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Review

Current perspectives on the role of CD8+ T cells in systemic sclerosis

Patrizia Fuschiotti

Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh School of Medicine, S709 BST, 200 Lothrop Street, Pittsburgh, PA 15261, USA

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ABSTRACT

Despite long-standing recognition of the importance of T cells in systemic sclerosis (SSc; scleroderma), the role of $CD8^+$ T cells in disease pathogenesis has not been well studied. Our work has shown that over-production of the pro-fibrotic cytokine IL-13 by peripheral blood effector/memory $CD8^+$ T cells is critical for predisposing patients to more severe forms of cutaneous fibrosis. Moreover, IL-13-producing $CD8^+$ T cells induce a pro-fibrotic phenotype in normal and SSc dermal fibroblasts, and exhibit a strong cytotoxic activity *ex vivo*. We also found that $CD8^+$ T cells are predominantly abundant in the skin lesions of patients in the early stages of diffuse cutaneous (dc)SSc compare to late-stage disease patients. Isolation of $CD8^+$ T cells from the lesional skin of early active dcSSc patients, established that they are skin-resident, express cytolytic molecules and co-express extremely high levels of IL-13 and IFNγ. Other recent studies corroborate these findings and together strongly suggest that $CD8^+$ T cells contribute to SSc pathogenesis through the production of high levels of cytokines with pro-inflammatory and pro-fibrotic function as well as by exhibiting a cytotoxic activity.

1. Introduction

Systemic sclerosis (SSc; scleroderma) is a highly debilitating connective tissue disease, characterized by inflammation, vasculopathy, and cutaneous and visceral fibrosis [1]. SSc is very heterogeneous in its clinical presentation, extent and severity of skin and internal organ involvement [2,3]. Patients are characterized into two main clinical subsets on the basis of the degree of cutaneous fibrosis and patterns of organ involvement [2–4]: diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc). Rapidly progressive fibrosis of the skin, lungs, and other internal organs characterizes dcSSc, while vascular manifestations, with generally mild skin and internal organ fibrosis are the most characteristic features of lcSSc. The complexity of SSc and its variable clinical course prevent a full understanding of disease pathogenesis. Tissue fibrosis is the most prominent clinical manifestation of SSc and results from the interaction of immune mediators and other growth factors with responsive tissue resident fibroblasts, leading to increased deposition of extracellular matrix proteins in affected tissues [5,6]. Macrophages and T lymphocytes represent the predominant cell types of the inflammatory infiltrate in the fibrotic tissues of patients [7–9] and produce cytokines that contribute to the inflammatory and fibrotic processes of SSc [10–12].

2. Detection of CD8+ T cells in affected SSc tissue

Despite the importance of T cells in SSc pathogenesis being well established, the phenotype and function of specific T-cell subsets involved in the different phases of the disease are not well defined. Multiple studies implicate CD4+ T-cell subsets such as Th1, Th17, Th22 in the inflammatory phase of SSc, while Th2 cells were shown to contribute to tissue fibrosis [13–20], dysregulation of T regulatory cells has also been observed [17]. However, few studies have focused on CD8+ T cells in SSc. Genome-wide expression profiling of skin biopsies from distinct scleroderma subsets previously identified expression of genes associated with $CD8^+$ T cells (e.g: CD8A, GraK, GraH, and GraB) in lesional skin from the "inflammatory" subset of scleroderma patients [21,22]. This subgroup contained biopsies from patients with dcSSc, lcSSc, and localized scleroderma, which were characterized by a unified gene expression signature indicative of an early inflammatory response [22]. A CD8+ T-cell signature in SSc skin was confirmed by more recent genome-wide gene expression profiling [23,24]. Microarray data showing specifically the relationship between pro-fibrotic, macrophage, and CD8+ T-cell gene expression in SSc skin is depicted in Fig. 1A. The correlation between CD8+ T-cell gene expression in SSc skin samples and skin thickness, measured by modified Rodnan Skin Score (MRSS) [25], is reported in Fig. 1B. By immunohistochemical analyses of T-cell infiltrates in the lesional skin of dcSSc patients at different disease-stages, we showed that CD8+ T cells are predominant in the early stage (disease duration less than 3 years) [2] of SSc compared to CD4+ T cells [26], while more CD4+ lymphocytes are found in late-stage disease (disease duration more than 6 years) [2], suggesting that CD8+ T cells contribute to the initial phase of SSc while CD4+ T cells to its

E-mail address: paf23@pitt.edu.

