nature reviews rheumatology
https://doi.org/10.1038/s41584-024-01109-5

Review article

Check for updates

Granzyme serine proteases in inflammation and rheumatic diseases

Alexandre Aubert[1], Karen Jung[1], Sho Hiroyasu[2], Julian Pardo[3,4] & David J. Granville[1,5]

Abstract

Granzymes (granule-secreted enzymes) are a family of serine proteases that have been viewed as redundant cytotoxic enzymes since their discovery more than 30 years ago. Predominantly produced by cytotoxic lymphocytes and natural killer cells, granzymes are delivered into the cytoplasm of target cells through immunological synapses in cooperation with the pore-forming protein, perforin. On internalization, granzymes can initiate cell death through the cleavage of intracellular substrates. However, evidence now also demonstrates the existence of non-cytotoxic, pro-inflammatory, intracellular and extracellular functions that are granzyme specific. Under pathological conditions, granzymes can be produced and secreted extracellularly by immune cells as well as by non-immune cells. Depending on the granzyme, accumulation in the extracellular milieu might contribute to inflammation, tissue injury, impaired wound healing, barrier dysfunction, osteoclastogenesis and/or autoantigen generation.

Sections

Introduction

Granzymes: a family of pleiotropic serine proteases

Granzymes in rheumatic diseases

Granzymes as therapeutic targets

Unmet needs and future perspectives

Conclusions

[1]International Collaboration on Repair Discoveries (ICORD) Centre; British Columbia Professional Firefighters' Burn and Wound Healing Group, Vancouver Coastal Health Research Institute; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada. [2]Department of Dermatology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan. [3]Fundación Instituto de Investigación Sanitaria Aragón (IIS Aragón), Biomedical Research Centre of Aragon (CIBA); Department of Microbiology, Radiology, Paediatrics and Public Health, University of Zaragoza, Zaragoza, Spain. [4]CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain. [5]Centre for Heart Lung Innovation, Providence Research, University of British Columbia, Vancouver, BC, Canada. e-mail: dgranville@icord.org

Key points

- Granzymes are serine proteases with both cytotoxic and non-cytotoxic functions; phenotypic and mechanistic characterization of granzymes in rheumatic diseases is required to delineate their specific roles.

- The five human granzymes have unique substrate specificities and functional roles as determined by granzyme-specific cleavage preferences, location of accumulation (intracellular or extracellular), and exposure to substrates in tissues.

- Extracellular granzyme activity can contribute to tissue injury, inflammation, autoimmunity, epithelial and endothelial barrier dysfunction, bullae formation, impaired wound healing, and degenerative or pathological aging.

- In addition to cytoplasmic proteins involved in apoptosis, granzyme B substrates include extracellular matrix proteins, hemidesmosomal or desmosomal proteins, pro-inflammatory cytokines, cell surface receptors and autoantigens.

- Granzyme A and granzyme B are elevated in synovial fluid, tissues and plasma of people with rheumatoid arthritis; in an arthritis model, *Gzma*$^{-/-}$ mice have lower disease severity than wild type.

- CD8$^+$ T cells expressing granzyme B and granzyme K are enriched in the peripheral blood and inflamed tissues of people with rheumatoid arthritis, systemic lupus erythematosus or Sjögren syndrome.

Introduction

Granzymes constitute a family of serine proteases long assumed to exert redundant roles as cytotoxic initiators of target-cell death. Granzymes were first characterized as intracellular proteases produced by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, and internalized by target cells with the pore-forming protein perforin to promote caspase-dependent and caspase-independent apoptosis. Over the past decade, increasing evidence has emerged in support of non-cytotoxic roles for these proteases in various pathological contexts. Under conditions associated with aging, chronic injury and/or dysregulated immunity and/or inflammation (Box 1), granzymes can accumulate in the extracellular milieu, and (depending on the tissue, localization and access to substrates) contribute to pathogenesis, irrespective of their ability to induce cell death, a concept that is still controversial for some granzymes[1,2].

The human genome encodes five granzymes (GzmA, GzmB, GzmH, GzmK and GzmM), each possessing unique cleavage preferences, substrate specificities and functionalities (Table 1). Although *GZMA*, *GZMB*, *GZMK* and *GZMM* possess direct orthologues in mice, duplication of mouse *Gzmb* and *Gzmh* during evolution created paralogues, for a total of ten granzyme-encoding genes in the mouse genome. Much of our understanding pertaining to granzymes is derived from the use of genetic knockout mouse models alongside in vitro and ex vivo studies using purified enzymes and tissues. Recent advancements in our understanding of granzymes in disease have prompted an examination of their roles in rheumatic diseases.

Granzymes: a family of pleiotropic serine proteases

A persistent challenge in granzyme research is the complexity of translating lessons learned from mouse models to human pathological conditions, notably because human and mouse GzmA and GzmB might differ in their substrate specificities and preferred cleavage sequences. This suggestion is mostly based on results from studies of cell death in which mouse GzmB preferentially cleaved caspase-3 in the apoptotic signalling cascade whereas human GzmB more efficiently truncated the pro-apoptotic Bcl-2 family member Bid (BH3 interacting-domain death agonist)[3,4]. Both caspase-3 and Bid are key pro-apoptotic proteins, and their cleavage activates downstream apoptotic cascades. However, it is important to note that these findings were derived from biochemical studies performed with recombinant proteins produced in various cell types (*Escherichia coli*, yeast, human YT cells) and the limitations of in vitro modelling (potentially resulting from variation in post-translational modification or protein refolding) were not always considered. Regardless, despite their cleavage preferences, mouse[5,6] and human GzmB[7] can both cleave caspase-3 and Bid, and both pathways are involved in GzmB-mediated apoptosis. Human and mouse GzmK also exhibit small differences in their optimal cleavage sites, but they have similar substrate specificities[8], as do human GzmH and its closest mouse orthologous protein, GzmC (although the wild type mouse GzmC is inactive and requires mutation to enable its cleavage activity)[9].

Other findings (unrelated to cell death) demonstrate similarities in the regulation of cell signalling pathways by human and mouse granzymes. For example, mouse and human GzmA can promote IL-1β, TNF and IL-6 production in mouse macrophages[10,11] and human monocytes[11], respectively. Additionally, immune cells from both species typically demonstrate a similar profile of granzyme expression, especially for GzmA and GzmB, with the notable exception of GzmB-producing B cells that have only been identified in humans[12]. These observations necessitate careful translation of findings between human and animal models and emphasize the importance of confirming, as much as possible, murine findings in human cells, tissues and models of disease.

Granzyme A

Human GzmA, the only granzyme that forms homodimers, is encoded by the *GZMA* gene, which is located at chromosome 5q11.2 in a region clustered with the gene encoding its closest homologue, GzmK[13]. Similar to GzmK, GzmA exhibits tryptase-like activity, cleaving substrates preferentially after arginine or lysine residues[8]. In contrast to GzmK, GzmA proteolytic activity is regulated by an exosite (a secondary regulatory site resulting from the dimeric structure)[14], whereby small changes in the cleavage site sequence translate to different proteolytic efficiencies between GzmA and GzmK[8]. GzmA optimally cleaves substrates with Arg, Ala and Gly in the P1, P1′ and P2′ positions[8]. Cleavage occurs between residues at P1 and P1′ and other residues are numbered outward relative to the cleavage site, usually from P4 to P4′.

Shortly after its discovery, a cytotoxic role for human GzmA was proposed based on its colocalization with perforin in the cytotoxic granules of NK and CD8$^+$ T cells. Despite results showing that perforin-mediated intracellular delivery of mouse GzmA results in double-stranded DNA fragmentation and cell death in vitro, *GzmA*$^{-/-}$ mice exhibited neither impairment of lymphocyte-mediated cytotoxicity nor predisposition to viral or bacterial infection (as reviewed elsewhere[15,16]). Results from a subsequent study showed that human GzmA promotes single-stranded DNA nicking by cleaving the

nucleosome assembly protein SET[17]. Furthermore, human GzmA contributes to the release of reactive oxygen species through the degradation of mitochondrial NDUFS3 (an iron-sulphur subunit of the NADH–ubiquinone oxidoreductase complex I)[15,16]. GzmA derived from mouse NK cells and CTLs, as well as recombinant human and mouse GzmA, can also convert non-inflammatory apoptosis into a pro-inflammatory form of cell death known as pyroptosis through the cleavage of gasdermin B[18], resulting in pore formation and the release of pro-inflammatory cytokines[19]. Collectively, mechanisms underlying GzmA-mediated cytotoxicity still require further elucidation (Fig. 1).

Beyond cytotoxicity, human GzmA can also be released extracellularly by CTLs in the absence of target-cell engagement[20]. In vitro analyses have identified several non-apoptotic substrates of mouse GzmA including extracellular matrix (ECM) proteins (fibronectin and collagen IV), coagulation proteins (pro-urokinase), thrombin receptors, cytokines (IL-1β) and cytoskeletal proteins (dystrophin, myosin and nebulin)[21]. However, validation of GzmA cleavage of these substrates in vivo requires further exploration, especially where human substrate cleavage was assessed using mouse GzmA rather than human GzmA.

In 2008, a pro-inflammatory role for extracellular GzmA was proposed. Extracellular human and mouse GzmA were shown to stimulate pro-inflammatory cytokine production by human monocytes and mouse macrophages, respectively[11]. These observations were confirmed in subsequent in vitro experiments[10,22] and in in vivo[23] studies demonstrating a reduction of inflammation and organ damage in response to bacterial and polymicrobial sepsis in *Gzma*-knockout mice without affecting the capacity to control bacterial load[10], suggesting intact cytotoxic function in the absence of GzmA. More recently, extracellular GzmA-mediated inflammation has been reported in mouse models of colitis[24], colorectal cancer[24] and rheumatoid arthritis (RA)[25], including correlative studies using samples from human patients. As extracellular GzmA is inhibited by antithrombin-III[26] and α2-macroglobulin[27] in plasma, further investigation into the regulation of the extracellular activity of human GzmA is required. However, intraperitoneal injection of serpinb6b, a mouse extracellular inhibitor of GzmA[28], produced similar results to GzmA deletion, and attenuated inflammation in disease models of abdominal sepsis and colorectal cancer in vivo[10,24].

Although some results suggest that cytokine production associated with human GzmA or GzmK is an artefact of lipopolysaccharide contamination[29], it should be emphasized that results obtained with recombinant GzmA produced in prokaryotic cells have been confirmed using GzmA expressed in eukaryotic systems[10,22]. Furthermore, inactive or inhibited GzmA produced in prokaryotic cells did not induce IL-6 production by macrophages, contradicting the hypothesis of a role for lipopolysaccharide contamination[10].

Granzyme B

Human GzmB is a 27.7-kDa monomeric aspase-like protease encoded by the *GZMB* gene, which is located at chromosome 14q11.2. Following its discovery in the mid-1980s, GzmB has been extensively studied for its role in CTL-mediated apoptosis (of infected and tumour cells), and it remains the best-characterized granzyme. Similar to other granzymes, once delivered into target-cell cytoplasm, GzmB cleaves substrates to induce both caspase-dependent and caspase-independent apoptosis, as reviewed elsewhere[30]. Preferentially cleaving at aspartic acid (and to a lesser extent, glutamate) residues[31], human GzmB is also involved in the conversion of non-inflammatory apoptosis into immune-stimulatory pyroptosis through the cleavage of gasdermin E[32] (Fig. 2).

Box 1

Conditions exacerbated by extracellular granzymes

- The effects of extracellular activities of granzymes on these conditions are demonstrated in vivo in mouse models using genetic deletion and/or pharmacological inhibitors

Granzyme A
- Rheumatoid arthritis[24], colitis[23], abdominal sepsis[10], colitis-associated colorectal cancer[23]

Granzyme B
- Atopic dermatitis[41], atherosclerosis[198], interface dermatitis[187], cardiac fibrosis[64], autoimmune blistering diseases[39], abdominal aortic aneurysm[48,49], pressure injuries[42], allergic asthma[62], diabetic wounds[184], inflammatory bowel disease[68], scarring (thermal injury)[56], vaginal epithelial ulceration[67], skin aging[50], photoaging[55]

Granzyme K
- Atopic dermatitis[92], thermal wound healing

In response to environmental stimuli or cytokine stimulation, GzmB expression can be induced in many cell types, including immune cells (T cells, B cells, NK cells, NKT cells, mast cells, macrophages, basophils and plasmacytoid dendritic cells) and non-immune cells (keratinocytes, retinal pigment epithelial cells, chondrocytes and pneumocytes), as reviewed elsewhere[33,34]. In addition to its perforin-mediated internalization, GzmB is released extracellularly by CTLs in the absence of target-cell engagement[20,35] and it is secreted by cells that do not form immunological synapses and/or that do not express perforin[34,36]. Based on these observations, increasing efforts have been devoted to understanding the extracellular functions of GzmB.

Extracellular GzmB concentrations are minimally detectable to absent in healthy individuals, but are elevated in plasma in RA and atherosclerosis[37,38]; synovial fluid in RA[38]; bronchoalveolar lavage fluid (BALF) in chronic obstructive pulmonary disorder[39]; blister fluid in bullous pemphigoid[40]; cerebrospinal fluid in multiple sclerosis[41]; and inflammatory skin lesions[42–44]. Notably, recombinant human GzmB retains its proteolytic activity when incubated with human plasma[45]. Furthermore, none of the serine proteinase inhibitors found in human BALF (such as alpha-antitrypsin, elafin and secretory leukocyte protease inhibitor) inhibit GzmB[46]. In fact, no endogenous extracellular inhibitors of GzmB have been identified in humans[45]. As such, GzmB accumulation in the extracellular milieu[34] coupled with unimpeded proteolytic activity[45,46] exacerbates inflammatory conditions through the cleavage of ECM molecules[43,47–61], coagulation proteins[62], cell-surface receptors[63,64], cell adhesion and/or dermal–epidermal hemidesmosomal proteins[42,58,65], and cytokines such as IL-1α and IL-18 (refs. 66,67) (Fig. 2).

