inflammation and autoimmunity due to microbial mimicry [56]. This hypothesis is, however, weakened by the fact that HLA-B*27 transgenic rats still developed colitis and arthritis in the absence of CD8+ T cells [57]. The homodimer hypothesis is based on the propensity of HLA-B*27 molecules to misfold and form heavy chain homodimers [58]. HLA-B*27 has been shown to form free heavy chains and homodimers on PBMCs. These free heavy chains and homodimers have been shown to be recognized by KIR and Leukocyte Ig-like receptor (LIR) receptors with higher affinity than HLA-B*27 [59]. Activation of these receptors can enhance T and NK cell responses. According to the misfolding hypothesis, the propensity of HLA-B*27 to misfold causes its aggregation in the ER, which in turn activates the unfolded protein response (UPR) and ER overload response, which induce proinflammatory responses [60–62]. Interestingly, UPR activation enhances IL-23 secretion, overexpression of the latter has been shown to induce inflammation at the enthesis [63], which is thought to be the primary site of inflammation that gives rise to ankylosis in AS [64].

In our recently published work, we have shown that mice lacking

the *ERAP1* gene develop key skeletal and intestinal features of ankylosing spondylitis [40]. Specifically, global investigations of ERAP1$^{-/-}$ mice confirmed that they developed spontaneous spinal inflammation, ankylosis at the lumbosacral region, osteoporosis of the trabecular bone, intestinal dysbiosis and increased susceptibility to chemically induced colitis. Moreover, ERAP1$^{-/-}$ mice had significantly reduced tDCs and Tr1 T cells in their spleens. Given the role of Tr1 cells in maintenance of tolerance, it is tempting to hypothesize that the reduction in the abundance of these cells in ERAP1$^{-/-}$ mice, may be the cause of dysbiosis, susceptibility to colitis and the spinal inflammatory infiltrates in these mice, and by inference in the human AS.

Exactly how ERAP1 plays a role in normal Tr1 cell biology, and how this could be contributing to the pathogenesis of AS is currently unclear. It is known that HLA-G is important for the development of tDCs, which in turn are required for Tr1 cell development [65]. In fact, patients with reduced HLA-G levels have been shown to have deficits in tDCs and Tr1 cells [66]. It has been noted that silencing of ERAP1 in trophoblastic cells reduces HLA-G expression levels [67]. Possibly, ERAP1 may play a role in Tr1 cell development via its function as a molecular ruler, a function that ultimately influences overall HLA-G surface expression levels. In agreement with this, we observed reduced levels of Qa-2, a functional homolog of HLA-G [68], in splenic tDCs and macrophages from ERAP1$^{-/-}$ mice [40]. Future studies of the interactions between ERAP1, HLA-G, and Tr1 cell biology are warranted. It is also possible that other ERAP1 functions such as receptor shedding activity, inflammasome pathway regulation, as well as induction of UPR and ER stress response as a result of reduced availability of peptides available for loading onto MHC-I complexes are all contributing to the human disease.

It is important to note that interpretation of the results from mouse studies and their translation to humans is complicated by the genetic and immunological difference between these two species. One such difference is the existence of single *ERAP1* gene in mice, compared to two distinct ERAP genes in humans, *ERAP1* and *ERAP2* which are thought to have resulted from the *ERAP* gene duplication and have been reported to form heterodimers which have enhanced peptidase function *in vitro* [69]. Additionally, the use of global ERAP1$^{-/-}$ mice makes interpretation of the results complicated. There are no documented cases of *ERAP1* deletions in humans, making it possible that complete deficiency or ERAP1 in humans would be lethal, therefore phenotypes observed in ERAP1$^{-/-}$ mice could be maximal compared to what would normally be observed in humans secondary to the AS-associated SNPs. Moreover, to specifically evaluate the function of ERAP1 in the mature immune system and eliminate the specific effect of ERAP1 in the shaping and development of the immune system, the use of inducible ERAP1$^{-/-}$ mice, where the *ERAP1* gene is turned off once the mice are immunologically mature would be useful. Altogether, one must keep these matters in mind when interpreting murine data and translating it to human diseases.

