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Granzyme-Producing CD4 T Cells in Cancer and Autoimmune Disease

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ABSTRACT
CD4 T cells play important roles in promoting protective immunity and autoimmune disease. A great deal of attention has been given to the differentiation and function of subsets of cytokine-producing CD4 T cells (i.e., Th1, Th2, and Th17 cells) in these settings. However, others have also observed the accumulation of granzyme-producing CD4 T cells in tumors and in autoimmune patients that are distinct from their cytokine-producing counterparts. Despite the relatively large numbers of granzyme-producing cells in diseased tissues, their roles in driving disease have remained enigmatic. This review will focus on the phenotype(s) and roles of granzyme-producing CD4 T cells in cancer and autoimmunity. We will also examine how granzyme-producing cells interact with current therapeutics and speculate how they may be targeted during disease. ImmunoHorizons, 2021, 5: 909–917.

INTRODUCTION
The CD4+ Th cells play a critical role in shaping the protective immune response to pathogens but also contribute to immunopathology and autoimmune disease when they become dysregulated. Traditionally, autoimmune disease has been associated with IFN-γ–producing Th1 cells, IL-4–producing Th2 cells, and IL-17–producing Th17 cells that accumulate in lesional tissues and act to amplify the local inflammatory response (1). Despite the importance of these cytokine-producing Th lineages in the inflammatory process, it has become clear that a distinct type of granzyme-producing Th cell emerges in the context of chronic viral infection, cancer, and autoimmunity disease. This review will focus on the phenotypic characteristics and functional roles of granzyme-producing CD4 T cells in distinct disease settings and how they may be targeted therapeutically in each setting.

Granzymes in cell death and disease
Granzymes are a class of serine proteases that are associated with killing of infected or malignant cells by cytotoxic immune cells (i.e., CD8+ T cells and NK cells). Although granzyme A (GrA) and granzyme B (GrB) are the most highly expressed granzymes by immune cells, there are a number of other granzymes (granzymes C, D, E, F, G, K, M, and N in mice and granzymes H, K, and M in humans) that are either only minimally expressed by immune cells or have lesser-known roles in disease. Because of this fact, we will focus on the role of GrA and GrB in these processes. GrA and GrB are highly selective for their protein substrates and recognize an ~3–4 aa sequence on each side of the cleavage site. For instance, GrA preferentially cleaves after the basic amino acids (Arg or Lys), and GrB cleaves after the acidic amino acids Asp or Glu (2). GrA and GrB often play distinct roles in the immune response, and this may in part be due to their unique substrate specificities.

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Abbreviations used in this article: CD, Crohn’s disease; GrA, granzyme A; GrB, granzyme B; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; IBD, inflammatory bowel disease; IgG4-RD, IgG4-related disease; PAR, protease-activated receptor; RNA-seq, RNA-sequencing; SSc, systemic sclerosis; TME, tumor microenvironment; Treg, T regulatory cell; UC, ulcerative colitis.
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In T cells and NK cells, granzymes are stored within cytolytic granules that contain the pore-forming protein perforin and the proteoglycan serglycin. In this setting, serglycin enables the formation of large granzyme–perforin–serglycin complexes containing up to 30 granzyme/perforin proteins in a single complex (3, 4). During the cytolytic response, these complexes are released from these cytolytic granules and delivered to target cells via serglycin/perforin-mediated uptake or directly through perforin-formed pores (3, 5). After delivery, GrA localizes to the mitochondria, where it cleaves the electron transport chain–associated NDUSF3 protein and leads to enhanced reactive oxygen species production and loss of mitochondrial membrane potential (6). GrA also activates the endoplasmic reticulum–associated SET complex, resulting in downstream ssDNA nicks and a distinct caspase-independent “writhing” form of apoptosis (7, 8) or pyroptosis by cleaving cytoplasmic gasdermin B (Fig. 1) (9). GrB is also a potent inducer of apoptosis but does so by different mechanisms than GrA. Upon cytoplasmic delivery, GrB cleaves Bid, which leads to mitochondrial membrane permeabilization and release of cytochrome C. In contrast to GrA, GrB-mediated cell death is caspase dependent, and GrB directly cleaves cytoplasmic caspases that induce apoptosis via degradation of several nuclear proteins (Fig. 1) (10–12).