Q7

A perforin-independent extracellular role for mouse GzmB was first demonstrated in vivo in 2010 (ref. 49). In an experimental

model of abdominal aortic aneurysm, the absence of GzmB led to reduced aneurysmal rupture and increased survival, whereas perforin deficiency provided no protective effect, underscoring a perforin-independent role for extracellular GzmB in medial disruption through the cleavage of the ECM protein fibrillin-1. In addition, elevation of human GzmB co-localization with immune cells in abdominal and thoracic aortic aneurysm tissues was observed[49]. In a follow-up study[50], systemic administration of a mouse extracellular GzmB inhibitor (serpinA3N) prevented aneurysmal rupture by inhibiting GzmB-mediated decorin cleavage, and phenocopied GzmB deletion by improving adventitial collagen remodelling and circumferential strength. Human and mouse GzmB-dependent cleavage of decorin has been confirmed in many other in vivo models, including photoaging[56], pressure injury[43], age-impaired wound healing[51] and scarring[57]. As human GzmB-dependent cleavage of decorin releases bioactive transforming growth factor-β (TGF-β) from human smooth muscle cell-derived decorin and biglycan[52], this mechanism could link GzmB to scarring and fibrosis. In a similar manner, human GzmB cleavage of fibronectin can generate bioactive fragments that induce matrix metalloproteinase-1 (MMP-1) expression by dermal fibroblasts[56]. GzmB also induces the release of fibronectin-sequestered vascular endothelial growth factor (VEGF), which in turn contributes to pathological angiogenesis and endothelial permeabilization[55]. The latter observation has been confirmed in vivo in a mouse model of aging and pressure injury, in which GzmB-deficient mice exhibited a reduction of fibronectin cleavage in association with decreased vascular permeability, microhaemorrhaging and tissue injury, relative to GzmB-expressing mice[43]. In addition, human and mouse CTLs can release GzmB to cleave basement-membrane proteins around blood vessels, thereby facilitating diapedesis[48], promoting their transmigration and extravasation under inflammatory conditions. In the airways, NK cell-derived extracellular mouse GzmB activates proteinase-activated receptor 2 (PAR-2) in the epithelium to induce IL-25 production and a type 2 immune response, leading to allergic airway disease in offspring as a result of maternal exposure to diesel exhaust particles[63]. Human GzmB can also induce *IL25* transcription in vitro[63]. Collectively, the proteolytic activity of GzmB in the extracellular space triggers the activation of numerous downstream pro-inflammatory cascades.

Newly recognized roles for extracellular GzmB in epithelial dysfunction have prompted intense study as roles in the disruption of skin[43], airway[63], retinal[58], vaginal[68], gut[69] epithelial as well as vascular[65] barrier function have been revealed. In the context of dermatitis[42],

Table 1 | Overview of the human granzyme family

Granzyme	Cell sources	Cleavage specificity	Classification of direct substrates	Nullizygous mouse phenotype[a]	Refs.
Granzyme A	CD4+ and CD8+ T cells, NK cells, NKT cells, mast cells, pneumocytes, platelets	Tryptase-like (Arg, Lys)	Intracellular: Cell death proteins, cytoskeletal proteins, myelin protein Extracellular: ECM components, cell surface receptors, plasma proteins, coagulation proteins, cytokines	Healthy, normal haematopoiesis; unchanged cytotoxic lymphocyte activity; intact capacity to control viral and bacterial load as well as tumour progression	34,196
Granzyme B	CD4+ and CD8+ T cells, NK cells, NKT cells, B cells, macrophages, mast cells, neutrophils, basophils, plasmacytoid dendritic cells, haematopoietic progenitors, keratinocytes, retinal pigment epithelial cells, chondrocytes, pneumocytes, spermatocytes, trophoblasts, granulosa cells, platelets	Aspase-like (Asp>Glu)	Intracellular: Cell death proteins, viral replication (host and viral proteins) Extracellular: ECM components, cell adhesion/ basement membrane, cell surface receptors, plasma proteins, coagulation proteins, cytokines, autoantigens	Healthy, normal haematopoiesis; reduced rate of DNA fragmentation and cytotoxic lymphocyte activity; delayed clearance of some viral and bacterial infections	33,34,197
Granzyme H	NK cells » CD4+ and CD8+ T cells, mast cells	Chymase-like (Phe, Tyr)	Intracellular: Cell death proteins, viral replication (host and viral proteins)	No direct mouse orthologue identified for human *GZMH*; murine *Gzmc* believed to be most closely related	34,75,76
Granzyme K	NK cells, CD4+ and CD8+ T cells, CD56+ T cells, γδ T cells, M1 macrophages, MAIT cells, mast cells	Tryptase-like (Arg, Lys)	Intracellular: Cell death proteins Extracellular: Cell surface receptors, proteoglycans	Healthy, normal haematopoiesis; unchanged cytotoxic lymphocyte activity; capacity to clear viral and bacterial infections as well as tumour cells intact	34,82,83,198
Granzyme M	NK cells, CD3+CD56+ T cells, γδ T cells, CD4+ and CD8+ T cells	Met-ase-like (Leu, Met)	Intracellular Cell death proteins, viral replication (host and viral proteins) Extracellular: Plasma proteins	Healthy, normal haematopoiesis; unchanged anti-tumoural activity; increased susceptibility to murine cytomegalovirus infection (higher viral burden) though unchanged NK cell cytotoxic potential	34,103,105

[a]Phenotypes of nullizygous mouse strains assessed with different autoimmune and inflammatory models can be found in Box 1. ECM, extracellular matrix; MAIT cell, mucosal-associated invariant T cell; NK, natural killer.

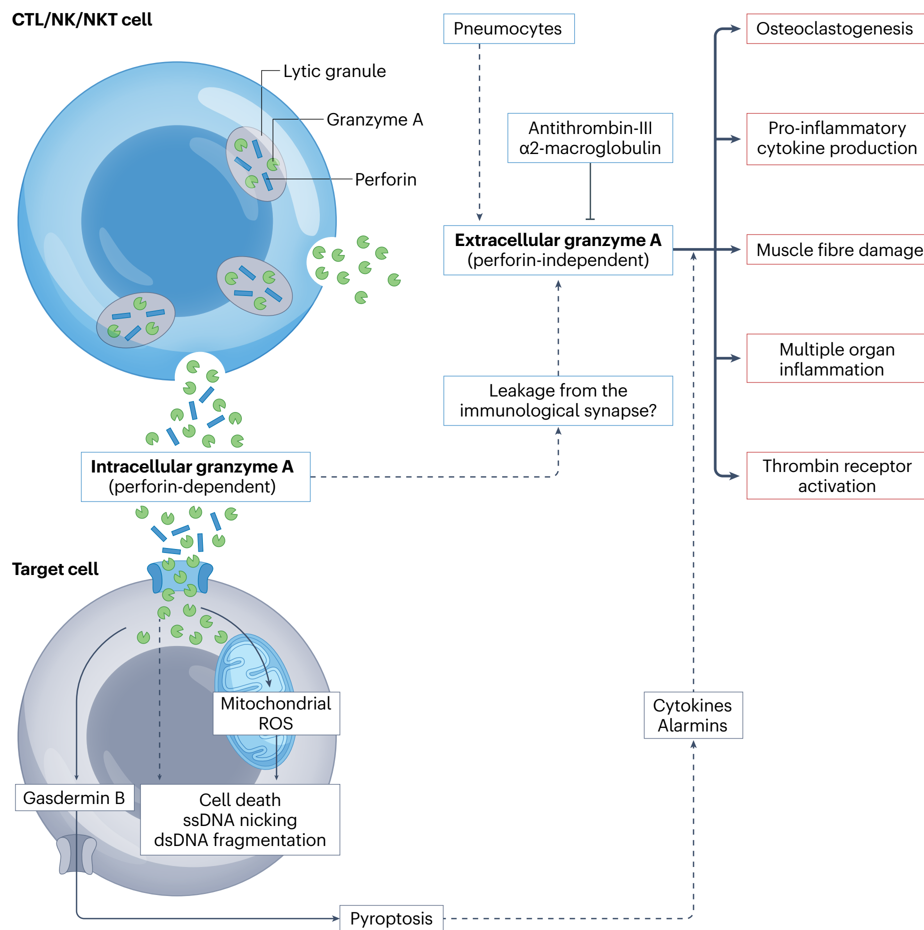

Fig. 1 | Proposed intracellular and extracellular functions of granzyme A. Intracellular functions: granzyme A is secreted by cytotoxic T lymphocytes (CTLs), natural killer (NK) and NKT cells and delivered into the cytoplasm of targeted cells through a perforin-dependent, pore-forming mechanism. On internalization, granzyme A cleaves intracellular substrates leading to cell death. Granzyme A cleavage of gasdermin B promotes pyroptosis, leading to pore formation and release of pro-inflammatory cytokines. Extracellular functions: granzyme A that leaks from the immunological synapse or is produced by non-cytotoxic cells is released into the extracellular space. Through the cleavage of extracellular substrates, extracellular granzyme A can contribute to osteoclastogenesis, pro-inflammatory cytokine production, muscle fibre damage, multiple-organ inflammation and thrombin receptor activation. In humans, granzyme A can be inhibited by antithrombin-III and α2-macroglobulin in the extracellular milieu. Solid and dotted lines denote published and postulated mechanisms, respectively. dsDNA, double-stranded DNA; ROS, reactive oxygen species; ssDNA, single-stranded DNA.

macular degeneration[58] and vascular disease[65], human and mouse GzmB both disrupt cell–cell junctions through the cleavage of cadherins, desmogleins, junctional adhesion molecule A and/or zonula occludens-1, promoting the loss of barrier integrity and subsequent trans-epithelial water loss[42], retinal pigment epithelial barrier disruption[58] or vascular leakage[55]. Independent of perforin, mouse GzmB also induces enterocyte shedding in Crohn's disease[69] and genital epithelial ulceration in HSV infection[68]. An important pathological role for extracellular GzmB in the degradation of hemidesmosomal dermal–epidermal junction proteins also exists. In three independent mouse models of autoimmune sub-epidermal blistering (pemphigoid) diseases, *Gzmb* knockout or GzmB topical inhibition prevented separation of the dermal–epidermal junction and blister formation through the cleavage of hemidesmosomal proteins[40].

A pivotal role for GzmB in autoantigen generation was proposed over two decades ago[70]. Since then, an extensive list of known human autoantigens has been identified as GzmB substrates[40,71,72]. However, despite much evidence supporting the generation of human autoantigens in vitro, progress in the field has been hindered by limitations in establishing predictive, spontaneously occurring murine models of autoimmune disease, as well as by the subtle differences between murine and human GzmB substrate specificities. These differences could provide a possible explanation for the lack of comparable GzmB-generated autoantigens in mice. GzmB-mediated autoantigen generation in rheumatic disease is discussed in more detail below.

Granzyme H

Human GzmH is encoded by the *GZMH* gene located on chromosome 14q11-q12, flanked by the *GZMB* gene and the gene encoding cathepsin G (*CTSG*)[73]. Although GzmH shares more than 70% amino acid similarity with GzmB[73], GzmH is distinguished by its chymase-like activity, preferentially cleaving polypeptides with bulky aromatic phenylalanine or tyrosine residues at the P1 position[74].

Human GzmH is highly expressed by NK cells and mast cells, and it is expressed at low levels by unstimulated $CD4^+$ and $CD8^+$ T cells[75,76]. Perforin-dependent, intracellular delivery of GzmH into target cells promotes caspase-dependent and caspase-independent apoptosis, as well as virus inactivation, through the cleavage of host and viral substrates[77]. Notably, the human La phosphoprotein, an autoantigen in RA (and a protein involved in viral RNA metabolism), was the first identified non-apoptotic substrate for human GzmH[78].

As the only known human granzyme without a direct mouse orthologue[73], GzmH function remains largely unexplored. Nonetheless, the use of proteomic approaches has enabled identification of GzmH production in NK cells of people with age-related atherosclerotic cerebral small-vessel disease, suggesting a role for NK cell-derived extracellular GzmH in a rat model of neuronal damage[79].