3.2. Behcet's disease

Behcet's disease (BD) is a multisystemic autoinflammatory disease on unknown etiology which presents with oral/genital ulcers, systemic vasculitis, ocular, gastrointestinal and skin inflammation, with an age of onset at 30–40 years of age [70]. It is strongly associated with *HLA-B*51* and less strongly with *HLA-A*26*, *HLA-B*15*, *HLA-B*57* and *HLA-B*27* [71]. Meanwhile, the presence of the *HLA-A*03* and *HLA-B*49* variants have been shown to be protective. Two *ERAP1* SNPs have been found to be associated with Behcet's, namely rs17482078 and rs10050860 [9]. Moreover, there is an epistatic effect between *ERAP1* rs17482078 and *HLA-B*57* [9,72]. Low activity ERAP1 variant rs17482078 co-expression with HLA-B*57 *in vitro* resulted in altered peptide generation with lower affinity for HLA-B*57 compared to other, ERAP1 variants, not associated with Behcet's [73]. Epistatic and functional interaction between ERAP1 variants and MHC-I in BD suggests that it is ERAP1's aminopeptidase activity that is also responsible for the underlying mechanism of the pathogenesis in this disease. Proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IL-23 are thought to play a major role in the pathogenesis of Behcet's based on GWAS results, findings of cytokines at the sites on inflammation and damage, and based on positive clinical responses in patients treated with cytokine blocking agents [70]. Meanwhile, IL-10 is thought to have a protective role in BD. ERAP1's role in activation of innate immune cells and activation of the inflammasome may be related to the enhanced cytokine levels observed in BD. PBMCs from patients with active Behcet's disease have been shown to have reduced IL-27 mRNA and reduced IL-27 levels in their sera supernatants from cultured PBMCs [74]. Supplementation of IL-27 promoted secretion of IL-10 by DCs and inhibited differentiation of Th17 cells. Based upon our findings in ERAP1 deficient mice, we propose that ERAP1's role in Tr1 T cell differentiation may also be at play in BD.

3.3. Inflammatory bowel disease

IBD is a chronic autoinflammatory disease affecting the intestinal tract and is precipitated due to complex interactions between environmental triggers, the microbiome, immune system development and responses, and genetic predispositions [75]. Its pathogenesis is thought to develop due to inappropriate immune dysregulation of the innate immune response against intestinal microbiota, which leads to increased permeability of the epithelium, and enhanced adaptive immune cell activation. IBD includes two distinct diseases, Crohn's and ulcerative colitis. Both Crohn's and ulcerative colitis have an estimated prevalence of 0.3 percent each in the US and western Europe. Patients develop inflammation of the gut and can experience symptoms such as abdominal pain, diarrhea, vomiting, rectal bleeding, weight loss, fevers, and anemia. Over 200 genetic loci active in both the innate and the adaptive immune responses have been found to be associated with ulcerative colitis and Crohn's disease patients. The very first gene which was found to be associated with Crohn's disease was nucleotide-binding oligomerization domain containing 2 (NOD2), which encodes for an intracellular protein which binds to muramyl dipeptide and activates NF-κB. This signaling activates autophagy, which is thought to play an important role in the pathogenesis of IBD, as DCs from Crohn's patients shave been shown to have autophagy functions [76]. NOD2 also contains N-terminal caspase activation and recruitment domains (CARDs) and is thought to be a key player in the activation of adaptive immunity. In addition to NOD2 signaling, inflammasome activation is thought to be an important contributor to the pathogenesis of IBD. In support of this, susceptibility to Crohn's disease has been shown to be associated with the presence of 3 different *NLRP3* variants (rs4353135, rs4266924, and rs10733113), and there is an increased NLRP3 expression in the biopsies of Crohn's patients and colon samples of mice subjected to trinitrobenzene sulfonic acid (TNBS)-induced colitis [4]. Also, increased NLRP3 activation and increased IL-1β production have been detected in both Crohn's and long-standing ulcerative colitis patients [5].