Despite the important roles of granzymes in inducing immune-mediated killing, mice lacking GrA or GrB expression are capable of clearing a number of infections (13–16), potentially because of the overlapping substrates and/or parallel programs of cell death through other orphan granzymes. Similarly, the human GZMB gene contains a number of single nucleotide polymorphisms, a set of which produce a mutant protein (e.g., Q48R; R245H) with altered subcellular localization (17). Similar to their murine counterparts, individuals with these GZMB mutations also develop normally, and conventional T cells from these individuals exhibited full killing capacity against transformed B cells. However, other studies indicated that R245H patient-derived NK and γδ T cells had reduced killing capacity against other tumor cell types (18, 19). These data suggest that granzyme functional redundancy may depend on immune cell type or target cell type specific in humans.

**Noncytolytic roles of granzymes in host immunity**

Mounting evidence suggests that granzymes are secreted from immune cells and have activity in the extracellular space. Both CD4 and CD8 T cells have been demonstrated to secrete granzymes after TCR ligation but do so by seemingly distinct mechanisms (20–22). Despite these differences, GrA and GrB are secreted in complex with proteoglycans that enable their extended activity in blood or tissues by preventing inactivation by extracellular serine protease inhibitors and rapid clearance from circulation (23, 24). During disease, however, extracellular granzyme levels are increased, and granzyme inhibitor levels are diminished (25, 26), indicating that inflammation shifts the balance toward active extracellular granzymes that may contribute to pathology. Interestingly, although there are dedicated inhibitors of GrA in circulation (SERPINC1 and α2-macroglobulin in humans and Serpinb6b in mouse), there are not well-defined inhibitors of GrB in the extracellular space (27). These data suggest that extracellular activity of GrA is more tightly regulated and therefore may have heightened inflammatory capacity.

In the extracellular space, granzymes promote extracellular matrix degradation (28–30) and the induction of inflammatory cytokines (31, 32). In extracellular matrix degradation, both GrA and GrB substrates are components of the extracellular matrix, and recent work shows that granzymes, particularly GrB, enhance T cell extravasation through tissue basement membranes (29) (Fig. 1). Extracellular granzymes have also been shown to promote inflammatory cytokine production through multiple mechanisms. Extracellular GrA and GrB have been shown to enter cells and to cleave pro–IL-18 and pro–IL-18 into their biologically active forms (33, 34). Further, GrA has been shown to enhance signaling through bacterial sensing TLRs on/in myeloid cells and augment their production of inflammatory cytokines (Fig. 1). For instance, GrA interacts with TLR2 and TLR4 and enhances the sensitivity of this receptor to bacterial lipopeptides and LPS (31, 35). Extracellular GrA is also taken up by plasmacytoid dendritic cells and promotes TLR9-mediated production of type I IFNs (36). Granzymes additionally cleave protease-activated receptors.
(PARs) on various cell types in the intestines and brain that alter cell behavior and promote cytokine production (37, 38).

Granzymes A and B play a myriad of roles in the immune response, and CD4 T cells are a biologically relevant source of granzymes in tumors and in autoimmune disease. In the next sections, we will examine granzyme-producing CD4 T cells across a spectrum of disease states. Within each disease, we will highlight their phenotypic features, their proposed roles in disease pathogenesis, and how these granzyme-producing cells interact with current or potential therapeutics.