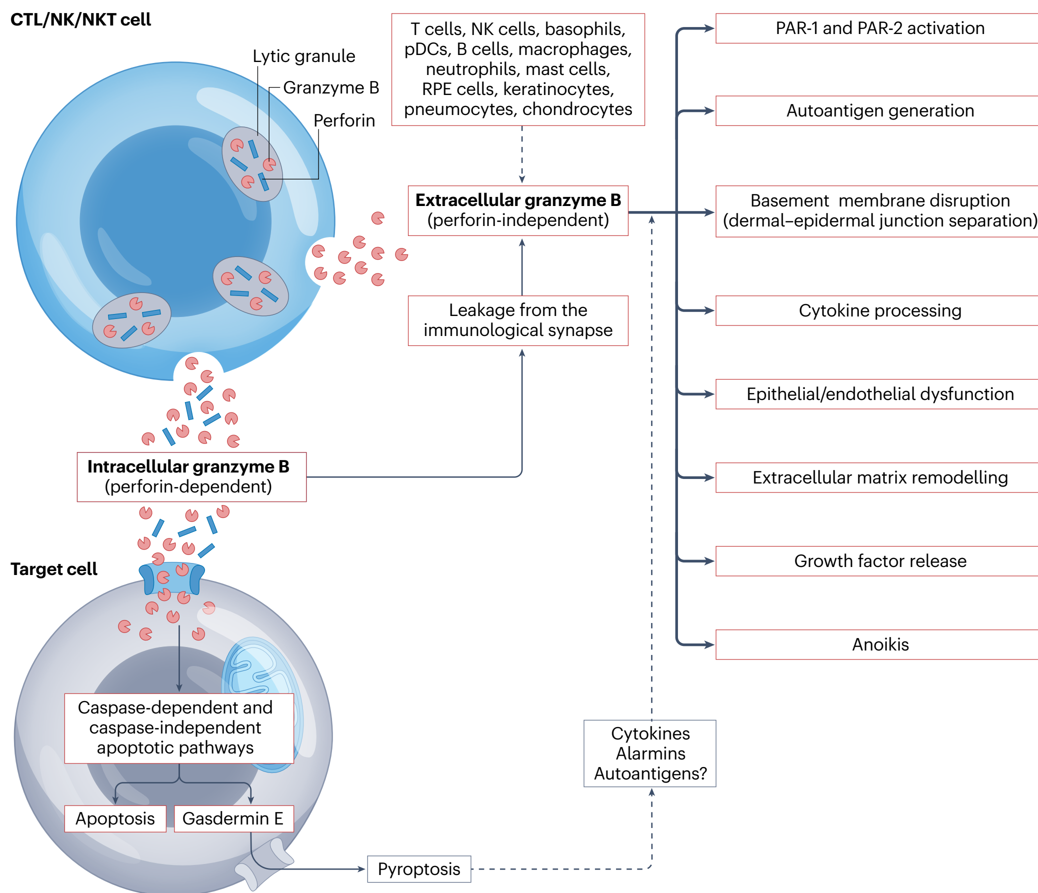

Fig. 2 | Proposed intracellular and extracellular functions of granzyme B. Intracellular functions: granzyme B is secreted by cytotoxic T lymphocytes (CTLs), natural killer (NK) and NKT cells and internalized into target cells in a perforin-dependent manner. On internalization, granzyme B cleaves intracellular substrates, leading to apoptosis. Granzyme B cleavage of gasdermin E promotes pyroptosis. Extracellular functions: granzyme B that leaks from the immunological synapse, is secreted by cytotoxic cells in the absence of target-cell engagement, and/or is produced by other cells, is released into the extracellular space. Through the cleavage of extracellular substrates, extracellular granzyme B contributes to anoikis, autoantigen production, dermal–epidermal junction separation, cytokine processing, epithelial barrier dysfunction, extracellular matrix remodelling and growth factor release. To date, no endogenous inhibitor of extracellular granzyme B has been identified in humans. Solid and dotted lines denote published and postulated mechanisms, respectively. pDC, plasmacytoid dendritic cell; RPE cells, retinal pigment epithelial cells.

Granzyme K

Human GzmK and GzmA were discovered simultaneously as the genes encoding both granzymes map close to each other at chromosome 5q11.2. Similar to GzmA, GzmK exhibits tryptase-like activity and cleaves after lysine and arginine amino acids[80]; however, human GzmK possesses a rigid active site in its resting state that is activated by specific substrate sequences[81], preferentially with Arg, Ser and Leu residues in the P1, P1′ and P2′ positions[8].

In humans, GzmK is expressed by CD56^{bright} NK cells and mucosal-associated invariant T (MAIT) cells, as well as subsets of CD4$^+$ T cells, CD8$^+$ T cells, CD56$^+$ T cells and γδ T cells[82,83]. Extracellularly, elevated soluble GzmK concentrations occur in the serum of people with viral infections[84], sepsis[85] or abdominal aortic aneurysm[86]. Soluble GzmK is also increased in the BALF of people with acute bronchopneumonia or allergic asthma triggered by allergen challenge[87]. In the first study investigating its non-cytotoxic role, mouse GzmK promoted the secretion of IL-1β from macrophages[88]. In subsequent studies, a role for extracellular human GzmK in the activation of PAR-1, PAR-2 and PAR-4 was demonstrated, leading to the release of pro-inflammatory cytokines IL-6, IL-8, CCL2 or CXCL1, depending on the cell type[88–94]. Mounting evidence supports that GzmK is a pro-inflammatory protease with the ability to induce pro-inflammatory cytokine production through both perforin-dependent (intracellular) and perforin-independent (extracellular) mechanisms (Fig. 3).

Although GzmK is not detectable in healthy skin, its expression is elevated in inflammatory skin tissue from people with burn injuries or atopic dermatitis. Using a mouse model of second-degree thermal injury, GzmK was found to contribute to scarring and delayed wound closure through the impairment of matrix remodelling, re-epithelialization and resolution of inflammation[89]. In a mouse model of dermatitis, GzmK impaired angiogenesis and increased microvascular damage[93].

GzmK is proposed to have a role in inflammaging, as demonstrated by the existence of a subset of *GZMK*$^+$ CD8$^+$ T cells that

expand with age in multiple human and mouse tissues, linking GzmK secretion to: increased circulating pro-inflammatory cytokine concentrations (IL-6, IL-8 and TNF) observed in aging; the presence of age-related inflammation markers across tissues; and the induction of senescence-associated secretory phenotype (SASP) components (IL-6, CCL2 and CXCL1) in fibroblasts[94]. A caveat to the interpretation of the SASP-induction experiments is that the cells were treated with a truncated recombinant protein consisting of residues 22–227 of the 263-amino acid mouse GzmK that was expressed in a prokaryotic system, rather than with native protein. Additional evidence of the involvement of GzmK in inflammaging is provided by the association of GzmK expression with aging in CD8$^+$ T cells[95,96]. Enrichment of *GZMK*$^+$ CD8$^+$ T cells is also observed in the synovial tissue and fluid of people with RA[92], BALF of people infected by SARS-CoV-2 (ref. 92), atherosclerotic plaques of aging individuals[97], inflamed bowel in people with Crohn's disease or ulcerative colitis[92], pleural fluids of people with tuberculosis[98], labial gland and peripheral mononuclear blood cells of people with Sjögren syndrome[99], and kidney and skin of people with systemic lupus erythematosus (SLE)[100,101]. As we continue to unravel the pro-inflammatory roles of GzmK in aging and inflammatory disorders, future investigations using genetic ablation or pharmacological inhibition are necessary to decipher whether intracellular and/or extracellular GzmK inhibition affects pathology.

Granzyme M

Human GzmM, which is encoded by the *GZMM* gene, located on chromosome 19p13.3, shares less than 40% amino acid sequence homology with other granzymes and is a Met-ase-like protease that preferentially cleaves substrates after methionine and leucine residues[102].

Human GzmM is constitutively highly expressed by NK cells, CD3$^+$CD56$^+$ T cells and γδ T cells[103]. Characterization of GzmM has focussed on its cleavage of intracellular substrates involved in cell death, as well as host and viral proteins implicated in viral replication[104], mostly in human models. Nevertheless, the consequences of extracellular GzmM accumulation remain largely unexplored.

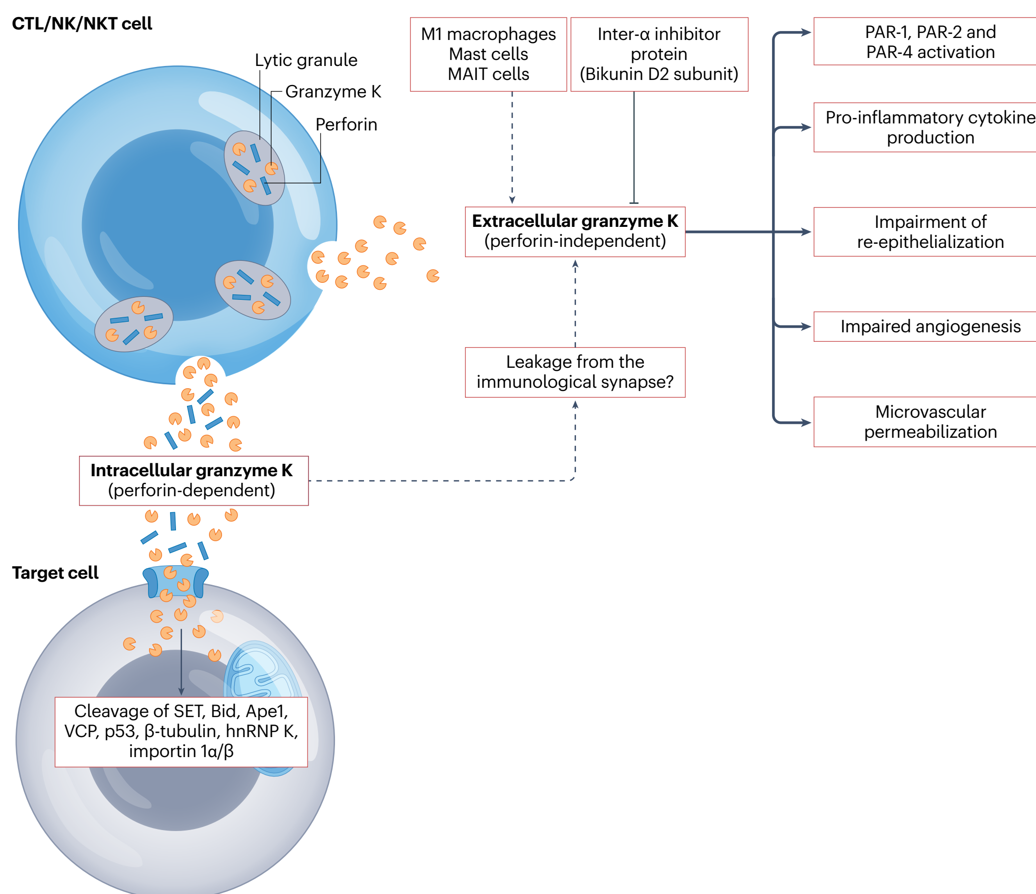

Fig. 3 | Proposed intracellular and extracellular functions of granzyme K. Intracellular functions: granzyme K is secreted by cytotoxic T lymphocytes (CTLs), natural killer (NK) and NKT cells and internalized into target cells in a perforin-dependent manner. On internalization, granzyme K cleaves intracellular substrates implicated in cell death and viral replication. Extracellular functions: granzyme K that leaks from the immunological synapse or is produced by non-cytotoxic cells as well as cells that do not express perforin, is released in the extracellular space. Through the cleavage of protease-activated receptors PAR-1, PAR-2 and PAR-4, extracellular granzyme K contributes to pro-inflammatory cytokine production and impairment of re-epithelialization, as well as pathological angiogenesis and microvascular permeabilization. In humans, granzyme K can be inhibited by the bikunin D2 subunit of inter-α inhibitor protein in the extracellular milieu. Solid and dotted lines denote published and postulated mechanisms, respectively. MAIT cells, mucosal-associated invariant T cells.

Although GzmM-deficient mice have normal NK cell and T cell development and NK cell-dependent anti-tumoural activity, they have a transient higher viral burden and increased infection susceptibility to cytomegalovirus[105]. GzmM also seems to have a pro-inflammatory role, and compared with wild type mice, *Gzmm*$^{-/-}$ mice exhibit reduced serum levels of IL-1α, IL-1β, TNF and IFNγ following lipopolysaccharide injection[106], as well as decreased liver secretion of MIP-1α, impairing recruitment of NK cells[107]. In contrast to GzmA, GzmB and GzmK, the role of GzmM in inflammatory cytokine production is poorly understood.

Granzymes in rheumatic diseases

Arthritis

RA is a chronic autoimmune disease characterized by severe inflammation of the joint synovium, resulting in cartilage degradation, bone erosion and autoantibody production. Although GzmA was first detected in biospecimens of people with RA >30 years ago[108], application of single-cell-based approaches combined with a greater understanding of granzyme biology has shed new light on the roles of granzymes in the pathogenesis of RA (Fig. 4).

GzmB in RA. Accumulating clinical evidence supports a pathological role for GzmB in RA. In people with RA, extracellular GzmB levels are elevated in plasma, synovial fluid and synovial tissue[38,109]. Serum GzmB levels correlate with multiple markers of disease activity and joint damage[110], in addition to serving as an independent predictor of radiographic erosions in rheumatoid factor-positive individuals[111]. Consistently, the presence of the rs8192916 single-nucleotide polymorphism in the *GZMB* gene is associated with higher *GZMB* mRNA expression and correlates with joint destruction in RA lesions[112]. Notably, reduction of GzmB serum levels occurs in people with RA who respond to the targeted anti-CD28 antibody therapy abatacept, an immunomodulator that is currently used for the management of RA symptoms[113], thereby indicating that GzmB is a potential biomarker for disease activity.

In RA tissues and fluids, GzmB is expressed by CD8$^+$ T cells[92,114,115], NK cells and NKT cells[116], macrophages[117], CD19$^+$ B cells[118,119], TNF-activated synovial cells[120] and articular chondrocytes[121]. In seropositive individuals harbouring anti-citrullinated protein antibodies (ACPAs, which are an early diagnostic marker for RA), the *GZMB*-expressing CD8$^+$ T cell subset was expanded compared with healthy individuals[92,115]. Similarly, stimulation of fresh whole blood from people with ACPA$^+$ RA with citrullinated antigens increased the proportion of *GZMB*$^+$ CD8$^+$ T cells and promoted degranulation, thereby enhancing cytotoxicity in vitro[115].