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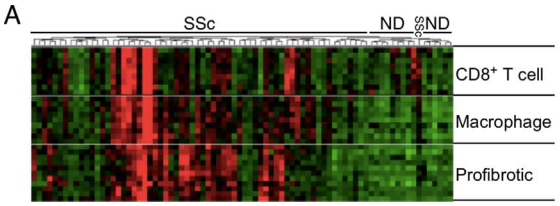

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R	gene	protein
0.18	CD8B	CD8b molecule
0.35	GZMK	granzyme K
0.32	GZMB	granzyme B
0.22	CD3D	CD3d molecule
0.15	CD3E	CD3e molecule

Fig. 1. CD8+ T-cell gene expression signature in SSc skin. Unsupervised clustering of microarray data [23,71]. A hierarchical relationship is suggested between $CD8^+$ T cells driving macrophage driving pro-fibrotic gene expression. (B) $CD8^+$ T cell correlations between gene expression and MRSS (R). (Data courtesy of Dr. R. Lafyatis).

maintenance. High numbers of CD8+ T cells were also found in the skin of patients with active localized scleroderma [26,27]. In contrast, only scant numbers of CD8+ T cells were found in normal skin, matched for anatomical site [26,28]. Other studies report on an increased frequency of CD8+ compared to CD4+ T cells in bronchoalveolar lavage fluid [29] and lungs [30] of SSc patients with pulmonary fibrosis. Moreover, CD8+ T cells in lung and peripheral blood of SSc patients, present an activated phenotype [31,32] and antigen-driven oligoclonal expansion [33–35]. Although antigen-specificity is not yet known, previous studies reported the presence of Topoisomerase-1-specific CD8+ T-cell responses in patients with SSc [36]. Thus, oligoclonal CD8+ T cells are numerous in the affected tissues of patients with active, inflammatory SSc and likely contribute to the pathogenesis of the disease.

3. Phenotype of SSc CD8+ T-cell subsets

Determination of cell surface expression of CD45RA and CD27 by multi-color flow cytometry allows identification of human CD8+ T-cell subsets [37]. We found that peripheral blood SSc CD8+ T cells are characterized by an increased proportion of effector (CD8+CD45RA+CD27-) and effector/memory (CD8+CD45RA-CD27-) cells compared to healthy controls [38]. Similar results were obtained by other groups [39,40]. In these studies, CD8+ T-cell subsets were identified by cell surface expression of CD62L and CCR7 [41,42]. Strikingly, we recently showed that CD8+ T cells in the lesional skin of patients with early active dcSSc uniformely present an effector/memory phenotype [28]. While we found an increased frequency of circulating SSc CD8+ T cells that express skin-homing receptors such as CLA and CCR10 [26], we demonstrated that CD8+ T cells in the lesional skin of dcSSc patients with active disease are skin-resident, as demonstrated by expression of the early acute activation marker CD69 and the αE integrin CD103 [28]. However, only few cells expressed CD103, such as skin-resident T cells confined to normal dermis [43]. This phenotype was further confirmed by immunohistochemical analysis of affected skin of dcSSc patients, showing that CD8+ T cells were restricted to the lower dermis and sub-dermal fat. In contrast, CD8+ T cells in peripheral blood were uniformly negative for CD69 and CD103 expression. While skin-resident memory T cells provide rapid *in situ* protection against most common pathogens [44,45], their dysregulation can contribute to autoimmune and inflammatory skin diseases [46], such as SSc.

In conclusion, SSc CD8+ T cells from the blood and skin of patients with active disease exhibit an effector/memory phenotype and an increased ability to migrate to site of inflammation. Importantly, we identified a specific subset of skin-resident CD8+ T cells in the lesional skin of dcSSc patients that likely play a critical role in the early stage of SSc skin disease.