CANCER

**CD4 T cells in anticancer immunity**

Traditionally, cytolytic CD8 T cells have been most commonly associated with successful antitumor immunity. However, with advances in single cell RNA-sequencing (RNA-seq) technology, it has become clear that several noncanonical (i.e., non-Th1 Th2, and Th17) Th cell subsets infiltrate into the tumor microenvironment (TME) and may contribute to the anticancer response. Within these noncanonical subsets are populations of cytolytic CD4 T cells that express high levels of granzymes and perforin that represent ~2–40% of the total CD4 T cell population in various cancer types (39). Intertumoral cytolytic CD4 T cells express similar levels of these cytolytic genes as compared with CD8 T cells in the same locale, indicating that these cells are likely a biologically relevant source of granzymes within the tumor. Further, this cytotoxic CD4 T cell signature is largely conserved across multiple tumor types (39, 40), suggesting that cytolytic CD4 T cell responses are not a product of specific tumor TME. Instead, it is likely that this is a hardwired antitumor Th cell program. As MHCI expression is induced on tumor cells by IFN-γ and TNF (39), it begs to reason that tumor cells may be a direct target of granzyme-producing CD4 T cells in this setting.

**Phenotypic features of granzyme-producing CD4 T cells in the TME**

In bladder cancer, single cell RNA-seq studies have identified several subtypes of granzyme-producing CD4 T cells. GZMB<sup>hi</sup> cells expressed high transcript levels of cytotoxic-associated (PRF1, NKG7, and GZMH) and exhaustion-associated (PDCD1, LAG3, and HAVCR2) transcripts as compared with other granzyme-expressing CD4 T cell subsets (40). GZMK<sup>hi</sup> cells expressed low levels of GZMB, GZMH, and PRF1, indicating a less cytolytic phenotype. Interestingly, GZMA was equivalently expressed between these subsets and also meaningfully expressed by Th17-like cells within the TME (40). It is possible that these cells share a common progenitor and represent different states of development within the tumor. Alternatively, these subsets may represent distinct cytotoxic lineages with unique functions within the TME. Bladder cancer may be an ideal model system to use to tackle these questions given the high diversity of granzyme-expressing cells within these tumors.

**Functional roles of granzyme-producing CD4 T cells in anticancer immunity**

There has been a paucity of studies that have manipulated T cell granzyme expression in human or mouse models of cancer. However, in our previous work, we demonstrated that CD4 T cell–derived GrA was not required for the beneficial graft-versus-leukemia effect in clearance of MLL-AF9 leukemia cells from bone marrow transplant recipient mice (41). These data suggest that GrA may not have direct antitumoricidal effects in this model. In contrast, in human gasdermin B<sup>+</sup> tumor cell lines, GrA acted to cleave gasdermin B and induce cancer cell death via pyroptosis (9). As gasdermin B is not expressed in mice, this may explain the lack of direct antitumor activity in our study and indicates that GrA may have a specific role in eliminating gasdermin B<sup>+</sup> tumor cells. In contrast to GrA, GrB-deficient CD4 T cells were less able to kill A20 lymphoma cells in a graft-versus-lymphoma response, suggesting that GrB may promote CD4 T cell–mediated tumor killing (42).

**Cancer immunotherapies bolster granzyme-producing CD4 T cells to promote antitumor immunity**

Tumors use multiple mechanisms to suppress anticancer immunity, including enhanced tumor T regulatory cell (Treg) activity and induction of immune checkpoint receptors that limit conventional T cell activity. Depletion of Tregs in tumor-bearing mice enhanced antitumor immunity and resulted in an increased population of GrB-producing conventional CD4 T cells within the tumor (43). Similarly, removal of Tregs from bulk CD4<sup>+</sup> tumor-infiltrating cell preparations enhanced the ability of granzyme-expressing CD4 T cells to kill autologous tumors after coculture (40). Checkpoint blockade therapy (i.e., anti–PD-L1 or anti-CTLA4) has also been shown to dramatically enhance antitumor immunity in some cases through reinvigoration of exhausted T cells within the tumor (44). Evidence of a strong pretreatment granzyme-producing CD4 T cell signature was a predictor of an enhanced response to checkpoint blockade (i.e., anti–PD-L1) therapy in bladder cancer patients (40), further implicating the importance of these cells in antitumor immunity.