Despite the proposed role for GzmB in autoantigen processing >20 years ago[70], peptidylarginine deiminase 4 (PAD4) remains the only substrate of human GzmB confirmed as an autoantigen in the context of RA[122]. PAD4 is an enzyme involved in protein citrullination, generating citrulline-containing epitopes that are responsible for the production of ACPAs. Additionally, PAD4 peptides are established autoantigens in RA[123], and the presence of PAD4 peptide-specific T cells is associated with high levels of C-reactive protein in RA[72]. As PAD4 localizes intracellularly but is believed to leak into the extracellular space after loss of membrane integrity during cell death[124], the precise location of PAD4 cleavage by GzmB is yet to be confirmed. Whether GzmB-induced gasdermin pore formation is involved in this release is unknown.

Extracellularly, GzmB contributes to cartilage degradation by cleaving the major articular proteoglycan aggrecan from chondrocyte-derived matrix[61], resulting in the release of its branched glycosaminoglycans[60]. It might be possible to extrapolate lessons learned from the proteolytic cleavage of GzmB substrates in other tissues to RA (Table 2). For instance, GzmB cleaves collagen IV[58], a minor network-forming collagen located at the pericellular matrix of articular chondrocytes[125]. Additionally, through direct proteolysis, fibronectin fragments generated by GzmB might contribute to bone erosion by promoting MMP-1 expression in fibroblasts[56]. Although the role of TGF-β is still debatable in the context of RA, GzmB might increase its bioavailability by cleaving decorin and/or biglycan[52].

Further investigations into the role of GzmB in autoantigen generation and ECM remodelling in the context of RA could be addressed through the use of animal models as well as novel N-terminal degradomics-based approaches[126].

GzmB in other arthritides. Expansion of GzmB-expressing NK cells occurs in the peripheral blood of people with pauciarticular or polyarticular juvenile idiopathic arthritis relative to systemic juvenile idiopathic arthritis[127]. Multiple subsets of *GZMB*$^+$ NK cells and γδ T cells are also present in people with gouty arthritis and they correlate with disease severity[128]. In parallel, GzmB is mostly absent or undetectable in biospecimens from people with OA (synovial fluid and plasma)[38], reactive arthritis (synovial fluid and plasma)[38,109], psoriatic arthritis (synovial fluid)[129] and Behçet's disease (synovial fluid)[129].

GzmK in RA. Emerging roles for GzmK in inflammaging (SASP induction)[94] and inflammatory disease[89,91,93] have generated increased interest around GzmK in RA and other rheumatic conditions. Dissociation of leukocyte-rich synovial tissue followed by single-cell RNA sequencing (scRNA-seq) revealed three CD8$^+$ T cell subpopulations with distinct granzyme expression profiles in people with RA: *GZMK*$^+$ T cells, *GZMK*$^+$*GZMB*$^+$ T cells and *GNLY*$^+$ (encoding granulysin) *GZMB*$^+$ CTLs, with intracellular GzmK protein detectable in the majority of synovial CD8$^+$ T cells[114]. These CD8$^+$ T cell subsets were also observed in the synovial fluid and tissues of people with RA[92], with approximately 70% of the CD8$^+$ T cell population in RA synovial fluid and tissue being characterized by high expression of GzmK and intermediate expression of GzmB and perforin-1 (ref. 92). The predominance of *GZMK*-expressing cells in RA synovial tissue is further demonstrated by the observation of higher GzmK concentrations in synovial fluid (≃175 pg/ml) than in serum (≃75 pg/ml) collected from people with RA[130]. Altogether, these observations suggest that granzyme expression is compartmentalized in RA people, with *GZMK*$^+$ CD8$^+$ T cells localizing to the synovial tissue (and *GZMB*$^+$ CD8$^+$ T cells enriched in the circulation).

Unique CD8$^+$ T cell subsets stratified on the basis of differential GzmK and GzmB expression might have distinct phenotypes. *GZMB*$^+$*GZMK*$^+$ CD8$^+$ T cells express less GzmB and perforin-1 and might therefore have lower cytotoxic potential than the *GZMB*$^+$ CD8$^+$ T cell cluster[92]. In contrast to the *GZMB*$^+$ CD8$^+$ T cell population that expresses cytotoxic molecules[115], and in line with their localization, *GZMK*$^+$ CD8$^+$ T cells show higher expression of genes associated with tissue residency, cell proliferation and pro-inflammatory cytokine stimulation[92]. Instead of inducing apoptosis, human GzmK stimulates synovial fibroblast production of IL-6 and CCL2 in a perforin-independent manner[92]. Human GzmK also potentializes IFNγ-dependent production of IL-6 and CCL2 by synovial fibroblasts[92]. These observations strongly suggest that GzmK and GzmB are expressed by distinct human CD8$^+$ T cell populations in a tissue-specific manner and exert dichotomous functions in RA.

Collectively, GzmK could be an important mediator of local inflammation in RA joints through its extracellular functions (Table 2).

Fig. 4 | Potential involvement of granzymes in rheumatoid arthritis. In the pathogenesis of rheumatoid arthritis (RA), extracellular granzyme A contributes to bone erosion through the potentiation of TNF-dependent osteoclast differentiation from progenitor cells. Alongside granzyme A, extracellular granzyme B might promote cartilage degradation through the proteolysis of key cartilage extracellular matrix (ECM) components. Granzyme B-mediated fibronectin fragmentation might generate bioactive fragments involved in matrix metalloproteinase-1 (MMP-1) induction, leading to bone erosion. In parallel, intracellular or extracellular granzyme B can induce chondrocyte apoptosis or anoikis and exposure of peptidylarginine deiminase 4 (PAD4) cryptic epitopes, promoting cartilage degradation and autoantigen generation, respectively. Granzyme B-mediated autoantigen generation can stimulate autoantibody production. CD8$^+$ T cell-derived granzyme B and granzyme A directly contribute to the RA amplification phase by promoting bone erosion, cartilage degradation and autoantigen generation, but the role of granzyme H in RA is unknown. Extracellular granzyme K can contribute to local inflammation by stimulating pro-inflammatory cytokine production (of IL-6 and CCL2) by synovial fibroblasts in a perforin-independent manner. Solid and dotted lines denote published and postulated mechanisms, respectively. Gzm, granzyme; MMP-1, matrix metalloproteinase-1; NK, natural killer.

GzmK-mediated synovial inflammation might be a consequence of its ability to cleave and activate PAR-1 and PAR-2, a phenomenon previously linked to IL-6 production by human epithelial[91] and endothelial cells[90]. As IL-6 has been linked to T cell infiltration in inflamed tissues[131], extracellular GzmK might contribute to immune cell chemoattraction into the synovium in people with RA.

GzmA in RA. More than 30 years ago, elevated concentrations of GzmA were reported in plasma[38], synovial fluid[38,108] and synovial tissues[116,132,133] from people with RA. Currently, documented cell sources of GzmA in arthritic lesions include CD8$^+$ T lymphocytes[92,114,115,132,134] and CD4$^+$ T lymphocytes[134], as well as NK cells and NKT cells[116,132,133,135]. GzmA was also detected in serum and joint extracts from two different mouse models in which inflammatory arthritis was induced by type II collagen injection[25] or Chikungunya virus infection[136].

To date, GzmA is the only granzyme that has been investigated using genetic deletion in an in vivo experimental model of arthritis[25]. Using the collagen-induced arthritis model, GzmA$^{-/-}$ animals had attenuated disease severity compared with wild type or perforin-deficient mice, suggesting that the protease contributes to RA through a perforin-independent extracellular mechanism[25]. At late-stage RA (6 weeks post-induction), GzmA$^{-/-}$ mice show reductions of tissue damage in paws and knees, pannus formation, bone and cartilage erosion and osteoclastogenesis compared with wild type mice. In vitro, treatment of osteoclast progenitors with mouse GzmA stimulates production of TNF, promoting osteoclast maturation and differentiation[25].

Table 2 | Non-cytotoxic granzyme substrates relevant to rheumatic disease

Granzyme	Substrate	Tissue and cellular localization	Potential role in pathology	Refs.
Granzyme B	Peptidylarginine deiminase 4 (PAD4)	Intracellular (and extracellular?)	Structural changes in PAD4; exposure of immunogenic cryptic epitopes; recruitment of PAD4-peptide-specific T cells	72,122
	Unidentified intracellular substrate(s)	Intracellular	Chondrocyte apoptosis	199
	Aggrecan	Extracellular (cartilage ECM)	Cartilage degradation	61
	Cartilage proteoglycan	Extracellular (cartilage ECM)	Release of glycosaminoglycans; cartilage degradation	60
	Collagen IV	Extracellular (articular chondrocyte pericellular matrix)	Impairment of chondrocyte homeostasis; cartilage degradation	48,58
	Fibronectin	Extracellular (cartilage ECM)	Fibronectin fragmentation resulting in cartilage degradation; MMP-1 production leading to bone erosion, release of VEGF from fibronectin	56
	Decorin and biglycan	Extracellular (cartilage ECM)	Impaired collagen fibrillogenesis (decorin); release of bioactive TGF-β	52
	AHNAK, α-fodrin, B23, CENP-B and -C, fibrillarin, HERV-K10, Ku-70, La/SSB, lamin B, M3R, NOR-90/UBF, NuMA, PARP1, RNA pol I and II, SRP-72, topo-1, U1-70 kDa, Ufd2p, XRCC4	Intracellular (cytosol, nucleus, nuclear membrane) and extracellular (plasma membrane)	Exposure of immunogenic cryptic epitopes; autoantibody production; immune tolerance breakdown	71
	α6β4 integrin, collagen VII, collagen XVII, desmoglein-1 and -3, E- and VE-cadherin, filaggrin, ICAM-1, JAM-A, laminin-332 (laminin-5), laminin-511 (laminin-10), fibrillin-1, occludin, vitronectin, ZO-1, pro-IL-1α, pro-IL-18	Intracellular (cytosol) and extracellular (plasma membrane, ECM, basement membrane)	Epithelial/endothelial barrier dysfunction/ permeability; microvascular leakage; basement membrane disruption; bullae formation; ECM remodelling; cytokine processing/activation	33,34
Granzyme A	Aggrecan	Extracellular (cartilage ECM)	Cartilage degradation	61
	Unidentified cell surface receptor(s)	Extracellular	Stimulation of TNF production by osteoclast precursors; osteoclastogenesis; bone erosion	25
	Collagen IV	Extracellular (articular chondrocyte pericellular matrix)	Impairment of chondrocyte homeostasis; cartilage degradation	138
	Fibronectin	Extracellular (cartilage ECM)	Fibronectin fragmentation leading to cartilage degradation	139
	Lamin A, B and C	Intracellular (nuclear membrane)	Nuclear lamina disruption (apoptosis); exposure of immunogenic cryptic epitopes (autoantibody production)	200
	Pro-IL-1β	Intracellular (cytosol)	Cytokine processing/activation	34
Granzyme K	Protease activated receptors 1, 2 and 4	Extracellular	IL-6 and CCL2 production by synovial fibroblasts; potentiation of IFNγ-dependent IL-6 and CCL2 production by synovial fibroblasts; local inflammation and chemoattraction	90–93

ECM, extracellular matrix.

This finding has been confirmed in vivo in a mouse model of osteoclastogenesis related to chronic apical periodontitis[137].

Additional mechanisms for GzmA in RA could be attributed to its perforin-independent role in ECM remodelling, because mouse GzmA, similar to GzmB, cleaves collagen IV[138] and fibronectin[139] (Table 2). Notably, aggrecan is also cleaved by human GzmA, although this proteolytic activity is 2,000-fold lower than that of human GzmB[61]. Evidence indicates that human GzmA can be inhibited in the extracellular space by antithrombin-III[26] and α2-macroglobulin[27], but notably the protease retains most of its proteolytic activity in BALF[46]. Consequently, further investigation of GzmA extracellular activity in the biospecimens of people with RA is required[139].

GzmA in other arthritides. GzmA is detectable in the plasma and synovial fluid of people with OA[38,133], as well as in synovial fluid from individuals with reactive arthritis[38,108]. Notably, GzmA concentrations in OA are lower than those in people with RA. Additionally, enrichment of NK cells expressing GzmA (but not GzmB or perforin) is observed in the synovial fluid of people with OA[135], suggesting a potential but as yet unexplored role for GzmA in OA.

GzmH and GzmM in RA. In general, pathophysiological roles of GzmH and GzmM are poorly understood. Increased *GZMH* mRNA expression was detected in $CD8^+$ T cells from people with $ACPA^+$ RA after stimulation with citrullinated antigens[115]. Further investigations are required to elucidate the relevance of these granzymes with respect to arthritis.

Systemic lupus erythematosus

SLE is an autoimmune disorder characterized by the presence of pathogenic anti-nuclear antibodies, chronic inflammation and tissue damage affecting multiple organs, most commonly skin (in cutaneous lupus erythematosus (CLE)) and kidneys (in lupus nephritis (LN))[140].

Several studies have reported elevation of extracellular GzmB concentrations in the peripheral blood of people with SLE[141–143]. These concentrations, along with the presence of GzmB-expressing CD4$^+$ and CD8$^+$ T cells, correlate positively with disease susceptibility and severity[141,144,145]. Expansion of GzmB$^+$ CD8$^+$ T cells occurs in chronic CLE[101,146], including discoid lupus erythematosus[147], tumid lupus erythematosus[148] and lupus erythematosus profundus[101,146–152]. In LN, although infiltration of *GZMB*$^+$ CD8$^+$ (but not *GZMB*$^+$ CD4$^+$) T cells occurs in the kidneys of patients[100,101,153], the frequency of GzmB-expressing MAIT cells is increased in the peripheral blood, most notably in people with intractable disease[154]. As MAIT cells can also infiltrate the kidneys[155], GzmB secretion from these cells could contribute to tissue inflammation and damage in people with LN. Together, these observations suggest a pathological role for GzmB in SLE (as well as CLE and LN).

