Clonal expansion of $CD4^+$ cytotoxic T lymphocytes in patients with IgG_4-related disease

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Background: IgG_4-related disease (IgG_4-RD) is a systemic condition of unknown cause characterized by highly fibrotic lesions with dense lymphoplasmacytic infiltrates. $CD4^+$ T cells constitute the major inflammatory cell population in IgG_4-RD lesions.

Objective: We used an unbiased approach to characterize $CD4^+$ T-cell subsets in patients with IgG_4-RD based on their clonal expansion and ability to infiltrate affected tissue sites.

Methods: We used flow cytometry to identify $CD4^+$ effector/memory T cells in a cohort of 101 patients with IgG_4-RD. These expanded cells were characterized by means of gene expression analysis and flow cytometry. Next-generation sequencing of the T-cell receptor β chain gene was performed on $CD4^+SLAMF7^+$ cytotoxic T lymphocytes (CTLs) and $CD4^+GATA3^+$ T_H2 cells in a subset of patients to identify their clonality. Tissue infiltration by specific T cells was examined by using quantitative multicolor imaging.

Results: $CD4^+$ effector/memory T cells with a cytolytic phenotype were expanded in patients with IgG_4-RD. Next-generation sequencing revealed prominent clonal expansions of these $CD4^+$ CTLs but not $CD4^+GATA3^+$ memory T_H2 cells in patients with IgG_4-RD. The dominant T cells infiltrating a range of inflamed IgG_4-RD tissue sites were clonally expanded $CD4^+$ CTLs that expressed SLAMF7, granzyme A, IL-1β, and TGF-β1. Clinical remission induced by rituximab-mediated B-cell depletion was associated with a reduction in numbers of disease-associated $CD4^+$ CTLs.

Conclusions: IgG_4-RD is prominently linked to clonally expanded IL-1β– and TGF-β1–secreting $CD4^+$ CTLs in both peripheral blood and inflammatory tissue lesions. These active, terminally differentiated, cytokine-secreting effector $CD4^+$ T cells are now linked to a human disease characterized by chronic inflammation and fibrosis. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Fibrosis, IgG_4-related disease, IgG_4, $CD4^+$ cytotoxic T cells, T_H2 cells, rituximab, IL-1β*

IgG_4-related disease (IgG_4-RD) is a chronic inflammatory syndrome with a poorly understood pathogenesis. This disease can affect virtually every organ system of the body and is characterized by tumefactive lesions, storiform fibrosis, obliterative phlebitis, and the presence of IgG_4-secreting plasma cells in affected tissues.[1-3] IgG_4 itself is generally considered a noninflammatory immunoglobulin because of its limited ability to fix complement and bind activating Fc receptors.[4,5] There is very limited evidence that the autoantibodies described thus far are of the IgG_4 subclass, and it is unclear whether they are involved in disease pathogenesis.[6] On the other hand, T cells are the most abundant cells in the lymphoplasmacytic infiltrate in IgG_4-RD lesions and are thought to be the drivers of IgG_4-RD pathogenesis.[2,3]

The analysis of circulating T_H1 and T_H2 cells has led to conflicting results in patients with IgG_4-RD. One study reported a T_H1 skew in peripheral blood T cells in patients with autoimmune pancreatitis, whereas other studies on IgG_4-related sialadenitis showed an increase in numbers of cells expressing T_H2 cytokines in peripheral blood.[7-10] Some T_H2-type cytokines, as well as M2 macrophages, have been found in IgG_4-RD lesions, suggesting that this disease can be caused by T_H2 cells.[11] We have recently reported that atopic manifestations are seen in about 40% of patients with IgG_4-RD, a proportion that is within the range seen in the population at large.[12] We also performed studies on GATA-3–expressing circulating T_H2 cells in patients with IgG_4-RD. Circulating $GATA\text{-}3^+$ IL-4–, IL-5–, and IL-13–secreting T_H2 cells were only found in patients with IgG_4-RD with a history of atopy.[13]

Fibrosis is a prominent feature of many chronic inflammatory disorders, including rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus, and IgG_4-RD, among others. Many distinct triggers are known to contribute to fibrosis, but a detailed understanding of this pathologic process has proved elusive.[14] Both innate and adaptive immune mechanisms can drive fibrotic responses,[15] but it is unclear what constitutes the tipping point between physiologic wound healing and pathologic fibrosis. Activated macrophages secrete cytokines, such as TNF-α

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Abbreviations used
CTL: Cytotoxic T lymphocyte
Eomes: Eomesodermin
Foxp3: Forkhead box protein 3
IgG_4-RD: IgG_4-related disease
MyD88: Myeloid differentiation primary response gene–88
Runx3: Runt-related transcription factor 3
T-bet: T-box transcription factor
TCR: T-cell receptor
T_{EM}: Effector/memory T
ThPOK: T_H-inducing POZ/Krueppel-like factor

and IL-1β, which activate fibroblasts and induce overproduction of extracellular matrix proteins.[15] In the murine model of bleomycin-induced pulmonary fibrosis, inflammasome activation and IL-1 receptor/myeloid differentiation primary response gene–88 (MyD88) signaling are critical aspects of the profibrotic activity of IL-1β.[16] Transient expression of IL-1β alone in the rat lung has been shown to result in tissue damage and progressive fibrosis.[17] Transgenic overexpression of IL-1β in the murine pancreas also results in fibrotic pancreatitis.[18]

