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"In Patients Receiving Glucocorticoids For Igg4-Related Diseases, Elevated Circulating th1 And tfh1 Cell Numbers Are Linked To Disease Activity."

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1. Background

The purpose of this study is to investigate the alterations and importance of circulating Th and Tfh cell subsets in patients with IgG4-RD receiving glucocorticoid treatment. Techniques. 22 healthy controls (HC) and 39 IgG4-RD patients receiving glucocorticoid treatment were included. Following the separation of peripheral blood mononuclear cells, circulating Th and Tfh cell subsets were analyzed by flow cytometry in accordance with surface and intranuclear markers. The disease activity was measured using the responder index (RI) score of IgG4-RD.Analysis of correlations between the numbers in the Th/Tfh subset and clinical markers were done. The effectiveness of the Th and Tfh subsets in differentiating between IgG4-RD patients in remission and active patients was assessed using the receiver operating characteristic (ROC) curve. Conclusions. Significantly more circulating Th1, Th17, Tfh1, and Tfh17 cells were present in active IgG4.patients with RD in contrast to HC. In individuals with IgG4-RD, Th1 and Tfh1 counts were strongly linked with serum IgG4 levels. In the meantime, there was a positive correlation between IgG4-RD RIscores and the total numbers of Th1 and Tfh1 cells in circulation. Th1 and Tfh1 had AUCs of 0.8276 and 0.7310, Tfh2 had AUCs of 0.5862 and Tfh17 had AUCs of 0.6810.In conclusion. During glucocorticoid treatment, increased blood IgG4 levels in individuals with active IgG4-RD are correlated with increased circulating Th1 and Tfh1 subsets. These subsets may be crucial in the development of IgG4-RD disease and may serve as possible biomarkers for tracking the disease's activity.

2. Introduction

The chronic immune-mediated inflammatory illness known as IgG4-related disease (IgG4-RD) is typified by tumefactive lesions, a dense lymphoplasmacytic infiltration that is rich in IgG4-positive plasma cells, storiform fibrosis, and often high blood IgG4 concentrations [1]. The bulk of infiltrating cells are tiny lymphocytes, primarily made up of T cells dispersed widely throughoutthe lesion and mixed in with cells of the plasma [2]. Activated CD4 + T cells are the main kind of infiltrating T cells in the lesion, and they are crucial to the progression of IgG4-RD [3].Prior research has demonstrated that follicular helper T (Tfh) cells and CD4 + cytotoxic T lymphocytes (CTLs) thatThe majority of CD4 + T lymphocytes at disease locations in IgG4-RD were seen in infiltrating tissue lesions [4–8]. Patients with IgG4-RD were shown to have increased circulating CD4 + CTLs and Tfh cells [5, 6,9]. Th1 cells (CXCR3 + CCR6 -), Th2 plus naïve CD4 + cells (CXCR3 - CCR6 -, CXCR3-CCR6 - CCR4+ cells represent Th2, and CXCR3 - CCR6 - CCR4 - cells belong to naïve CD4), and Th17 cells (CXCR3 -CCR6+) are the three subtypes of circulating CD4 T helper (Th) [10, 11]. CD4 + CTLs, that isexpress ThPOK, Runx3, and T-bet transcription factors, and they primarily vary from Th1 cells [12, 13]. As a result, patients with IgG4-RD may have enhanced Th1 cells in their peripheral blood due to the proliferation of circulating CD4 + CTLs. Tfh cells are a unique subset of CD4 + T cells that provide important cues to B cells so they can differentiate intohigh-affinity antibodies are secreted by plasma cells in the germinal center [14, 15]. Tfh1 (CXCR3 + CCR6-), Tfh2 (CXCR3 -CCR6-), and Tfh17 (CXCR3 - CCR6 +) cells are three subtypes of peripheral blood CD4+ CXCR5 + T cells that are thought to be circulating pools of memory Tfh cells. These cells have varying capacities to aid in the differentiation of B cell subsets [16, 17]. The function of circulating CD4 + T cell subsets in IgG4-RD has been examined in a few studies. But the majority ofPrior research has concentrated on patients with untreated IgG4-RD. The association between circulating CD4 + T cell subsets and disease activity in patients with treated IgG4-RD has not been thoroughly studied.The purpose of this research is to ascertain the association between serum IgG4 levels, circulating Th and Tfh cell subsets, and disease activity in

patients with IgG4-RD receiving glucocorticoids. Our goal is to elucidate the modifications and practical implications of circulating Th and Tfh cell subsets in patients with treated IgG4-RD.