GZMK$^+$ CD8$^+$ T cells infiltrate the kidneys and skin of individuals with LN and CLE[100,101]. Circulating cytotoxic *GZMH*$^+$ CD8$^+$ T cell and *GZMK*$^+$ CD8$^+$ T cell populations can be identified in people with SLE through scRNA-seq[156], revealing clonal expansion of a *GZMH*$^+$ CD8$^+$ T cell subset that constitutes up to 50% of all lymphocytes and exhibits heterogeneous expression of cytotoxic, exhaustion and type 1 interferon-stimulated-gene signatures[156]. These *GZMH*$^+$ CD8$^+$ T cells, which also express *GZMB*, are expanded in people with SLE compared with healthy individuals[156], warranting further investigation into the potential pathogenic role of GzmH in SLE.

Environmental triggers that are associated with the exacerbation of SLE (including ultraviolet irradiation, viral infection, smoking and air pollution) also increase granzyme concentrations in stimulated organs or peripheral blood[39,95,157–159]. Moreover, upregulation of type I interferon signalling (an established mediator of disease progression)[160] induces *GZMB* expression in CD8$^+$ T cells through stimulation of the JAK–STAT signalling pathway in both humans and mice[161,162]. Given the ability of GzmB to promote perforin-dependent apoptosis and perforin-independent anoikis[163], GzmB likely contributes to cell death in SLE, thereby leading to disease progression and exacerbation. Additionally, GzmB cleaves numerous intracellular proteins that are recognized SLE autoantigens in vitro, including AHNAK, HERV-K10, Ku-70, La, Lamin B, PARP1, SRP-72, U1-70kDa and XRCC4 (Table 2). As such, GzmB might trigger immune-tolerance breakdown and contribute to disease onset by unmasking cryptic epitopes[71]. However, in mice, GzmB is dispensable for autoantibody production in a pristane-induced model of lupus in vivo[164]. As human and mouse GzmB exhibit discrepancies in substrate preferences as well as cleavage specificities[4], this finding might not translate to humans.

In the pristane-induced model of lupus, a protective role for GzmB was suggested, as GzmB-deficient mice exhibited higher disease severity than wild type counterparts[164]. A GzmB protective role might involve regulatory B cells, as both the quantity and quality of GzmB-producing regulatory B cells are impaired in the peripheral blood of people with active SLE, with the numbers of these cells correlating negatively with clinical severity[165,166]. This observation is in line with the reported protective role of CD19$^+$ B cell-derived GzmB in the context of RA[118]. In summary, the precise implications of granzymes in SLE pathology remains to be elucidated. Whereas GzmB and GzmK from effector T cells and MAIT cells might have pathological roles in SLE, regulatory B cell-derived GzmB could be protective.

Sjögren syndrome

Sjögren syndrome is one of the most common autoimmune diseases, and is characterized by progressive, immune-mediated damage of the exocrine glands (primarily lacrimal and salivary glands), resulting in ocular and oral dryness[167]. As Sjögren syndrome is often accompanied by other autoimmune diseases such as SLE and systemic sclerosis (SSc), the disease presenting on its own is referred to as primary Sjögren syndrome (pSS).

Granzyme (GzmA, GzmB and GzmK)-producing CD8$^+$ T cells are expanded in both lesional exocrine glands and peripheral blood of people with pSS, according to results from studies using scRNA-seq, immunohistochemistry and flow cytometry, and multi-omic studies[99,168–170]. In damaged labial glands in pSS, scRNA-seq identified elevated infiltration of resident memory *GZMB*$^+$*GZMK*$^+$ CD8$^+$ T cells[99]. A distinct aging-associated population of circulating CD8$^+$ T cells co-expressing GzmB and CX3CR1 is present in pSS[169], consistent with findings linking circulating GzmB-expressing T cells and aging[84]. In people with pSS, although *GZMB*$^+$ CD4$^+$ T cells are also expanded in the peripheral blood and correlate positively with disease severity[171], their precise contribution to pathogenesis is unclear.

As CD8$^+$ CTLs are the predominant cell type producing GzmB in pSS lesions, GzmB might contribute to tissue damage through both intracellular (apoptotic) and extracellular mechanisms (Fig. 2). Similar to SLE, GzmB might contribute to autoantibody production through the generation of cryptic autoantigenic epitopes[71]. Specifically, the presence of a 27-kDa fragment of the La protein, which can be generated by GzmB cleavage in vitro, is observed in the serum of people with pSS[172]. Further investigations should delineate the in vivo role of GzmB in the induction of autoantigens, including in relation to its established substrates α-fodrin, M3R, NuMA and La[70,71].

Systemic sclerosis

SSc is a rare autoimmune disease presenting vasculopathy, immune abnormalities and prominent multi-organ fibrosis, with skin involvement a hallmark feature[173].

Granzyme (GzmA, GzmB and/or GzmK)-expressing CD8$^+$ T cells[174–176] are observed in the peripheral blood, skin and lungs of people with SSc. Expansion of CD4$^+$ CTLs expressing GzmA and GzmB in the peripheral blood and skin lesions of patients also occurs[177]. These effector CD4$^+$ CTLs (CD28^{low}CD57^{hi}CD4$^+$), which are clonally expanded in the blood of people with pSS, have elevated expression of *GZMA*, *GZMB*, *GZMM*, *GZMH* and *PFN1*, and cell numbers correlate with skin and lung fibrosis[177]. Consistently, a CD16$^+$CD56^{dim} NK cell population expressing GzmB is enriched in the lungs of people with SSc[176] (although reportedly not in the circulation[178,179]). Finally, elevation of GzmA and GzmB expression also occurs in CD27$^+$ γδ T cells[180], a T cell subpopulation that is expanded in people with pSS, and that demonstrates cytotoxicity against endothelial cells in vitro[181]. As this cytotoxicity is inhibited by anti-GzmA antibodies, these observations support a role for GzmA in pSS tissue damage.

In vitro mechanistic and immunohistochemistry-based studies support the potential roles of CD4$^+$ CTLs, effector CD8$^+$ T cells and γδ T cells in vasculopathy and tissue fibrosis in SSc via granzyme-mediated targeted endothelial cell killing[175,177,181]. As the roles of granzymes were assessed through a perforin-dependent, pro-apoptotic perspective in earlier studies, it remains to be determined whether non-cytotoxic granzyme mechanisms pertaining to vascular permeability and fibrosis contribute to these events. Granzymes might also contribute to SSc initiation and flares through the generation of cryptic autoantigenic epitopes, as suggested in earlier in vitro studies[70,71,182,183]. As such, our understanding of granzymes in SSc requires more research linking observations to mechanisms and causation.

Granzymes as therapeutic targets

Proteases represent one of the largest families of enzymes in humans and an important class of proteins for drug development[184]. Among the proteases, granzymes remain one of the few protease families for which therapeutics have not been developed despite mounting in vivo and ex vivo evidence supporting their pathogenic role in numerous human pro-inflammatory disorders. Current systemic and topical granzyme inhibitors are discussed below.

Systemic inhibitors

Although to our knowledge no publications have reported the systemic use of granzyme-specific small-molecule inhibitors or monoclonal antibodies, serpins (a superfamily of irreversible serine protease inhibitors) have been administered as investigative tools to attenuate the extracellular activity of some mouse granzymes in vivo.

In 2006, serpin A3N was identified as a potent and irreversible extracellular inhibitor of both human and mouse GzmB[185]. Serpin A3N is a serine protease inhibitor that is endogenously expressed in mice. Often associated with its human paralogue α1-antichymotrypsin, which is encoded by the *SERPINA3* gene, serpin A3N is encoded by 1 of 13 closely-related mouse genes generated through diversification and duplication events and has no human orthologue[186]. Of the 13 clade 3 A serpins encoded by the mouse genome, serpin A3N is the only serpin with the capacity to inhibit human and mouse extracellular GzmB[186]. Serpin A3N has demonstrated therapeutic efficacy in inhibiting extracellular GzmB in mouse models of abdominal aortic aneurysm[50], diabetic wound healing[187], ischaemic stroke[188], multiple sclerosis[189] and interface dermatitis[190]. Unfortunately, translation of these findings to humans is limited by serpin A3N immunogenicity (owing to its mouse origin) and lack of specificity[186]. In an alternative approach to GzmB inhibition, intravenous injection of GzmB siRNA attenuated late or chronic experimental autoimmune encephalomyelitis in vivo through a PAR-1-dependent mechanism[191].

A serpin-based approach has been used to target the extracellular activity of GzmA. Systemic administration of the mouse-derived GzmA inhibitor serpinb6b improved survival in an in vivo mouse model of sepsis by reducing TLR4-dependent expression of IL-6 and TNF by macrophages[10,192]. Serpinb6b also reduces gut inflammation and development of colitis-induced colorectal cancer in vivo[24]. In vitro reduction of GzmA-induced pro-inflammatory cytokine production by human monocytes and mouse macrophages was similarly achieved using antithrombin-III[10] or serpinb6b, which are specific endogenous inhibitors of human and mouse GzmA, respectively[193].

Compound 20 is a reversible granzyme B inhibitor[194]. Although it is effective in vitro, efficacy of Compound 20 has not been demonstrated in vivo, possibly because of pharmacokinetic and solubility challenges.

Topical inhibitors

To date, VTI-1002 is to our knowledge the only specific extracellular GzmB inhibitor developed for potential clinical application that has demonstrated efficacy in vivo. VTI-1002 is a potent, first-in-class, small-molecule inhibitor of human GzmB with minimal activity against other proteases[57]. Formulated as a gel, VTI-1002 can penetrate the stratum corneum and exhibits no adverse events when topically applied to mice daily for 30 days[57]. Although optimized for human GzmB, VTI-1002 also inhibits mouse GzmB[57]. Through the inhibition of extracellular GzmB, VTI-1002 effectively reduces disease severity in mouse models of scarring[57], autoimmune blistering (bullous pemphigoid and epidermolysis bullosa acquisita)[40], atopic dermatitis[42] and vaginal epithelial ulceration[68].

Unmet needs and future perspectives

Despite being discovered more than 30 years ago, a considerable gap remains between the in situ detection of granzymes (intracellular or extracellular) and the understanding of their mechanisms of action with respect to injury, inflammation and rheumatic disease. One explanation for this gap is the long-standing view of granzymes only as functionally overlapping, cytotoxic proteases secreted by CTLs and NK cells and internalized into target cells through a perforin-dependent process, a concept that has been refined following the advent of scRNA-seq and the recent identification of $GZMB^{+}$, $GZMK^{+}$ and $GZMB^{+}GZMK^{+}$ $CD8^{+}$ T cell subsets. As novel, non-cytotoxic, perforin-dependent intracellular as well as perforin-independent extracellular roles of granzymes emerge and continue to reshape the field, the contributions of granzymes to immune-mediated pathogenesis should be re-examined.

Although recent findings have brought increased attention to the field, they have also raised additional challenges and questions. Notably, the use of specific granzyme knockout approaches does not distinguish between intracellular and extracellular proteolytic activities. Extracellular pharmacological approaches are beginning to address this issue for GzmA and GzmB, but further studies (and tools) are required to elucidate the extracellular roles and substrates of other granzymes. Here, it should be reiterated that the relevance of results obtained using mouse models should be confirmed (at least in vitro) in human models, as differences are observed between some human and mouse granzyme substrate specificities and enzymatic activities, the biological relevance of which is currently unclear. In vivo confirmation of physiologically relevant substrates coupled with better functional characterization in health and disease will provide greater clarity. The dysregulation and/or addition of a single protease can considerably affect other proteolytic, signalling and/or metabolic pathways within the tightly regulated and interconnected protease web[195]. As such mechanisms are delineated, the full involvement of granzymes within this network will be revealed.

Moving forward, we anticipate that single-cell transcriptomic and proteomic approaches will continue to identify the presence of perforin-deficient, granzyme-positive subsets of lymphocytes in rheumatic and other diseases. However, research will ultimately have to shift towards understanding the functional roles of granzymes and move beyond the mere observation of their expression to understanding the role of these proteases in pathogenesis. This change will be accomplished through the development of better research tools, including antibodies, in vitro, ex vivo and in vivo granzyme-specific activity assays, and intracellular and extracellular inhibitors. Characterization of granzymes in larger animals, such as pigs or non-human primates, will be an important step for preclinical research and development. These tools would enable further progress and investigation into granzymes, which at present remain one of the largest and most poorly understood families of immune-secreted proteases in the human proteome.

Conclusions

The observation that granzymes are elevated in conditions associated with increasing age, autoimmunity and/or acute or chronic inflammation is not new. As the knowledge pertaining to the roles of granzymes continues to expand beyond cytotoxicity into injury, inflammation, epithelial dysfunction, impaired healing, aging and other pathological events, a re-evaluation of these proteases (in particular GzmB) and their potential as therapeutic targets is warranted.