GRAFT-VERSUS-HOST DISEASE

Graft-versus-host disease (GVHD) is a detrimental alloimmune response disease that occurs in ~40–70% patients that receive hematopoietic stem cell transplant (HSCT) as a treatment for blood cancers. Both CD4 and CD8 T cells contribute to various aspects of GVHD; however, CD4 T cells are thought to be a major contributor to acute GVHD (i.e., disease that occurs within the first 100 d of HSCT). Acute GVHD commonly manifests within the liver and intestines where donor CD4 T cells produce inflammatory cytokines and cause tissue destruction.
Similar to colitis (see below), GVHD is often considered a Th1-like disease as IFN-γ-producing Th1 cells are abundant in GVHD target organs (i.e., gut and liver) in mouse models of disease. In some cases, however, HSCT with IFN-γ-deficient T cells or neutralizing IFN-γ with Abs actually augmented GVHD severity (45, 46). These data suggest that other types of Th cells play a role in disease (47–49).

**Granzyme-producing conventional CD4 T cells in acute GVHD**

Patients with acute GVHD have elevated serum levels of GrA and GrB (50). GrA and GrB are also produced in pre-HSCT MLRs with donor and host lymphocytes. Interestingly, GrA production in these MLR cultures correlated with increased numbers of CD4 T cells and with the number of MHCII donor–host mismatches, indicating that CD4 T cells are a potential source of GrA in pretransplant MLRs (51). Further, GrA, but not GrB, production in these cultures was predictive of severe acute GVHD development post-HSCT (51). Recent work from our laboratory has identified a subset of GrA-producing conventional CD4 T cells in the intestines of mice with acute GVHD (41). GrA+ CD4 T cells accounted for 30–50% of the total intestinal CD4 T cell population and did not meaningfully coproduce other Th lineage cytokines or transcription factors (i.e., IL-4, IL-17, and FOXP3). However, similar with granzyme-producing cells in other autoimmune diseases (see below), these cells did partially coexpress IFN-γ (41). GrA+ CD4 T cells did not express high levels of GrB or perforin and maintained high levels of the transcription factor ThPOK, suggesting that intestinal GrA+ T cells may have limited cytotoxic ability. Importantly, CD4 T cell–derived GrA was required for intestinal damage and the development of acute GVHD (41). In our studies, we noted that GrA+ CD4 T cells were associated with the loss of colon crypt bases (41). This is significant as these basal crypt cells have previous been reported to express MHCII (52) and may be a direct target of GrA-producing cells. GrA-expressing CD4 T cells were also recently identified at high levels in the peripheral blood of a chronic GVHD patient (53), stressing the potential importance of these cells in driving human disease.

A separate population of GrB-producing CD4 T cells were also enriched in GVHD target organs after HSCT (54). However, in contrast to GrA, HSCT with GrB-deficient CD4 T cells resulted in exacerbated disease, suggesting that GrB protects mice from acute GVHD. Mechanistically, CD4 T cell–derived GrB suppressed systemic cytokine production and intestinal inflammation. Further, GrB played a critical role in maintaining activation-induced cell death in donor T cells (54), thereby invoking a mechanism by which GrB+ CD4 T cells recognize and kill other donor T cells and thus maintain an anti-inflammatory state. These divergent functions of GrA and GrB were also observed in mouse models of colitis (see below), suggesting that these functions may be conserved across intestinal disease states.

**Targeting GrA+ CD4 T cell differentiation as an acute GVHD therapeutic**

Our previous work indicated that CD4 T cell–derived GrA plays a pathogenic role in acute GVHD. However, the ability to target GrA directly is limited by the availability of specific granzyme inhibitors (41). As an alternative approach, it may be possible to specifically target factors that contribute to the differentiation of GrA-producing conventional CD4 T cells. This approach has shown efficacy in Th17-mediated diseases, in which blockade of cytokines that drive Th17 differentiation (i.e., IL-23) is effective in treating colitis and psoriasis patients (55, 56). We demonstrated that IL-6– and IL-21–induced STAT3 signaling was required for the differentiation of GrA-producing CD4 T cells in mice (41). These data suggest that directly targeting STAT3-activating cytokines (i.e., IL-6 or IL-21) or JAK (57) involved in STAT3 phosphorylation may eliminate GrA+ CD4 T cell differentiation and thereby limit disease. Indeed, anti-IL-6R (tocilizumab) therapy and the JAK1/2 inhibitor ruxolitinib were recently shown to be effective in mouse models of GVHD and in GVHD patients (58–60). Neither study, however, directly examined effects of these treatments on granzyme-producing CD4 T cells.