3. Results

The clinical features of individuals with IgG4-RD. 39 patients were engaged in the study with IgG4-RD, of which 25 had definite, 12 had probable, and 2 had questionable diagnoses. Supplementary Table 1 describes the clinical and demographic characteristics of the patients. 38 (97.4%) of those subjects had more than two organs implicated. Submandibular glands (13 cases, 33.3%), lacrimal glands (7 cases, 17.9%), parotid gland (1 case, 2.6%), pancreas (10 cases, 25.6%), bile ducts (1 case, 2.6%), lymph nodes (2 cases, 5.1%), and retroperitoneum (3 cases, 3.6%), were among the frequently involved initial organ sites.7.7%), mesentery (1 case; 2.6%), and sinus (1 case).Among these IgG4-RD patients, the median RI score was 5.Based on RI scores, these patients were split into two groups: IgG4-RD patients in remission (n = 10) and active (n = 29).IgG4, IgG, IgE, C3, C4, and CRP levels in the serum of patients with active IgG4-RD, patients with remission IgG4-RD, and HC were measured and compared. Serum IgG4 concentrations in active IgG4-RD patients were considerably greater than in HC and remission patients (3.01 g/L versus 0.46 g/L and 0.85 g/L; P < 0:0001 and P = 0:0153, respectively), as indicated in Supplementary Figure 1.Similarly, in active IgG4-RD, IgE was much higher.patients (103.40 IU/mL versus 24.94 IU/mL and 38.13 IU/mL; P = 0:0001 and P = 0:0483, respectively) in comparison to HC and remission patients. On the other hand, patients with active IgG4-RD had substantially lower serum C4 levels than those with HC (0.209 g/L versus 0.268 g/L, P = 0:0032). Additionally, we looked at lymphocyte concentrations and CD4 + T cell percentages. We discovered that active IgG4-RD patients had higher lymphocyte concentrations and CD4 + T cell percentages than HCor remission patients (not shown).

Th1 and Th17 Cells Are Increasing in Patients with Active IgG4-RD. Next, using surface markers, we assessed Th cell levels in IgG4-RD patients and HC. Because we wish to rule out the influence of Foxp3 + regulatory T cells, circulating T cells were characterized as CD3 + CD4 + CXCR5- Foxp3- cells. Within the CD3 + CD4 +-CXCR5- Foxp3 - Th cells, Th1, Th2 plus naïve CD4 and Th17 cell subsets were identified as CXCR3 + CCR6 - cells, CXCR3 - CCR6+ cells and CXCR3 - CCR6+ cells, respectively. As illustrated in Figure 1, there was a significant increase in the absolute number (per µL) of circulating Th1 cells in active IgG4-RD patients when compared to HC and remission patients (111.9 cells/µL versus 76.4).cells/µL and 57.4 cells/µL, respectively; P = 0:0047 and 0:0376). Additionally, patients with active IgG4-RD had significantly larger absolute numbers (per µL) and frequencies of circulating Th17 cells than did HC (76.4 cells/µL compared 30.6 cells/µL, 0.107 versus 0.070; P = 0:0007 and P = 0:0067, respectively). Remission IgG4-RD patients had significantly less circulating Th2 plus naïve CD4 cells per microliter (197.9 cells/µL) than both active patients and healthy control (400.9 and 374.6 cells/µL, respectively; P = 0:0264 and P = 0:0474). A significant difference was seen between the frequency of circulating Th2 plus naïve CD4 cells in active IgG4-RD and HC (0.611 versus 0.745; P = 0:0007).In patients with active IgG4-RD, there was an increase in Tfh1 and Tfh17 cells. The circulating Tfh cell alterations in these IgG4-RD patients were next examined. Foxp3-positive CD3+ CD4 + CXCR5+ Tfh cells were identified as circulating Tfh cells. Because we wish to rule out the influence of follicular regulatory T cells, Foxp3 +cells were also disregarded. Within Tfh1 + CD4 + CXCR5 + Foxp3 - Tfh cells, Tfh1, Tfh2, and Tfh17 cell subsets were identified as CXCR3 + CCR6 - cells, CXCR3 - CCR6 - cells, and CXCR3 - CCR6+ cells, respectively. As demonstratedIn Figure 2, there was a substantial increase in the absolute number (per µL) of circulating Tfh1 cells in active IgG4-RD patients when compared to HC (26.2 cells/µL versus 17.4 cells/µL; P = 0:0172). Furthermore, in patients with active IgG4-RD compared to HC, there was a significant increase in both the absolute numbers (per microliter) and frequencies of circulating Tfh17 cells (25.8 cells/µL against 10.4 cells/µL, 0.226 versus 0.145; P = 0:0009 and P = 0:0027, respectively).