References

1. Joeckel, L. T. & Bird, P. I. Are all granzymes cytotoxic in vivo? *Biol. Chem.* **395**, 181–202 (2014).
2. Voskoboinik, I., Whisstock, J. C. & Trapani, J. A. Perforin and granzymes: function, dysfunction and human pathology. *Nat. Rev. Immunol.* **15**, 388–400 (2015).
3. Cullen, S. P., Adrain, C., Lüthi, A. U., Duriez, P. J. & Martin, S. J. Human and murine granzyme B exhibit divergent substrate preferences. *J. Cell Biol.* **176**, 435–444 (2007).
4. Kaiserman, D. et al. The major human and mouse granzymes are structurally and functionally divergent. *J. Cell Biol.* **175**, 619–630 (2006).
5. Pardo, J. et al. Granzyme B-induced cell death exerted by ex vivo CTL: discriminating requirements for cell death and some of its signs. *Cell Death Differ.* **15**, 567–579 (2008).
6. Thomas, D. A., Scorrano, L., Putcha, G. V., Korsmeyer, S. J. & Ley, T. J. Granzyme B can cause mitochondrial depolarization and cell death in the absence of BID, BAX, and BAK. *Proc. Natl Acad. Sci. USA* **98**, 14985–14990 (2001).
7. Sánchez-Martínez, D. et al. Human NK cells activated by EBV^{+} lymphoblastoid cells overcome anti-apoptotic mechanisms of drug resistance in haematological cancer cells. *Oncoimmunology* **4**, e991613 (2015).
8. Plasman, K., Demol, H., Bird, P. I., Gevaert, K. & Van Damme, P. Substrate specificities of the granzyme tryptases A and K. *J. Proteome Res.* **13**, 6067–6077 (2014).
9. Plasman, K. et al. Conservation of the extended substrate specificity profiles among homologous granzymes across species. *Mol. Cell Proteom.* **12**, 2921–2934 (2013).
10. Garzón-Tituaña, M. et al. Granzyme A inhibition reduces inflammation and increases survival during abdominal sepsis. *Theranostics* **11**, 3781–3795 (2021).
11. Metkar, S. S. et al. Human and mouse granzyme A induce a proinflammatory cytokine response. *Immunity* **29**, 720–733 (2008).
12. Hagn, M. et al. Human B cells secrete granzyme B when recognizing viral antigens in the context of the acute phase cytokine IL-21. *J. Immunol.* **183**, 1838–1845 (2009).
13. Hink-Schauer, C., Estébanez-Perpiñá, E., Kurschus, F. C., Bode, W. & Jenne, D. E. Crystal structure of the apoptosis-inducing human granzyme A dimer. *Nat. Struct. Biol.* **10**, 535–540 (2003).
14. Bell, J. K. et al. The oligomeric structure of human granzyme A is a determinant of its extended substrate specificity. *Nat. Struct. Biol.* **10**, 527–534 (2003).
15. Lieberman, J. Granzyme A activates another way to die. *Immunol. Rev.* **235**, 93–104 (2010).
16. Pardo, J. et al. The biology of cytotoxic cell granule exocytosis pathway: granzymes have evolved to induce cell death and inflammation. *Microbes Infect.* **11**, 452–459 (2009).
17. Beresford, P. J. et al. Granzyme A activates an endoplasmic reticulum-associated caspase-independent nuclease to induce single-stranded DNA nicks. *J. Biol. Chem.* **276**, 43285–43293 (2001).
18. Zhou, Z. et al. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. *Science* **368**, eaaz7548 (2020).
19. Liu, X., Xia, S., Zhang, Z., Wu, H. & Lieberman, J. Channelling inflammation: gasdermins in physiology and disease. *Nat. Rev. Drug Discov.* **20**, 384–405 (2021).
20. Isaaz, S., Baetz, K., Olsen, K., Podack, E. & Griffiths, G. M. Serial killing by cytotoxic T lymphocytes: T cell receptor triggers degranulation, re-filling of the lytic granules and secretion of lytic proteins via a non-granule pathway. *Eur. J. Immunol.* **25**, 1071–1079 (1995).
21. Nakamura, K., Arahata, K., Ishiura, S., Osame, M. & Sugita, H. Degradative activity of granzyme A on skeletal muscle proteins in vitro: a possible molecular mechanism for muscle fiber damage in polymyositis. *Neuromuscul. Dis.* **3**, 303–310 (1993).
22. Campbell, R. A. et al. Granzyme A in human platelets regulates the synthesis of proinflammatory cytokines by monocytes in aging. *J. Immunol.* **200**, 295–304 (2018).
23. Arias, M. A. et al. Elucidating sources and roles of granzymes A and B during bacterial infection and sepsis. *Cell Rep.* **8**, 420–429 (2014).
24. Santiago, L. et al. Extracellular granzyme A promotes colorectal cancer development by enhancing gut inflammation. *Cell Rep.* **32**, 107847 (2020).
25. Santiago, L. et al. Granzyme A contributes to inflammatory arthritis in mice through stimulation of osteoclastogenesis. *Arthritis Rheumatol.* **69**, 320–334 (2017).
26. Masson, D. & Tschopp, J. Inhibition of lymphocyte protease granzyme A by antithrombin III. *Mol. Immunol.* **25**, 1283–1289 (1988).
27. Simon, M. M., Tran, T., Fruth, U., Gurwitz, D. & Kramer, M. D. Regulation of mouse T cell associated serine proteinase-1 (MTSP-1) by proteinase inhibitors and sulfated polysaccharides. *Biol. Chem. Hoppe Seyler* **371**, 81–87 (1990).
28. Kaiserman, D. et al. Identification of serpinb6b as a species-specific mouse granzyme A inhibitor suggests functional divergence between human and mouse granzyme A. *J. Biol. Chem.* **289**, 9408–9417 (2014).
29. Aybay, E. et al. Extended cleavage specificities of human granzymes A and K, two closely related enzymes with conserved but still poorly defined functions in T and NK cell-mediated immunity. *Front. Immunol.* **14**, 1211295 (2023).
30. Boivin, W. A., Cooper, D. M., Hiebert, P. R. & Granville, D. J. Intracellular versus extracellular granzyme B in immunity and disease: challenging the dogma. *Lab. Invest.* **89**, 1195–1220 (2009).
31. Thornberry, N. A. et al. A combinatorial approach defines specificities of members of the caspase family and granzyme B: functional relationships established for key mediators of apoptosis. *J. Biol. Chem.* **272**, 17907–17911 (1997).
32. Zhang, Z. et al. Gasdermin E suppresses tumour growth by activating anti-tumour immunity. *Nature* **579**, 415–420 (2020).
33. Aubert, A., Lane, M., Jung, K. & Granville, D. J. Granzyme B as a therapeutic target: an update in 2022. *Expert Opin. Ther. Targets* **26**, 979–993 (2022).
34. Richardson, K. C., Jung, K., Pardo, J., Turner, C. T. & Granville, D. J. Noncytotoxic roles of granzymes in health and disease. *Physiology* **37**, 323–348 (2022).
35. Prakash, M. D., Bird, C. H. & Bird, P. I. Active and zymogen forms of granzyme B are constitutively released from cytotoxic lymphocytes in the absence of target cell engagement. *Immunol. Cell Biol.* **87**, 249–254 (2009).
36. Jung, K., Pawluk, M. A., Lane, M., Nabai, L. & Granville, D. J. Granzyme B in epithelial barrier dysfunction and related skin diseases. *Am. J. Physiol. Cell Physiol.* **323**, C170–c189 (2022).
37. Skjelland, M. et al. Plasma levels of granzyme B are increased in patients with lipid-rich carotid plaques as determined by echogenicity. *Atherosclerosis* **195**, e142–e146 (2007).
38. Tak, P. P. et al. The levels of soluble granzyme A and B are elevated in plasma and synovial fluid of patients with rheumatoid arthritis (RA). *Clin. Exp. Immunol.* **116**, 366–370 (1999).
39. Hodge, S., Hodge, G., Nairn, J., Holmes, M. & Reynolds, P. N. Increased airway granzyme B and perforin in current and ex-smoking COPD subjects. *COPD* **3**, 179–187 (2006).
40. Hiroyasu, S. et al. Granzyme B inhibition reduces disease severity in autoimmune blistering diseases. *Nat. Commun.* **12**, 302 (2021).
41. Malmestrom, C. et al. Relapses in multiple sclerosis are associated with increased CD8^{+} T-cell mediated cytotoxicity in CSF. *J. Neuroimmunol.* **196**, 159–165 (2008).
42. Turner, C. T. et al. Granzyme B contributes to barrier dysfunction in oxazolone-induced skin inflammation through E-cadherin and FLG cleavage. *J. Invest. Dermatol.* **141**, 36–47 (2021).
43. Turner, C. T. et al. Granzyme B mediates impaired healing of pressure injuries in aged skin. *NPJ Aging Mech. Dis.* **7**, 6 (2021).
44. Russo, V. et al. Granzyme B is elevated in autoimmune blistering diseases and cleaves key anchoring proteins of the dermal-epidermal junction. *Sci. Rep.* **8**, 9690 (2018).
45. Kurschus, F. C. et al. Killing of target cells by redirected granzyme B in the absence of perforin. *FEBS Lett.* **562**, 87–92 (2004).
46. Tremblay, G. M., Wolbink, A. M., Cormier, Y. & Hack, C. E. Granzyme activity in the inflamed lung is not controlled by endogenous serine proteinase inhibitors. *J. Immunol.* **165**, 3966–3969 (2000).
47. Buzza, M. S. et al. Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. *J. Biol. Chem.* **280**, 23549–23558 (2005).
48. Prakash, M. D. et al. Granzyme B promotes cytotoxic lymphocyte transmigration via basement membrane remodeling. *Immunity* **41**, 960–972 (2014).
49. Chamberlain, C. M. et al. Perforin-independent extracellular granzyme B activity contributes to abdominal aortic aneurysm. *Am. J. Pathol.* **176**, 1038–1049 (2010).
50. Ang, L. S. et al. Serpina3n attenuates granzyme B-mediated decorin cleavage and rupture in a murine model of aortic aneurysm. *Cell Death Dis.* **2**, e209 (2011).
51. Hiebert, P. R. et al. Granzyme B contributes to extracellular matrix remodeling and skin aging in apolipoprotein E knockout mice. *Exp. Gerontol.* **46**, 489–499 (2011).
52. Boivin, W. A. et al. Granzyme B cleaves decorin, biglycan and soluble betaglycan, releasing active transforming growth factor-β1. *PLoS One* **7**, e33163 (2012).
53. Hendel, A. & Granville, D. J. Granzyme B cleavage of fibronectin disrupts endothelial cell adhesion, migration and capillary tube formation. *Matrix Biol.* **32**, 14–22 (2013).
54. Hiebert, P. R., Wu, D. & Granville, D. J. Granzyme B degrades extracellular matrix and contributes to delayed wound closure in apolipoprotein E knockout mice. *Cell Death Differ.* **20**, 1404–1414 (2013).
55. Hendel, A., Hsu, I. & Granville, D. J. Granzyme B releases vascular endothelial growth factor from extracellular matrix and induces vascular permeability. *Lab. Invest.* **94**, 716–725 (2014).
56. Parkinson, L. G. et al. Granzyme B mediates both direct and indirect cleavage of extracellular matrix in skin after chronic low-dose ultraviolet light irradiation. *Aging Cell* **14**, 67–77 (2015).
57. Shen, Y. et al. Topical small molecule granzyme B inhibitor improves remodeling in a murine model of impaired burn wound healing. *Exp. Mol. Med.* **50**, 1–11 (2018).
58. Matsubara, J. A. et al. Retinal distribution and extracellular activity of granzyme B: a serine protease that degrades retinal pigment epithelial tight junctions and extracellular matrix proteins. *Front. Immunol.* **11**, 574 (2020).
59. Obasanmi, G. et al. Granzyme B contributes to choroidal neovascularization and age-related macular degeneration through proteolysis of thrombospondin-1. *Lab. Invest.* **103**, 100123 (2023).
60. Ronday, H. K. et al. Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. *Rheumatology* **40**, 55–61 (2001).
61. Froelich, C. J. et al. Human granzyme B degrades aggrecan proteoglycan in matrix synthesized by chondrocytes. *J. Immunol.* **151**, 7161–7171 (1993).
62. Buzza, M. S. et al. Antihemostatic activity of human granzyme B mediated by cleavage of von Willebrand factor. *J. Biol. Chem.* **283**, 22498–22504 (2008).
63. Qian, Q. et al. Maternal diesel particle exposure promotes offspring asthma through NK cell-derived granzyme B. *J. Clin. Invest.* **130**, 4133–4151 (2020).
64. Loeb, C. R. K., Harris, J. L. & Craik, C. S. Granzyme B proteolyzes receptors important to proliferation and survival, tipping the balance toward apoptosis. *J. Biol. Chem.* **281**, 28326–28335 (2006).
65. Shen, Y. et al. Granzyme B deficiency protects against angiotensin II-induced cardiac fibrosis. *Am. J. Pathol.* **186**, 87–100 (2016).
66. Afonina, I. S. et al. Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1α. *Mol. Cell* **44**, 265–278 (2011).