Numerous studies have implicated the type 2 cytokines IL-4, IL-5, and IL-13 in driving progressive fibrosis.[19] IL-4 can directly induce mouse and human fibroblasts to synthesize extracellular matrix proteins.[19-21] IL-13, which is secreted by T_H2 cells, mediates fibrotic remodeling in a TGF-β1–dependent or independent manner in experimental lung fibrosis and might contribute to the pathogenesis of idiopathic pulmonary fibrosis, systemic sclerosis, dermatitis-induced skin fibrosis, and liver fibrosis associated with persistent infections.[22-27] M2 macrophages that have been triggered by IL-4 and IL-13 can induce other cells to produce IL-4, IL-10, IL-13, and TGF-β1 and thus contribute to fibrosis. M2 macrophages involved in wound healing also secrete large amounts of TGF-β1,[28] a cytokine that has also been linked to the genesis of fibrosis. Although fibrosis is generally linked to what is presumably the uncontrolled activity of T_H2 cells, M2 macrophages, and fibroblasts, the prominent role in fibrosis of IL-1β, a cytokine typically made by M1 macrophages, suggests that such T_H2-biased models might be applicable to a subset of fibrotic diseases. The presumption that a number of fibrotic diseases have an underlying T_H2 origin might be an oversimplification of an otherwise complex and poorly understood pathogenic process.

Because expansions of circulating T_H2 cells were not observed in patients with IgG_4-RD without atopy, we undertook an unbiased approach using next-generation sequencing to study the clonality of effector $CD4^+$ T cells in patients with active untreated IgG_4-RD. Our goal was to identify any specific $CD4^+$ effector subpopulation that is clonally expanded in subjects with this disease. We report here that $CD4^+$ T cells with a cytotoxic T-lymphoid phenotype are clonally expanded in patients with IgG_4-RD. Furthermore, these unusual $CD4^+$ T cells can synthesize and secrete IFN-γ, IL-1β, and TGF-β1 after T-cell receptor (TCR) or Toll-like receptor triggering. In addition to these cells being expanded in the blood, they are also found as the dominant $CD4^+$ T-cell population within diseased tissue sites, where they also synthesize cytokines. Their numbers decrease concomitantly with a clinical response to rituximab therapy, suggesting a contributory role for these $CD4^+SLAMF7^+$ cytotoxic T cells in the pathogenesis of this systemic fibrotic disease. We have also observed $CD4^+$ cytotoxic T lymphocyte (CTL) expansions in a smaller cohort with systemic sclerosis. Therefore our data suggest that IFN-γ–, IL-1β–, and TGF-β–secreting $CD4^+$ CTLs contribute to the pathogenesis of IgG_4-RD and are likely to be of broader relevance in other inflammatory fibrotic disorders.

METHODS

Patients

We evaluated peripheral blood from 101 patients with IgG_4-RD and selected tissue samples from a subset of those followed at the Massachusetts General Hospital. This study was approved by the Partners Institutional Review Board. All subjects provided written informed consent. Data pertaining to demographics, prior treatment, and laboratory findings at baseline evaluations were derived from the Massachusetts General Hospital Autoimmune Disease Center of Excellence for IgG_4-RD and the medical record. All patients had biopsy specimens obtained from at least 1 organ that were reviewed and confirmed at our center. For certain analyses, only patients with active untreated disease at sampling were included. Confirmation of the IgG_4-RD diagnosis was predicated on both specific histopathologic features and an increased number of $IgG4^+$ plasma cells (or increased $IgG4^+/IgG^+$ ratio) in affected tissues.[29] Data from patients with IgG_4-RD were compared with those from 35 healthy control subjects (age range, 32-70 years).

Tissue sections from 5 patients with IgG_4-related dacryoadenitis and sialadenitis (ie, IgG_4-related Mikulicz disease; mean age, 68.6 ± 4.12 years) and 5 patients with Sjögren syndrome (mean age, 50.8 ± 9.14 years) obtained from the Department of Oral and Maxillofacial Surgery, Kyushu University Hospital, Fukuoka, Japan, were also studied.

A subset of 74 patients with IgG_4-RD who met the definitions of the European Academy of Allergy and Clinical Immunology[30] were categorized based on their atopic history (see Table E1 in this article's Online Repository at www.jacionline.org). Seventeen patients with systemic sclerosis (age range, 24-85 years; median, 51 years; see Table E2 in this article's Online Repository at www.jacionline.org) were also chosen for this study.

Flow cytometric methods

See the Methods section in this article's Online Repository at www.jacionline.org for detailed laboratory methods. Briefly, flow cytometry was performed by incubating cells in staining buffer (BioLegend, San Diego, Calif) containing optimized concentrations of fluorochrome-conjugated antibodies. Surface staining was performed at 4°C for 30 minutes. For intracellular staining of transcription factors (T-box transcription factor [T-bet], GATA-3, and forkhead box protein 3 [Foxp3]), cytokines (IFN-γ and IL-4), and cytolytic molecules (granzyme B and perforin), cells were fixed and permeabilized with the Foxp3 staining kit (eBioscience, San Diego, Calif), according to the manufacturer's guidelines. Cells were then stained in permeabilization buffer at 4°C for 45 minutes. Stained samples were analyzed on a BD LSR II and sorted on a BD FACSAria II (BD Biosciences, San Jose, Calif).

Methods for next-generation sequencing

Next-generation sequencing analysis of the TCR Vβ repertoire was undertaken with the immunoSEQ platform (Adaptive Biotechnologies, Seattle, Wash), which was designed to target an output of 200,000 assembled output sequences.[31,32]

Gene expression analysis

The nCounter human immunology panel (NanoString Technologies, Seattle, Wash) was used to quantify the gene expression of effector/memory T (T_{EM}) $CD4^+$ cells. Targets were reverse transcribed and preamplified for 14 cycles by using the standard multiplexed target enrichment protocol for 458 immunology-related target genes that were previously validated to yield a linear response. The amplified products were hybridized in solution to color-coded nCounter probes, captured on an nCounter Cartridge for high-resolution digital scanning, and analyzed on the GEN2 Digital Analyzer.