4. Role of CD8 + CD28-T cells in SSc

We [28] and others [19] found an increased frequency of CD8+CD28- T cells in the peripheral blood of patients with SSc. Loss of CD28 expression by human CD8+ T cells occurs with age and during chronic inflammatory conditions [47,48]. We demonstrated that CD8+CD28- T-cell expansion in blood and lesional skin of SSc patients is independent of patient age and correlates with the extent of skin fibrosis, an early and specific manifestation of SSc. Human CD8+CD28- T cells are defined as antigen-specific, oligoclonally expanded, terminally differentiated senescent T cells [49]. They exhibit functional heterogeneity, ranging from immunosuppressive to effector. Our studies indicate that circulating and skin-resident SSc CD8 + CD28-T cells present an effector/memory phenotype and are cytotoxic [28]. Conversely, it has also been reported that peripheral blood SSc CD8 + CD28-T cells may exert an *in vitro* suppressor activity [19]. While we could not detect any production of immunosuppressive cytokines such as IL-10 and TGFβ by *ex vivo* SSc CD8+CD28- lymphocytes [28], recent *in vitro* studies by Negrini et al. suggest that abnormal expression of CD39 and CD127 molecules, may impair maturation of SSc CD8+ Treg from their CD8+CD28- precursors [50]. Although the existence and relevance of such an inhibitory subset has yet to be examined *in vivo*, the cytotoxic and the suppressor subsets can both coexist as functional heterogeneity in CD8+CD28-T cells has been previously shown [47].

Persistent common viral infections, such as human cytomegalovirus (hCMV), are implicated in the expansion of the CD8+CD28- T-cell population with age [47,51]. Previous studies have shown that latent hCMV infection may contribute to progression of SSc through its ability to infect endothelial cells [52]. Additional evidence for the association between hCMV and SSc comes from the prevalence of anti-hCMV antibodies in patients affected by the disease [53]. In the absence of any medical indication of hCMV infection, we did not test for it. However, we performed preliminary experiment in several SSc patients and found no production of effector cytokines such as IFNγ by SSc CD8+CD28- lymphocytes when they were stimulated *in vitro* with hCMV pp65–recombinant protein, an immunodominant target of CD8+ T-cell responses to hCMV [54] (data not shown). While these studies suggest that CD8+CD28- T-cell accumulation is a response to a yet unidentified antigen, more in-depth studies are necessary do establish the exact role of hCMV or other chronic infections in SSc CD8+ CD28- expansions.

Multiple reports claim that CD8+CD28- T cells present features of cellular senescence such as shortened telomeres, reduced proliferation, and resistance to apoptosis [55–57]. Increasing evidence, however, indicates that this subpopulation is highly heterogeneous regarding its proliferative and apoptotic potential [47,58]. In order to establish whether SSc CD8+ CD28- T cells presented features of cellular senescence, we analyzed their proliferative and apoptotic capacities. CFSE-labeled freshly isolated CD8+ T cells from SSc patients and age-matched controls were stimulated by anti-CD3 antibody with or without exogenous IL-2 and the dilution of the dye was studied in subsets of T cells by flow cytometry. As previously observed [47], we found that CD8+CD28- lymphocytes have a limited proliferative ability when stimulated by anti-CD3 alone over 5 days of culture. In contrast, CD8+CD28- cells proliferate at a comparable rate to their CD8+CD28+ counterparts in response to IL-2 (Fig. 2A–C). Interestingly, we found that the proliferation rate of SSc CD8+CD28- T cells is comparable to that of age-matched healthy controls. Similar results were obtained when cells were stimulated with anti-CD3 and IL-15 [59] (data not shown). This is in agreement with recent studies showing that CD8+CD28- T cells are able to proliferate under certain conditions, such as in the presence of IL-2 [47] or IL-15 [59] and/or in response to specific co-stimulatory signals, such as OX40, 4-1BB, ICOS [58–60]. Significantly, high levels of IL-15 have been found in the serum of SSc patients [61], and we observed an increased expression of IL-15Rα by