On the other hand, compared to HC and remission patients, the frequencies of circulating Tfh2 cells were significantly lower in active IgG4-RD patients (0.424 vs 0.612 and 0.572; P < 0:0001 and P = 0:0091, respectively).In IgG4-RD Patients, Circulating Th1 Cells Have a Positive Correlation with IgG4 Levels and RI Scores. The correlations between Th cell subsets and serum IgG4 and RI scores in IgG4-RD patients were examined in order to ascertain the relationships between Th cell subsets and clinical indications. Figure 3 illustrates the positive correlation between serum IgG4 levels and IgG4-RD RI scores and the absolute number (per µL) of Th1 cells (r = 0:8134 and 0.4457; P <P = 0:0045 and 0:0001, in that order). Analyses were also conducted on the correlations between Tfh cell subsets and serum IgG4 and RI scores in individuals with IgG4-RD, respectively (Figure 4).It is noteworthy to mention that there was a positive correlation between the quantity of Tfh1 cells and both serum IgG4 levels and IgG4-RD RIscores (r = 0:6424 and 0.3568; P < 0:0001 and P = 0:0257, respectively).Journal of Immunology Research, Numbers of Th1 and Tfh1 Cells May Be Employed as Potential Biomarkers to Track IgG4-RD Disease Activity. We further investigated whether Th1 and Tfh1 subgroup numbers may be employed as possible markers for IgG4-RD diseaseactivity monitoring since they are significantly higher in inactive IgG4-RD patients and have significant positive correlations with disease activity.

We employed Tfh1 and Th1 cell counts, as illustrated in, to differentiate between remis and active IgG4-RD.IgG4-RD and produced curves for ROC analysis. For Th1 cells, the areas under the curve (AUC) were 0.8276, and for Tfh1 cells, they were 0.7310. The effectiveness of diagnosing with different cell subsets was also examined. The Th2 plus naïve CD4 cells had an area under the curve (AUC) of 0.7586, whereas Th17 cells had an AUC of 0.6517. The Tfh2 cells had an AUC of 0.5862, and the Tfh17 cells had an AUC of 0.6810.

4. Discussion

In this study, we looked at how circulating Th and Tfh cell subsets

changed and how important they were in IgG4-RD patients receiving glucocorticoids. In patients with active IgG4-RD, we discovered that circulating Th1 and Tfh1 subsets were elevated and correlated with serum IgG4 levels and RI scores. These findings may have a significant function in IgG4-RD and have the potential to serve as biomarkers for tracking the disease's activity while treatment is being administered. Elevated serum IgG4 levels are a common characteristic of IgG4-RD [18, 19]. Additionally, serum IgE concentrations rise.in certain individuals [20]. Additionally, we discovered in this study that patients with active IgG4-RD had significantly higher serum IgG4 and IgE concentrations. On the other hand, HC had significantly higher serum C4 levels than active IgG4-RD patients. The decrease in blood C4 levels seen in these patients may suggest that certain antibodies bind to the target antigen and that the sickness is frequently accompanied by an activated complement system.It has been suggested that expanded CD4 + CTLs are crucial to the pathophysiology of IgG4-RD [4, 20–23].CD4 + CTLs mostly undergo Th1 cell differentiation. Consequently, we looked into circulating Th subsets in IgG4-RD. Patients with active IgG4-RD had considerably higher levels of circulating Th1 cells. Consistent with our findings, Ohta et al. and Higa-shioka et al. have previously shown elevated circulating Th1 cells in individuals with IgG4-related sialadenitis [24, 25].