The *ERAP1* SNP, rs30187, has been shown to be associated with Crohn's disease [13]. More recently a study investigating a small Turkish cohort detected *ERAP1* variant rs26653 to be associated with IBD [77]. Additionally, a recent study reported an association between the *HLA-C*07* allele and *ERAP1* SNP rs30187 in a Spanish cohort of patients with IBD [78]. Interestingly, there is a long known clinical link between AS and IBD, where 5–10% of AS patients also have inflammatory bowel disease [48] and up to 46% of patients with spondyloarthritis have microscopic gut inflammation [79]. We also recently confirmed that ERAP1$^{-/-}$ mice have increased susceptibility to dextran sodium sulfate (DSS)-induced colitis, where the ERAP1$^{-/-}$ mice challenged with DSS had increased diarrhea, weight loss, rectal bleeding, and mortality and developed ulceration and severe inflammation of their colons [40]. Additionally, IL-10 deficient mice

develop spontaneous enterocolitis, suggesting their role in the pathogenesis of IBD [80]. Tr1 cells are thought to play a role in the pathogenesis of IBD because of their role in immune tolerance of intestinal microbiota. Tr1 cell therapy has shown some success in clinical trials involving patients with refractory Crohn's disease, where 75% of patients had a reduction of the disease activity index 8 weeks after "Tr1 like" ovalbumin-specific cell infusions [81]. However, the remission rate was reduced from 38% to 25% between weeks 5 and 8, suggesting that higher doses or additional rounds of Tr1 infusion may be needed for sustained efficacy.

There are multiple mechanisms via which ERAP1 might be contributing to the pathogenesis of IBD, given that both the innate and the adaptive immune responses are involved in the pathogenesis of IBD. ERAP1's multiple functions including its role in antigen presentation and peptide immunodominance which may determine tolerance of the intestinal microbiota, its role in the regulation of the inflammasome activation, its role in the Tr1 cell biology and cytokine receptor shedding may all contribute to the development of this complex multifactorial disease.

3.4. Insulin dependent diabetes mellitus

IDDM is a polygenic autoimmune disease, which involves the destruction of insulin-producing β-pancreatic cells, resulting in insulin deficiency and as a result, hyperglycemia [82]. While the exact etiology of immune destruction of β-cells is likely multifactorial, there is evidence of pre-proinsulin specific CTLs *in vitro* [83,84] and β-cell specific CTLs *in vivo* in the circulation of recently diagnosed IDDM patients [85], as well as β-cell specific CTL infiltration in the pancreas of IDDM patients [86,87]. The major genes associated with IDDM are MHC class II genes *HLA-DQB1* and *HLA-DRB1*, however, similar to AS, HLA class I gene polymorphisms have also been found to be associated with increased risk of developing IDDM, namely *HLA-A*24:02* and *HLA-B*39:06* [88]. Interestingly, the rs30187 SNP in the *ERAP1* gene was found to also be associated with IDDM [7]. ERAP1 has been shown to be important for optimization of pre-proinsulin peptides for their loading and surface expression on IDDM-associated HLA's [89]. Knockdown of *ERAP1* gene expression resulted in a reduction of MIP-1β expression and killing of the pre-proinsulin peptide expressing cells by CTLs. Meanwhile, overall MHC-I surface levels were unchanged and there was no difference in the overall expression levels of ERAP1 between IDDM patients and controls. Interestingly, IDDM patients have been shown to have IFN-γ-skewed responses to pancreatic islet peptides, versus IL-10 responses elicited from T cells from non-diabetic individuals [90]. A recent study showed that intestinal Tr1 cells were capable of migrating to the periphery and inhibit diabetogenic T cells in non-obese diabetic mice via IL-10 secretion in the pancreas and delayed the disease development [91]. It may be that ERAP1 contributes to the pathogenesis of IDDM via its aminopeptidase function by altering the immunopeptidome, thereby affecting whether pre-proinsulin peptides are recognized by the CTLs and trigger β-cell killing. It may also be plausible that ERAP1 variants contribute to the development of IDDM via reduction of pre-proinsulin specific Tr1 cells, thereby reducing protection from β-cell killing by autoreactive CTLs, intriguing possibilities that require further investigation.