67. Omoto, Y. et al. Granzyme B is a novel interleukin-18 converting enzyme. *J. Dermatol. Sci.* **59**, 129–135 (2010).
68. Lim, Y. S. et al. NK cell-derived extracellular granzyme B drives epithelial ulceration during HSV-2 genital infection. *Cell Rep.* **42**, 112410 (2023).
69. Hu, M. D. et al. γδ intraepithelial lymphocytes facilitate pathological epithelial cell shedding via CD103-mediated granzyme release. *Gastroenterology* **162**, 877–889.e877 (2022).
70. Casciola-Rosen, L., Andrade, F., Ulanet, D., Wong, W. B. & Rosen, A. Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. *J. Exp. Med.* **190**, 815–826 (1999).
71. Darrah, E. & Rosen, A. Granzyme B cleavage of autoantigens in autoimmunity. *Cell Death Differ.* **17**, 624–632 (2010).
72. Darrah, E. et al. Proteolysis by granzyme B enhances presentation of autoantigenic peptidylarginine deiminase 4 epitopes in rheumatoid arthritis. *J. Proteome Res.* **16**, 355–365 (2017).
73. Haddad, P. et al. Structure and evolutionary origin of the human granzyme H gene. *Int. Immunol.* **3**, 57–66 (1991).
74. Wang, L. et al. Structural insights into the substrate specificity of human granzyme H: the functional roles of a novel RKR motif. *J. Immunol.* **188**, 765–773 (2012).
75. Sedelies, K. A. et al. Discordant regulation of granzyme H and granzyme B expression in human lymphocytes. *J. Biol. Chem.* **279**, 26581–26587 (2004).
76. Rönnberg, E. et al. Granzyme H is a novel protease expressed by human mast cells. *Int. Arch. Allergy Immunol.* **165**, 68–74 (2014).
77. Waterhouse, N. J. & Trapani, J. A. H is for helper: granzyme H helps granzyme B kill adenovirus-infected cells. *Trends Immunol.* **28**, 373–375 (2007).
78. Romero, V., Fellows, E., Jenne, D. E. & Andrade, F. Cleavage of La protein by granzyme H induces cytoplasmic translocation and interferes with La-mediated HCV-IRES translational activity. *Cell Death Differ.* **16**, 340–348 (2009).
79. Yu, D. et al. Natural killer cells disrupt nerve fibers by granzyme H in atheriosclerotic cerebral small vessel disease. *J. Gerontol. A Biol. Sci. Med. Sci.* **78**, 414–423 (2023).
80. Bovenschen, N. et al. Granzyme K displays highly restricted substrate specificity that only partially overlaps with granzyme A. *J. Biol. Chem.* **284**, 3504–3512 (2009).
81. Hink-Schauer, C. et al. The 2.2-A crystal structure of human pro-granzyme K reveals a rigid zymogen with unusual features. *J. Biol. Chem.* **277**, 50923–50933 (2002).
82. Bratke, K., Kuepper, M., Bade, B., Virchow, J. C. Jr & Luttmann, W. Differential expression of human granzymes A, B, and K in natural killer cells and during CD8^{+} T cell differentiation in peripheral blood. *Eur. J. Immunol.* **35**, 2608–2616 (2005).
83. Duquette, D. et al. Human granzyme K Is a feature of innate T cells in blood, tissues, and tumors, responding to cytokines rather than TCR stimulation. *J. Immunol.* **211**, 633–647 (2023).
84. Bade, B. et al. Detection of soluble human granzyme K in vitro and in vivo. *Eur. J. Immunol.* **35**, 2940–2948 (2005).
85. Rucevic, M. et al. Altered levels and molecular forms of granzyme K in plasma from septic patients. *Shock* **27**, 488–493 (2007).
86. Li, T., Yang, C., Jing, J., Sun, L. & Yuan, Y. Granzyme K — a novel marker to identify the presence and rupture of abdominal aortic aneurysm. *Int. J. Cardiol.* **338**, 242–247 (2021).
87. Bratke, K. et al. Granzyme K: a novel mediator in acute airway inflammation. *Thorax* **63**, 1006–1011 (2008).
88. Joeckel, L. T. et al. Mouse granzyme K has pro-inflammatory potential. *Cell Death Differ.* **18**, 1112–1119 (2011).
89. Turner, C. T. et al. Granzyme K expressed by classically activated macrophages contributes to inflammation and impaired remodeling. *J. Invest. Dermatol.* **139**, 930–939 (2019).
90. Sharma, M. et al. Extracellular granzyme K mediates endothelial activation through the cleavage of protease-activated receptor-1. *FEBS J.* **283**, 1734–1747 (2016).
91. Kaiserman, D. et al. Granzyme K initiates IL-6 and IL-8 release from epithelial cells by activating protease-activated receptor 2. *PLoS One* **17**, e0270584 (2022).
92. Jonsson, A. H. et al. Granzyme K^{+} CD8 T cells form a core population in inflamed human tissue. *Sci. Transl. Med.* **14**, eabo0686 (2022).
93. Turner, C. T. et al. Granzyme K contributes to endothelial microvascular damage and leakage during skin inflammation. *Br. J. Dermatol.* **189**, 279–291 (2023).
94. Mogilenko, D. A. et al. Comprehensive profiling of an aging immune system reveals clonal GZMK^{+} CD8^{+} T cells as conserved hallmark of inflammaging. *Immunity* **54**, 99–115.e112 (2021).
95. Verschoor, C. P. et al. NK- and T-cell granzyme B and K expression correlates with age, CMV infection and influenza vaccine-induced antibody titres in older adults. *Front. Aging* **3**, 1098200 (2022).
96. Tyrrell, D. J. et al. Clonally expanded memory CD8^{+} T cells accumulate in atherosclerotic plaques and are pro-atherogenic in aged mice. *Nat. Aging* **3**, 1576–1590 (2023).
97. Fernandez, D. M. et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat. Med.* **25**, 1576–1588 (2019).
98. Cai, Y. et al. Single-cell immune profiling reveals functional diversity of T cells in tuberculous pleural effusion. *J. Exp. Med.* **219**, e20211777 (2022).
99. Xu, T. et al. Single-cell profiling reveals pathogenic role and differentiation trajectory of granzyme K^{+}CD8^{+} T cells in primary Sjögren's syndrome. *JCI Insight* **8**, e167490 (2023).
100. Arazi, A. et al. The immune cell landscape in kidneys of patients with lupus nephritis. *Nat. Immunol.* **20**, 902–914 (2019).
101. Dunlap, G. S. et al. Single-cell transcriptomics reveals distinct effector profiles of infiltrating T cells in lupus skin and kidney. *JCI Insight* **7**, e156341 (2022).
102. de Poot, S. A. H. et al. Human and mouse granzyme M display divergent and species-specific substrate specificities. *Biochem. J.* **437**, 431–442 (2011).
103. Sayers, T. J. et al. The restricted expression of granzyme M in human lymphocytes. *J. Immunol.* **166**, 765–771 (2001).
104. de Poot, S. A. & Bovenschen, N. Granzyme M: behind enemy lines. *Cell Death Differ.* **21**, 359–368 (2014).
105. Pao, L. I. et al. Functional analysis of granzyme M and its role in immunity to infection. *J. Immunol.* **175**, 3235–3243 (2005).
106. Anthony, D. A. et al. A role for granzyme M in TLR4-driven inflammation and endotoxicosis. *J. Immunol.* **185**, 1794–1803 (2010).
107. Baschuk, N. et al. NK cell intrinsic regulation of MIP-1α by granzyme M. *Cell Death Dis.* **5**, e1115 (2014).
108. Nordstrom, D. C. et al. Granzyme A-immunoreactive cells in synovial fluid in reactive and rheumatoid arthritis. *Clin. Rheumatol.* **11**, 529–532 (1992).
109. Smeets, T. J., Dolhain, R. J., Breedveld, F. C. & Tak, P. P. Analysis of the cellular infiltrates and expression of cytokines in synovial tissue from patients with rheumatoid arthritis and reactive arthritis. *J. Pathol.* **186**, 75–81 (1998).
110. Qiao, J. et al. Elevated serum granzyme B levels are associated with disease activity and joint damage in patients with rheumatoid arthritis. *J. Int. Med. Res.* **48**, 0300060520962954 (2020).
111. Goldbach-Mansky, R. et al. Raised granzyme B levels are associated with erosions in patients with early rheumatoid factor positive rheumatoid arthritis. *Ann. Rheum. Dis.* **64**, 715–721 (2005).
112. Knevel, R. et al. A genetic variant in granzyme B is associated with progression of joint destruction in rheumatoid arthritis. *Arthritis Rheuma.* **65**, 582–589 (2013).
113. Colombo, E., Scarsi, M., Piantoni, S., Tincani, A. & Airo, P. Serum levels of granzyme B decrease in patients with rheumatoid arthritis responding to abatacept. *Clin. Exp. Rheumatol.* **34**, 37–41 (2016).
114. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
115. Moon, J.-S. et al. Cytotoxic CD8^{+} T cells target citrullinated antigens in rheumatoid arthritis. *Nat. Commun.* **14**, 319 (2023).
116. Tak, P. P. et al. Granzyme-positive cytotoxic cells are specifically increased in early rheumatoid synovial tissue. *Arthritis Rheum.* **37**, 1735–1743 (1994).
117. Kim, W. J., Kim, H., Suk, K. & Lee, W. H. Macrophages express granzyme B in the lesion areas of atherosclerosis and rheumatoid arthritis. *Immunol. Lett.* **111**, 57–65 (2007).
118. Xu, L. et al. Impairment of granzyme B-producing regulatory B cells correlates with exacerbated rheumatoid arthritis. *Front. Immunol.* **8**, 768 (2017).
119. Cui, D. et al. Changes in regulatory B cells and their relationship with rheumatoid arthritis disease activity. *Clin. Exp. Med.* **15**, 285–292 (2015).
120. Kageyama, Y., Kobayashi, H., Kato, N. & Shimazu, M. Etanercept reduces the serum levels of macrophage chemotactic protein-1 in patients with rheumatoid arthritis. *Mod. Rheumatol.* **19**, 372–378 (2009).
121. Horiuchi, K., Saito, S., Sasaki, R., Tomatsu, T. & Toyama, Y. Expression of granzyme B in human articular chondrocytes. *J. Rheumatol.* **30**, 1799–1810 (2003).
122. Darrah, E. et al. Erosive rheumatoid arthritis is associated with antibodies that activate PAD4 by increasing calcium sensitivity. *Sci. Transl. Med.* **5**, 186ra165 (2013).
123. Halvorsen, E. H. et al. Serum IgG antibodies to peptidylarginine deiminase 4 in rheumatoid arthritis and associations with disease severity. *Ann. Rheum. Dis.* **67**, 414–417 (2008).
124. Kinloch, A. et al. Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. *Arthritis Rheum.* **58**, 2287–2295 (2008).
125. Luo, Y. et al. The minor collagens in articular cartilage. *Protein Cell* **8**, 560–572 (2017).
126. Weng, S. S. H. et al. Sensitive determination of proteolytic proteoforms in limited microscale proteome samples. *Mol. Cell Proteom.* **18**, 2335–2347 (2019).
127. Zhou, J., Tang, X., Ding, Y., An, Y. & Zhao, X. Natural killer cell activity and frequency of killer cell immunoglobulin-like receptors in children with different forms of juvenile idiopathic arthritis. *Pediatr. Allergy Immunol.* **24**, 691–696 (2013).
128. Wang, M. et al. Single-cell analysis in blood reveals distinct immune cell profiles in gouty arthritis. *J. Immunol.* **210**, 745–752 (2023).
129. Canete, J. D. et al. Distinct synovial immunopathology in Behcet disease and psoriatic arthritis. *Arthritis Res. Ther.* **11**, R17 (2009).
130. Shan, L. et al. Increased intra-articular granzyme M may trigger local IFN-λ1/IL-29 response in rheumatoid arthritis. *Clin. Exp. Rheumatol.* **38**, 220–226 (2020).
131. McLoughlin, R. M. et al. IL-6 trans-signaling via STAT3 directs T cell infiltration in acute inflammation. *Proc. Natl Acad. Sci. USA* **102**, 9589–9594 (2005).
132. Young, L. H. et al. Expression of cytolytic mediators by synovial fluid lymphocytes in rheumatoid arthritis. *Am. J. Pathol.* **140**, 1261–1268 (1992).
133. Kummer, J. A. et al. Expression of granzymes A and B in synovial tissue from patients with rheumatoid arthritis and osteoarthritis. *Clin. Immunol. Immunopathol.* **73**, 88–95 (1994).
134. Nanki, T. et al. Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* **46**, 2878–2883 (2002).
135. Jaime, P. et al CD56^{+}/CD16^{−} natural killer cells expressing the inflammatory protease granzyme A are enriched in synovial fluid from patients with osteoarthritis. *Osteoarthritis Cartilage* **25**, 1708–1718 (2017).