**INFLAMMATORY BOWEL DISEASE**

Ulcerative colitis (UC) and Crohn’s disease (CD) are forms of inflammatory bowel disease (IBD) that are characterized by distinct patterns of inflammation within different regions of the gastrointestinal tract (61). CD4+ T cells are present at elevated numbers in the lesional tissue of UC patients (61), and transfer of highly purified CD4 T cells is sufficient to drive colitis in mouse models of disease (62). Although it is clear that Th cells are involved in colitis development, the types of Th cells that mediate pathology are less clear. In IBD patients, there is a tremendous diversity of Th cell types present within the inflamed tissue that include Th1 and Th17 cells. However, granzyme-producing cells have also been identified in lesional tissue of UC patients (63), and recent single cell RNA-seq studies have identified a pool of granzyme-producing Th helper cells that are expanded in CD patients (61). These data have been corroborated in mouse models of disease in which granzyme-producing Th cells, or their precursors, are elevated in diseased tissues (41, 64, 65). Because of the correlation of these cells with enhanced intestinal pathology, understanding how these cells function and differentiate is highly worthwhile.

**Granzyme-producing cells in colitis**

Within the intestines of ileal CD patients, a number of T cell types produced granzymes, including populations with an enhanced CD4:CD8 expression ratio (e.g., most likely CD4 T cells). Within these CD4-skewed populations, GZMA and GZMB are the most highly expressed granzymes, whereas the cytolytic-associated gene PRF1 (perforin) is only minimally expressed. This potentially indicates a less cytolytic role for these cells in 1

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1. [https://doi.org/10.4049/immunohorizons.2100017](https://doi.org/10.4049/immunohorizons.2100017)
disease. In UC patients, GrA-expressing Th cells are also enriched within lesional tissues and express the integrin CD103 that interacts with E-cadherin on intestinal epithelial cells (63). These CD103⁺ cells make up ~5–10% of the intestinal CD4 T cells in UC patients (63), indicating that these CD103⁺ cells may be a relevant source of GrA in the UC colon. Interestingly, ITGAE, the gene encoding CD103, is also more highly expressed by granzyme-producing cells ileal CD patients as compared with non-granzyme-producing Tregs and T follicular helper-like cells (61). These data suggest that granzyme-producing cells within the inflamed intestines may have a shared T resident memory–like phenotype and that CD103–E cadherin interactions may play a role in the functions of these cells. Indeed, E-cadherin stimulation of CD103⁺ T cells triggers granzyme release (M.D. Hu, N.B. Golovchenko, T.J. Kelly, J.agos, M.R. Zeglinski, E.M. Bonder, I. Sandrock, I. Prinz, D.J. Granville, A.J.M. Watson, et al., manuscript posted on bioRxiv, DOI: 10.1101/2021.07.22.453366), leading to the degradation of the epithelial barrier. As E-cadherin and MHCII are highly expressed on subsets of intestinal epithelial cells and on inflammatory dendritic cells, it begs to reason that these cells may aid in triggering granzyme release during colitis.

Conflicting roles of granzyme-producing CD4 T cells in IBD pathogenesis

Both GrA and GrB are elevated in the serum of patients with severe or active UC and CD (66, 67), suggesting that they may be involved in the disease process. In UC, GrA promoted intestinal disease and the development of colorectal cancer by inducing inflammatory cytokine production (i.e., IL-6 and TNF α) by myeloid cells (67). In this mouse model of disease (dextran sulfate sodium–induced colitis), there is little CD4 T cell involvement, and NK cells were the primary producers of GrA within the intestinal tract. However, given the elevated presence of GrA⁺ Th cells in human UC patients, it stands to reason that Th cells may meaningfully contribute to human disease via a similar mechanism. Using a CD4 T cell–dependent mouse model of disease, our laboratory has shown that GrA-producing cells are elevated in the intestines and are required for IBD development (S. Park and M.R. Olson, unpublished data), therefore providing a proof-of-principle that GrA production by CD4 T cells is capable of promoting intestinal disease.