3.5. Multiple sclerosis

MS is an autoimmune disease, which affects the central nervous system, where the inflammation and demyelination of the nerves occur with the involvement of both T and B cells, causing motor defects, hemiparesis, visual deficits, ataxia and cognitive impairments [92]. It is estimated to affect 2–3 million people worldwide [93]. MHC-II *HLA-DR1* is thought to have the strongest genetic link with MS [94]. Inflammasomes have been shown to play a role in neurological diseases including MS, where inflammasome activation promotes IL-1β secretion and Th1 and Th17 migration into CNS. IL-1β secreting monocytes have been shown in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS and in the cerebrospinal fluid of MS patients. Given that IL-1β promotes Th17 differentiation, inflammasome activation is thought to aggravate EAE and MS via induction of differentiation and recruitment of Th17 cells to the CNS [95]. The rs30187 *ERAP1* variant has been shown to be associated with MS [13]. Given, that ERAP1 does not have functionality in the MHC-II antigen presenting pathway, it is possible that ERAP1 promotes MS pathogenesis via inflammasome activation. In our work, we showed that addition of the rs30187 ERAP1 variant to PBMCs in-vitro enhanced IL-1β, TNF-α, and IL-6 secretion and activation of NK, DCs, and T cells in caspase-1 dependent manner [30].

It is also plausible that ERAP1's involvement in the pathogenesis of MS is via our recently identified potential role for ERAP1 in Tr1 cell biology [40]. "Tr1-like" cells from MS patients have been shown to produce reduced levels of IL-10 compared to healthy patients [96]. Moreover, CD4+ T cells from MS patients have a reduced propensity to differentiate into Tr1 cells compared to CD4+ T cells from healthy donors [97]. The IL-10 signaling pathway was found to be inhibited in the MS patients due to reduced STAT3 phosphorylation via SOCS3 mediated inhibition [97]. In EAE, intracranial injection of ovalbumin-specific Tr1 cells prevented neurological symptoms in an IL-10 dependent manner [98]. EAE is induced by myelin oligodendrocyte glycoprotein injection [99]. When mice are injected intravenously with soluble myelin antigen, the EAE is ameliorated via induction of conventional Tregs and Tr1 cells and subsequent reduction of myelin-specific T cells. Depletion of tDCs from myelin antigen treated EAE mice, resulted in the loss of tolerance, suggesting that the IL-10 and IL-27 producing tDCs are the key players in the induction of tolerance and prevention of EAE. In line with this, IL-27 administration prevents EAE development and it has been suggested that the therapeutic benefits of IFN-β treatment of MS are related to the IL-27 production by DCs [100].

3.6. Psoriasis

Psoriasis is a chronic autoimmune disease of the skin, characterized by scaly plaque formation, and is associated with psoriatic arthritis. It affects an estimated 2–3% of the population [101]. Psoriasis is thought to develop due to over-proliferation of keratinocytes in response to inflammatory infiltration and damage mediated by innate immune cells such as macrophages and DCs, adaptive immune cells such as T cells, and proinflammatory cytokines such as TNFα, all of which have been found in psoriatic lesions [102]. Several genes involved in the inflammasome pathway have been shown to be associated with psoriasis, including rs10733113 polymorphism in *NLRP3* and rs2043211 polymorphism in caspase recruitment domain-containing protein 8 (*CARD8*) [103]. Increased caspase-1 activation and absent in melanoma 2 (AIM-2) inflammasome expression have been reported in psoriatic lesions compared to unaffected skin [104]. Psoriasis has a strong association with *HLA-Cw6*, and interestingly, a strong association with *ERAP1* in *HLA-Cw6* positive individuals [10]. Two GWAS studies found associations between *ERAP1* SNPs and psoriasis. The Genetic Analysis of Psoriasis Consortium and the Welcome Trust Case Control Consortium 2 identified the *ERAP1* rs27525 SNP [10] and another GWAS in Han Chinese population reported the *ERAP1* rs151823 variant to be associated with psoriasis [105]. Given the epistatic effects of *ERAP1* and *HLA-Cw6*, it is likely that ERAP1's role in antigen presentation serves as the underlying mechanism for its role in the pathogenesis of psoriasis, where specific ERAP1 variants influence the peptide immunodominance in a manner where autoreactive CTLs infiltrate the psoriatic lesions.