136. Wilson, J. A. et al. RNA-Seq analysis of chikungunya virus infection and identification of granzyme A as a major promoter of arthritic inflammation. *PLoS Pathog.* **13**, e1006155 (2017).
137. Jia, T. et al. CRISPR/Cas13d targeting GZMA in PARs pathway regulates the function of osteoclasts in chronic apical periodontitis. *Cell Mol. Biol. Lett.* **28**, 70 (2023).
138. Simon, M. M., Kramer, M. D., Prester, M. & Gay, S. Mouse T-cell associated serine proteinase 1 degrades collagen type IV: a structural basis for the migration of lymphocytes through vascular basement membranes. *Immunology* **73**, 117–119 (1991).
139. Simon, M. M., Prester, M., Nerz, G., Kramer, M. D. & Fruth, U. Release of biologically active fragments from human plasma-fibronectin by murine T cell-specific proteinase 1 (TSP-1). *Biol. Chem. Hoppe Seyler* **369**, 107–112 (1988).
140. Crow, M. K. Pathogenesis of systemic lupus erythematosus: risks, mechanisms and therapeutic targets. *Ann. Rheum. Dis.* **82**, 999–1014 (2023).
141. Shah, D., Kiran, R., Wanchu, A. & Bhatnagar, A. Soluble granzyme B and cytotoxic T lymphocyte activity in the pathogenesis of systemic lupus erythematosus. *Cell Immunol.* **269**, 16–21 (2011).
142. Kok, H. M. et al. Systemic and local granzyme B levels are associated with disease activity, kidney damage and interferon signature in systemic lupus erythematosus. *Rheumatology* **56**, 2129–2134 (2017).
143. Chen, L. et al. IKZF1 polymorphisms are associated with susceptibility, cytokine levels, and clinical features in systemic lupus erythematosus. *Medicine* **99**, e22607 (2020).
144. Daca, A. et al. Two systemic lupus erythematosus (SLE) global disease activity indexes — the SLE disease activity index and the systemic lupus activity measure —demonstrate different correlations with activation of peripheral blood CD4$^+$ T cells. *Hum. Immunol.* **72**, 1160–1167 (2011).
145. Blanco, P. et al. Increase in activated CD8+ T lymphocytes expressing perforin and granzyme B correlates with disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* **52**, 201–211 (2005).
146. Patel, J. et al. Multidimensional immune profiling of cutaneous lupus erythematosus in vivo stratified by patient response to antimalarials. *Arthritis Rheumatol.* **74**, 1687–1698 (2022).
147. Abdou, A. G., Shoeib, M., Bakry, O. A. & El-Bality, H. Immunohistochemical expression of granzyme B and perforin in discoid lupus erythematosus. *Ultrastruct. Pathol.* **37**, 408–416 (2013).
148. Fogagnolo, L. et al. Cytotoxic granules in distinct subsets of cutaneous lupus erythematosus. *Clin. Exp. Dermatol.* **39**, 835–839 (2014).
149. Wenzel, J. et al. CXCR3-mediated recruitment of cytotoxic lymphocytes in lupus erythematosus profundus. *J. Am. Acad. Dermatol.* **56**, 648–650 (2007).
150. Wenzel, J. et al. Scarring skin lesions of discoid lupus erythematosus are characterized by high numbers of skin-homing cytotoxic lymphocytes associated with strong expression of the type I interferon-induced protein MxA. *Br. J. Dermatol.* **153**, 1011–1015 (2005).
151. Grassi, M., Capello, F., Bertolino, L., Seia, Z. & Pippione, M. Identification of granzyme B-expressing CD-8-positive T cells in lymphocytic inflammatory infiltrate in cutaneous lupus erythematosus and in dermatomyositis. *Clin. Exp. Dermatol.* **34**, 910–914 (2009).
152. Lovato, B. H. et al. IL-1β and IL-17 in cutaneous lupus erythematous skin biopsies: could immunohistochemicals indicate a tendency towards systemic involvement? *Bras. Dermatol.* **99**, 66–71 (2023).
153. Zhou, M. et al. JAK/STAT signaling controls the fate of CD8$^+$CD103$^+$ tissue-resident memory T cell in lupus nephritis. *J. Autoimmun.* **109**, 102424 (2020).
154. Litvinova, E. et al. MAIT cells altered phenotype and cytotoxicity in lupus patients are linked to renal disease severity and outcome. *Front. Immunol.* **14**, 1205405 (2023).
155. Murayama, G. et al. A critical role for mucosal-associated invariant T cells as regulators and therapeutic targets in systemic lupus erythematosus. *Front. Immunol.* **10**, 2681 (2019).
156. Perez, R. K. et al. Single-cell RNA-seq reveals cell type-specific molecular and genetic associations to lupus. *Science* **376**, eabf1970 (2022).
157. Hernandez-Pigeon, H. et al. UVA induces granzyme B in human keratinocytes through MIF: implication in extracellular matrix remodeling. *J. Biol. Chem.* **282**, 8157–8164 (2007).
158. van Leeuwen, E. M. et al. Emergence of a CD4$^+$CD28$^-$ granzyme B$^+$, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. *J. Immunol.* **173**, 1834–1841 (2004).
159. Aguilera, J. et al. Granzymes, IL-16, and poly(ADP-ribose) polymerase 1 increase during wildfire smoke exposure. *J. Allergy Clin. Immunol. Glob.* **2**, 100093 (2023).
160. Tsokos, G. C., Lo, M. S., Costa Reis, P. & Sullivan, K. E. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* **12**, 716–730 (2016).
161. Lu, C. et al. Type I interferon suppresses tumor growth through activating the STAT3-granzyme B pathway in tumor-infiltrating cytotoxic T lymphocytes. *J. Immunother. Cancer* **7**, 157 (2019).
162. Newby, B. N. et al. Type 1 interferons potentiate human CD8$^+$ T-cell cytotoxicity through a STAT4- and granzyme B-dependent pathway. *Diabetes* **66**, 3061–3071 (2017).
163. Turner, C. T., Lim, D. & Granville, D. J. Granzyme B in skin inflammation and disease. *Matrix Biol.* **75–76**, 126–140 (2019).
164. Graham, K. L. et al. Granzyme B is dispensable for immunologic tolerance to self in a murine model of systemic lupus erythematosus. *Arthritis Rheum.* **52**, 1684–1693 (2005).
165. Rabani, M. et al. IL-21 dependent granzyme B production of B-cells is decreased in patients with lupus nephritis. *Clin. Immunol.* **188**, 45–51 (2018).
166. Bai, M. et al. Impaired granzyme B-producing regulatory B cells in systemic lupus erythematosus. *Mol. Immunol.* **140**, 217–224 (2021).
167. Brito-Zeron, P. et al. Sjögren syndrome. *Nat. Rev. Dis. Prim.* **2**, 16047 (2016).
168. Kaneko, N. et al. Cytotoxic CD8$^+$ T cells may be drivers of tissue destruction in Sjögren's syndrome. *Sci. Rep.* **12**, 15427 (2022).
169. Akiyama, M., Yoshimoto, K. & Kaneko, Y. Significant association of CX3CR1+CD8 T cells with aging and distinct clinical features in Sjögren's syndrome and IgG4-related disease. *Clin. Exp. Rheumatol.* **41**, 2409–2417 (2023).
170. Tasaki, S. et al. Multiomic disease signatures converge to cytotoxic CD8 T cells in primary Sjögren's syndrome. *Ann. Rheum. Dis.* **76**, 1458–1466 (2017).
171. Wang, Q. et al. Correlation of peripheral CD4+GranzB+CTLs with disease severity in patients with primary Sjögren's syndrome. *Arthritis Res. Ther.* **23**, 257 (2021).
172. Huang, M. et al. Detection of apoptosis-specific autoantibodies directed against granzyme B-induced cleavage fragments of the SS-B (La) autoantigen in sera from patients with primary Sjögren's syndrome. *Clin. Exp. Immunol.* **142**, 148–154 (2005).
173. Pope, J. E. et al. State-of-the-art evidence in the treatment of systemic sclerosis. *Nat. Rev. Rheumatol.* **19**, 212–226 (2023).
174. Fuschiotti, P., Medsger, T. A. Jr & Morel, P. A. Effector CD8+ T cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. *Arthritis Rheum.* **60**, 1119–1128 (2009).
175. Ayano, M. et al. Increased CD226 expression on CD8$^+$ T cells is associated with upregulated cytokine production and endothelial cell injury in patients with systemic sclerosis. *J. Immunol.* **195**, 892–900 (2015).
176. Padilla, C. M. et al. Increased CD8$^+$ tissue resident memory T cells, regulatory T cells, and activated natural killer cells in systemic sclerosis lungs. *Rheumatology* **63**, 837–845 (2023).
177. Maehara, T. et al. Cytotoxic CD4$^+$ T lymphocytes may induce endothelial cell apoptosis in systemic sclerosis. *J. Clin. Invest.* **130**, 2451–2464 (2020).
178. Gumkowska-Sroka, O. et al. Cytometric characterization of main immunocompetent cells in patients with systemic sclerosis: relationship with disease activity and type of immunosuppressive treatment. *J. Clin. Med.* **8**, 625 (2019).
179. Horikawa, M. et al. Abnormal natural killer cell function in systemic sclerosis: altered cytokine production and defective killing activity. *J. Invest. Dermatol.* **125**, 731–737 (2005).
180. Henriques, A. et al. Subset-specific alterations in frequencies and functional signatures of γδ T cells in systemic sclerosis patients. *Inflamm. Res.* **65**, 985–994 (2016).
181. Kahaleh, M. B., Fan, P. S. & Otsuka, T. γδ Receptor bearing T cells in scleroderma: enhanced interaction with vascular endothelial cells in vitro. *Clin. Immunol.* **91**, 188–195 (1999).
182. Schachna, L. et al. Recognition of granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum.* **46**, 1873–1884 (2002).
183. Ulanet, D. B., Flavahan, N. A., Casciola-Rosen, L. & Rosen, A. Selective cleavage of nucleolar autoantigen B23 by granzyme B in differentiated vascular smooth muscle cells: insights into the association of specific autoantibodies with distinct disease phenotypes. *Arthritis Rheum.* **50**, 233–241 (2004).
184. Overall, C. M. & Dean, R. A. Degradomics: systems biology of the protease web. Pleiotropic roles of MMPs in cancer. *Cancer Metastasis Rev.* **25**, 69–75 (2006).
185. Sipione, S. et al. Identification of a novel human granzyme B inhibitor secreted by cultured Sertoli cells. *J. Immunol.* **177**, 5051–5058 (2006).
186. Horvath, A. J. et al. The murine orthologue of human antichymotrypsin: a structural paradigm for clade A3 serpins. *J. Biol. Chem.* **280**, 43168–43178 (2005).
187. Hsu, I. et al. Serpina3n accelerates tissue repair in a diabetic mouse model of delayed wound healing. *Cell Death Dis.* **5**, e1458 (2014).
188. Li, F. et al. Neuronal serpina3n is an endogenous protector against blood brain barrier damage following cerebral ischemic stroke. *J. Cereb. Blood Flow. Metab.* **43**, 241–257 (2023).
189. Haile, Y. et al. Granzyme B-inhibitor serpina3n induces neuroprotection in vitro and in vivo. *J. Neuroinflammation* **12**, 157 (2015).
190. Saito, A. et al. Blockade of granzyme B remarkably improves mucocutaneous diseases with keratinocyte death in interface dermatitis. *J. Invest. Dermatol.* **138**, 2079–2083 (2018).
191. Raveney, B. J. E. et al. Eomesodermin-expressing T-helper cells are essential for chronic neuroinflammation. *Nat. Commun.* **6**, 8437 (2015).
192. Uranga-Murillo, I. et al. Biological relevance of granzymes A and K during *E. coli* sepsis. *Theranostics* **11**, 9873–9883 (2021).
193. Sas, G., Pepper, D. S. & Cash, J. D. Investigations on antithrombin III in normal plasma and serum. *Br. J. Haematol.* **30**, 265–272 (1975).
194. Willoughby, C. A. et al. Discovery of potent, selective human granzyme B inhibitors that inhibit CTL mediated apoptosis. *Bioorg. Med. Chem. Lett.* **12**, 2197–2200 (2002).
195. Dufour, A. & Overall, C. M. Missing the target: matrix metalloproteinase antitargets in inflammation and cancer. *Trends Pharmacol. Sci.* **34**, 233–242 (2013).
196. Ebnet, K. et al. Granzyme A-deficient mice retain potent cell-mediated cytotoxicity. *EMBO J.* **14**, 4230–4239 (1995).
197. Heusel, J. W., Wesselschmidt, R. L., Shresta, S., Russell, J. H. & Ley, T. J. Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells. *Cell* **76**, 977–987 (1994).

198. Joeckel, L. T., Allison, C. C., Pellegrini, M., Bird, C. H. & Bird, P. I. Granzyme K-deficient mice show no evidence of impaired antiviral immunity. *Immunol. Cell Biol.* **95**, 676–683 (2017).
199. Saito, S., Murakoshi, K., Kotake, S., Kamatani, N. & Tomatsu, T. Granzyme B induces apoptosis of chondrocytes with natural killer cell-like cytotoxicity in rheumatoid arthritis. *J. Rheumatol.* **35**, 1932–1943 (2008).
200. Zhang, D., Beresford, P. J., Greenberg, A. H. & Lieberman, J. Granzymes A and B directly cleave lamins and disrupt the nuclear lamina during granule-mediated cytolysis. *Proc. Natl Acad. Sci. USA* **98**, 5746–5751 (2001).

Acknowledgements

D.J.G. is funded by grants-in-aid from the Canadian Institutes for Health Research (CIHR). A.A. is the recipient of the Arthritis Society Canada Training Postdoctoral Fellowship. J.P. is funded by CIBER (Centro de Investigación Biomédica en Red; CB21/13/00087), Instituto de Salud Carlos III, FEDER (Fondo Europeo de Desarrollo Regional), Gobierno de Aragón (Group B29_23R, and LMP139_21), Grant PID2020-113963RBI00 by MCIN/AEI/10.13039/501100011033, ASPANOA, and Carrera de la Mujer de Monzón.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

D.J.G. is a Co-Founder and Chief Scientific Officer of viDA Therapeutics, which owns patents for and is developing inhibitors targeting granzymes as therapeutics. The remaining authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Rheumatology* thanks Niels Bovenschen and the other, anonymous, reviewers for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© Springer Nature Limited 2024

QUERY FORM

Manuscript ID	
Author	

AUTHOR:

The following queries have arisen during the editing of your manuscript. Please answer by making the requisite corrections directly in the e.proofing tool rather than marking them up on the PDF. This will ensure that your corrections are incorporated accurately and that your paper is published as quickly as possible.

Query No.	*Nature of Query*
Q1:	Should affiliation 1 be separated into two separate affiliations? It seems to consist of two institutions.
Q2:	Should affiliation 3 be separated into two separate affiliations? It seems to consist of two institutions.
Q3:	Please note that affiliations have been re-numbered for sequential order.
Q4:	Please check your article carefully, coordinate with any co-authors and enter all final edits clearly in the eproof, remembering to save frequently. Once corrections are submitted, we cannot routinely make further changes to the article.
Q5:	Note that the eproof should be amended in only one browser window at any one time; otherwise changes will be overwritten.
Q6:	Author surnames have been highlighted. Please check these carefully and adjust if the first name or surname is marked up incorrectly, as this will affect indexing of your article in public repositories such as PubMed. Also, carefully check the spelling and numbering of all author names and affiliations, and the corresponding author(s) email address(es). Please note that email addresses should only be included for designated corresponding authors, and you cannot change corresponding authors at this stage except to correct errors made during typesetting.
Q7:	Do you mean alpha-1 antitrypsin?

Uncorrected proofs