GrB is reported to have both pro- and anti-inflammatory roles in IBD. Work from Takeuchi et al. (65) demonstrated the existence of a small population of CD4 T cells expressing the cytotoxic T cell marker CRTAM within uninflamed tissues. Interestingly, CRTAM downstream signaling promoted the induction of a subset of GrB- and perforin-expressing Th cells with cytolytic activity. During colitis, CRTAM⁺ cells were further enriched within the intestines, and GrB production by these cells was associated with enhanced disease (65). In contrast to these reports, Hoek et al. (64) demonstrated that Th cell–derived GrB inhibited the development of T cell–dependent colitis. In these studies, Th cell–derived GrB suppressed the differentiation of Th17 cells, and transfer of GrB-deficient T cells into RAG mice resulted in enhanced colitis, marked by the accumulation of IL-17–producing Th cells within the intestinal tract (64). These seemingly contradictory results may point to an important role of the microbiota in regulating the protective versus inflammatory nature of Th cell–derived GrB within this model system. Ivanov et al. (68) indicated that microbial composition played a dramatic role in regulating granzyme expression within the steady-state intestine. Therefore, it is possible that differing microbiota composition between animal facilities may dictate both GrB expression and function within disease.

Current and potential UC and CD therapeutics

As granzyme-producing Th cells are likely involved in disease progression, it is important to explore potential treatment options that target the recruitment and function(s) of these cells. CD103–E cadherin interactions are involved in UC pathogenesis, and CD103⁺ GrA⁺ Th cells accumulate within the inflamed colon of UC patients (63). A phase 2 randomized, double-blind, placebo-controlled trial of treating UC patients with etrolizumab, a drug that inhibits leukocyte trafficking by blocking CD103–E cadherin interactions, resulted in greater mucosal healing and UC remission (63). Importantly, efficacy of this treatment was predicted by GrA and CD103 expression patients prior to treatment. Although posttreatment samples were not evaluated, these data provide strong evidence that GrA⁺ Th cells contribute to UC and are viable therapeutic targets in IBD patients. Further work must be done to evaluate if other common IBD therapeutic strategies (i.e., TNF blockade) also alter granzyme⁺ Th cell populations or if these cells predict anti-TNF susceptibility or resistance.

SYSTEMIC AUTOIMMUNE DISEASE

Systemic sclerosis (SSc), IgG4-related disease (IgG4-RD), and primary Sjögren syndrome are a group of systemic, multiorgan autoimmune diseases. Although autoantibody-producing B cells are a hallmark of these diseases, inflammation and organ damage is also associated with the accumulation of CD4⁺ T cells in lesional tissues (69–71). Within these tissues, granzyme-producing CD4⁺ T cells are also elevated and may contribute to pathology. In this study, we will define the shared and unique characteristics of granzyme-producing cells in several disease states and potential functions of these cells in driving disease.

Granzyme-producing CD4 T cells are a hallmark of systemic autoimmune disease

In SSc, IgG4-RD, and primary Sjögren syndrome patients, granzyme-producing CD4 T cells were elevated in both the blood (~20–60% of total blood CD4 T cells in IgG4-RD patients) and several affected tissues (i.e., glands, liver, and skin) as compared with samples from healthy individuals. In SSc patients, GrA⁺
cells made up 40% of the total infiltrating CD4 T cells population in the skin (72), suggesting that they may be a driving factor of skin inflammation in SSc patients. Granzyme-producing cells in these patients resemble highly activated effector Th cells or T effector memory cells and exhibit reduced expression of CD62L and CD27 and elevated expression of the cytolytic-associated protein SLAMF7 and CX3CR1 (73, 74). Similar to our findings in GVHD, granzyme-producing CD4 T cells in IgG4-RD patients also exhibited some expression of IFN-γ and the Th1-associated transcription factor T-bet. However, granzyme-producing cells in IgG4-RD expressed several non-Th1 lineage–associated factors including TGF-β1, the T follicular helper cell–associated factor BCL6, and multiple myeloid-associated genes (i.e., IL1B, ITGAM, CD14, CSF3R, and CCL4) (75). This gene expression profile may be unique to granzyme-producing cells in systemic autoimmunity as this myeloid signature is not observed in tumor-infiltrating granzyme+ cells (see above).