4. Conclusions

ERAP1 is capable of enhancing innate immune responses via a

number of activities, including its ability to trim receptors such as TNFR1, IL6R, and IL2RII, having a role in NK cell maturation and activation, and via ERAP1 protein secretion outside the cells where they can activate macrophages via inflammasome and TLR dependent manner. Extracellular ERAP1 proteins also activate NK and T cells and enhance proinflammatory cytokine secretion in a polymorphism specific fashion. Additionally, ERAP1's ability to activate the inflammasome serves as an important bridge between the innate and adaptive immune systems. Additionally, via its aminopeptidase functions, ERAP1 itself directly affects adaptive immune responses by dictating which peptides are destined for antigen presentation and which are destroyed, thereby determining the composition of an individual's peptidome. ERAP1's roles in both the innate and adaptive immune systems likely explain its association with a number of diseases associated with overactive immune responses.

Peripheral self-tolerance is an important mechanism for the prevention of autoimmunity, by inhibiting and ensuring anergy of self-reactive lymphocytes which escaped negative selection [106]. Our most recent finding that ERAP1 function is important to maintain normal numbers of Tr1 regulatory T cells which ensure self-tolerance, highlights yet another way in which this protein may be important for the prevention of autoinflammation and autoimmunity [40]. Tr1 T cells have been implicated in multiple autoimmune diseases including IDDM [91] and MS [97], as well as autoinflammatory diseases such as IBD [80] and BD [74]. Of note, Tr1 cells have been shown to be able to suppress NLRP3 inflammasome activation and IL-1β secretion in an IL-10 dependent manner in co-culture experiments, suggesting that Tr1 infusion therapies carry a therapeutic potential for inflammasome-mediated diseases [107]. Other mechanisms of action of Tr1 cells include direct inhibition of T cell proliferation via secretion of IL-10 and TGF-β cytokines [108,109], as well as indirect mechanisms via inhibition of IL-2 and IFN-γ production by effector T cells [110]. Additionally, Tr1 cells can exhibit their suppressive function via their effects on antigen presenting cells (APCs) via downregulation of co-stimulatory receptors and attenuation of proinflammatory cytokine release [111]. Tr1 cells can also inhibit B cell functions and regulate antibody isotype switching, where Tr1 cells have been shown to promote IgG4 and prevent IgE antibody production [112]. Finally, Tr1 cells have been reported to inhibit T cell responses via cytolysis of myeloid APCs, a process mediated by granzyme B secretion [113,114]. Which of these Tr1 functions are ERAP1 dependent will be important to elucidate, as this understanding may lead to better understanding as to the pathogenesis of autoinflammatory and autoimmune diseases.

Infusion of Tr1 T cells has been problematic due to the difficulty of expanding these cells *in vitro*, however, a recently described lentiviral vector IL-10 expression system that promotes differentiation of conventional CD4+ T cells into Tr1-like cells will allow for scalability of Tr1 expansion [115]. Tr1 cell co-infusions have shown long-term prevention of graft-versus-host disease upon hematopoietic stem cell transplantation therapy in patients with hematological malignancies [95]. Tr1 cell therapy has also shown some success in clinical trials involving patients with refractory Crohn's disease, where 75% of patients had a reduction of the disease activity index with remission rate of 38% at 5 weeks and 25% at 8 weeks after infusion, suggesting that with higher dose or additional rounds of Tr1 infusions, the efficacy of this therapy can be improved [81]. While optimization is required before Tr1 cell therapy can be more widely utilized, it is a promising modality for cell therapy that could potentially be used in ERAP1-associated autoimmune and autoinflammatory diseases.

Multiple mechanisms seem to be at play in the pathogenesis of ERAP1-associated autoinflammatory and autoimmune diseases. ERAP1's versatile functions in both the innate and adaptive arms of the immune system make it a very interesting molecular participant that serves as the link between the two, and sheds insight as to the presence of common symptoms in the spectrum of the autoinflammatory and autoimmune diseases. Studies exploring the relationship between ERAP1 and Tr1 cells should also be explored in humans in the presence and absence of autoinflammation and autoimmunity. Given this understanding, ERAP1 may serve as a therapeutic target worth exploring in the search for new treatments for the several important diseases associated with ERAP1 to date.

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