Here, we characterized the Th subgroup using surface molecules in order to provide possible future diagnostic uses. Additionally, we discovered a strong correlation between the numbers of circulating Th1 cells and serum IgG4 levels, indicating that enlarged Th1 cells may play a role in the rise in serum IgG4. Similarly, Maehara et al. found a favorable correlation between blood IgG4 concentrations and the ratio of CD4 + CTLs in lesion tissues from individuals with IgG4-related dacryoadenitis and sialoadenitis [4]. Th1 cell or CD4 + CTL upregulation willincrease IFN-γ production. It is believed that an overabundance of IFN-γ causes a pathogenic buildup of Tfhcells, which in turn causes aberrant germinal centers to develop and the generation of autoantibodies [26].The favorable connection between Th1 cells and IgG4 could perhaps be explained by this indirect effect. Furthermore, we discovered that there was a positive correlation between the IgG4-RD RI scores and the quantity of Th1 cells in circulation. This indicates that Th1 cells in circulation are a reflection of IgG4-RD disease activity. The rationale might be because Th1 cells have the ability to create cytolytic molecules like granzyme and perforin as well as inflammatory cytokines like IFN-γ, which have been linked to disease activity.Prior research examined the function of Tfh cell subsets in IgG4-RD. Still, there is disagreement concerning the findings. According to research by Akiyama et al., serum IgG4 levels were linked to the quantity of Tfh2 cells, and activated circulating Tfh1 and Tfh2 cells were elevated in IgG4-RD [27, 28].IgG4-RD patients had considerably higher frequencies of circulating Tfh1 and Tfh2 cells, according to Chen et al. [8]. According to Grados et al., circulating T cells in patients with Th2/Tfh2 and Th17/Tfh17 polarized towardIgG4-RD [29]. According to a recent study, pancreatitis and sclerosing cholangitis connected to IgG4 were associated with enlarged circulating PD-1 + Tfh cell subsets, but only activated Tfh2 cells were linked to disease activity [30].

According to reports, there is a considerable increase in IL-4 + Tfh cells in lesion tissues and secondary lymphoid organs in IgG4-RD [7]. These IL-4+ Tfh cells express BATF instead of GATA-3, which has been identified as a master transcriptional factor of circulating Tfh2 [10]. In lesion tissues of IgG4-RD patients, CXCR3 and CCR6 were found to be elevated on CD4 + Tfh cells [4], indicating that increased Tfh in IgG4-RD may be attributed to CXCR3 + Tfh and CCR6 + Tfh cells.Here, we discovered that patients with active IgG4-RD had a noticeably greater level of circulating Tfh1 cells.Additionally, compared to HC, individuals with active IgG4-RD had significantly greater levels of both the quantity and percentage of circulating Tfh17 cells. On the other hand, patients with active IgG4-RD had a significantly lower proportion of circulating Tfh2 cells. Additionally, there was a positive correlation found between the quantity of circulating Tfh1 cells and serum IgG4 levels as well as IgG4-RDRI scores. This indicates that a higher Tfh1 cell count may be linked to raised IgG4 and may even contribute to IgG4-RD.The disparate inclusion criteria for patients are one of the causes of the contradictory conclusions. excluded from this study any individuals receiving glucocorticoid medication. Compared to individuals who started treatment recently or who stopped it later, the Th and Tfh subpopulations in these patients changed significantly during the course of treatment.It is challenging to gather new-onset patients because the majority of IgG4-RD patients are frequently in the process of beginning treatment, however our findings are more significant as references.since it represents the circumstances of the majority of hospitalized patients. Additionally, this is the first time that the alterations in Th and Tfh subsets in patients receiving glucocorticoids have been thoroughly examined. We believe that circulating T cell subsets may be impacted by glucocorticoids, and that changes in T cell subsets may potentially reflect treatment outcomes.Based on this, we also carried out a preliminary exploration of the Th and Tfh subsets' diagnostic value and employed ROC curves to assess how well these subsets worked to differentiate IgG4-RD patients' active from remission state during glucocorticoid treatment. Our findings showed that the AUC (area under the curve) was 0.8276. for Th1 and 0.7310 for Tfh1, indicating that Th1 and Tfh1 numbers may be useful in diagnosing and tracking the activity of IgG4-RD illness.