Mechanisms of CD4 T cell–derived granzymes in systemic autoimmune disease

SSc patients exhibited elevated numbers of apoptotic CD31+ endothelial cells within afflicted tissues. At least a proportion of these cells express MHCII/HLA-DR, indicating that they are potential targets for granzyme+ CD4 T cells (72). Similarly, granzyme-expressing CX3CR1+ cells exhibited potent killing of a MHCII+ leukemia cell line, an endothelial cell line, and patient-derived submandibular gland ductal cells directly ex vivo, whereas CX3CR1− cells did not. Both cell killing and granzyme secretion from patient-derived CD4+ T cells was enhanced by the addition of the CX3CR1 ligand to these ex vivo cultures, indicating that signaling through this receptor may be involved in their killing potential (73). In similar studies by Mattoo et al. (75), SLAMF7+ CD4 T cells isolated from IgG4-RD patients exhibited killing of allogeneic MHCII+B cell lines in culture, demonstrating their cytolytic potential. Despite these intriguing initial findings, defining which granzymes and downstream apoptotic pathways that are involved in these processes will require further study. Manipulation of target cell endogenous granzyme-selective inhibitors [SERPINCI/GrA or SERPINB9/GrB (76, 77)] may help elucidate these mechanisms. Because granzymes have additional roles in the disease process (i.e., promoting inflammation), it will be intriguing to see if these mechanisms also contribute to systemic autoimmunity or if the main role of these cells lies in tissue destruction via granzyme-mediated killing.

Granzyme-producing CD4 T cells as a target for therapeutics in systemic autoimmunity

In IgG4-RD, therapeutic depletion of B cells with an anti-CD20 Ab (rituximab) or treatment with glucocorticoids partially reduces disease severity (78, 79). Importantly, both of these treatment strategies also suppressed granzyme+ CD4 T cell numbers but not naive, Th2 cells, or Tregs after therapy (74). These data are significant as they imply that granzyme-producing cells are major perpetrators of disease and that targeting these cells may have therapeutic efficacy. Although specific granzyme inhibitors are still in the works, targeting the cytokine signals that drive the differentiation of these cells may be more feasible. As many Th2-associated cytokines are elevated in IgG4-RD and SSc, Th2-polarizing cytokines (i.e., IL-4) may serve as an initial target. We previously showed that IL-4, in combination with IL-6, is a potent inducer of Grα in vitro and that downstream STAT6 activation was required in vivo (41). Two case studies have recently suggested that blockade of the IL-4/IL-13 receptor with dupilumab had beneficial effects on IgG4-RD symptoms as part of a steroid dose-sparing regimen (80, 81). These data indicate that part of the success of dupilumab therapy may be through inhibiting the development of granzyme-producing CD4 T cells.

CONCLUSIONS

Granzyme-producing CD4 T cell responses are predictive of the development of severe GVHD (51), UC, and bladder cancer (40). Further, granzyme+ cells are impacted by therapeutics that are at least partially effective in treating autoimmune disease (74, 75). These data suggest that understanding the molecular mechanisms that lead to their development or recruitment to sites of disease may uncover novel therapeutic strategies for a number of diseases that do not currently have fully effective treatments. Further, the early detection of granzyme-producing CD4 T cells may also serve as predictive biomarkers in other disease settings and help direct the course of treatment. Although granzyme-producing CD4 T cells are associated with disease and are potential therapeutic targets, much less is understood about how CD4 T cell–derived granzymes function in cancer or disease. On the horizon, we need to define roles for individual granzymes during disease and anticancer responses. This will be greatly facilitated through the development of novel mouse tools (i.e., granzyme reporter mice and CD4 T cell–specific granzyme knock out mice) and granzyme-specific inhibitors.

DISCLOSURES

The authors have no financial conflicts of interest.

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