Flow cytometry is the method we employ in our research to find chemicals on peripheral blood cell surfaces.It will have a promising future if it is able to fulfill its objective of assessing patient care. To clarify the diagnostic value of Th1 and Tfh1, it is important to increase the sample size in the future due to its insufficiency.In conclusion, our research shows that circulating T cell polarization towardTh1/Tfh1 and Th17/Tfh17 following glucocorticoid treatment is a characteristic of active IgG4-RD. In individuals with IgG4-RD, circulating Th1 and Tfh1 levels positively correlate with serum IgG4 levels and disease activity. These biomarkers may be useful for tracking disease activity in IgG4-RD patients and may be significant along the disease's course.

5. Materials and Methods

Topics. Between December 2018 and May 2019, a total of 39 patients

with IgG4-RD (24 males, 15 females; median age: 62 years) and 22 healthy controls (HC) (13 males, 9 females; median age: 66 years) were recruited from Peking University People's Hospital's inpatient and outpatient departments. IgG4-RD was diagnosed in compliance with the 2011 comprehensive diagnostic criteria [31]. Every patient with IgG4-RD was undergoing glucocorticoid therapy.Predniso-lone dosages for the majority of patients have been standardized at 0.6 mg/kg/d for 2-4 weeks, followed by a reduction to 5 mg/d for 3-6 months, with the anticipated end of treatment.ment by a duration of 2-3 years. The evaluation of disease activity was conducted using the IgG4-RD responder index (RI) score [32]. Each affected organ was given a unique score, and the total RI was determined by adding the scores of each individual organ. Serum IgG4 levels were taken into account while calculating the RI scores in the current investigation. A score of ≥ 3 on the IgG4-RD RI indicated an active disease, while a value of <3 indicated remission [21]. The Peking University People's Hospital Ethics Committee gave its approval for this study, which was carried out in accordance with the Declaration of Helsinki's ethical guidelines.Measurement of Clinical Indicators. Using Siemens BN IINephelometer (Siemens Healthcare Diagnostics; Malburg, Germany) and Siemens reagents, the serum levels of IgG4 were determined by nephelometry. Beckman Coulter Immage 800 Nephelometer (Beckman Coulter Ireland Inc.; CA, USA) and Beckman Coulter reagents were used to measure the serum concentrations of IgG, C3, and C4. Using a Beckman Coulter Chemistry Analyzer AU58006 Journal of Immunology Research, CRP in serum was measured by immunoturbidimetry.Beckman Coulter reagents and (Beckman Coulter Ireland Inc.; CA, USA). Using a Cobase601 Electrochemiluminescence Immunoassay Analyzer (Roche; Mannheim, Germany), serum IgE levels were measured. To measure WBC and lymphocyte counts, Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan) was used.

minimal Cytometry. Following two PBS washes, human lymphocyte separation medium (Dakewei Biotech Co., Ltd.; Shenzhen, China) was used to separate peripheral blood mononuclear cells (PBMCs). The following antibodies were used to stain enriched peripheral blood monoclonal cells (PBMCs) for 30 minutes: CD3-APC, CD4-PerCP/Cy5.5, CXCR5-APC/Cy7, CXCR3-PE, and CCR6-PE/Cy7. A transcription factor staining buffer kit (ThermoFisher Scientific-eBioscience; San Diego, CA, USA) was used to stain cells intracellularly for Foxp3., as directed by the manufacturer. Cells were treated with anti-Foxp3allophycocyanin for 30 minutes following fixation and permeabilization. The source of all fluorescent antibodies was BioLegend, located in San Diego, California, USA.BD Biosciences, located in San Jose, California, USA, used Diva software to analyze samples on FACSCanto. The absolute count (per μL) of each subgroup was computed using the proportion of each subgroup of lymphocytes as determined by flow cytometry and the total number of lymphocytes in the full blood count.Data. Constant variables are displayed as medians with percentiles ranging from 25 to 75. We used the Kruskal-Wallis test to assess comparisons between many groups. Two groups were compared using the Mann-Whitney U test. In order to investigate the effectiveness of factors in assessing IgG4-RD disease activity, receiver operating characteristic (ROC) curve studies were carried out, and the AUC values were determined. The GraphPad Prism software V.7.0 (GraphPad Software; SanDiego, CA, USA) was used to calculate statistical significance. P values of less than 0.05 were regarded as significant in statistics.

Data Availability

Upon request, the data supporting the study's conclusions can be made available to Dr. Changsheng Xia, whose email address is xiachangsheng@bjmu.edu.cn.

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