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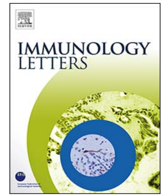

Expansion of circulating follicular T helper cells associates with disease severity in childhood atopic dermatitis

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ABSTRACT

Follicular helper T (T_{FH}) cells play crucial role in B-cell differentiation and antibody production. Although, atopic dermatitis (AD) is often associated with increased serum IgE levels, B-cell mediated responses have not been studied thoroughly. The aim of our study was to investigate the proportion of T_{FH}-like cells in the disease. Twelve children and 17 adults with AD as well as 14 healthy controls were enrolled in the study. The frequency of $CD4^+CXCR5^+ICOS^+PD\text{-}1^+$ T_{FH}-like cells and their IL-21 cytokine production were determined by flow cytometry. Immunohistochemical analysis was performed on skin biopsy specimens from AD patients for the detection of T_{FH} markers. The percentages and absolute numbers of circulating T_{FH}-like cells were significantly increased in children with AD compared to adult patients and healthy controls. IL-21 cytokine production of T_{FH}-like cells was also elevated and showed a strong positive correlation with paediatric patients' SCORAD index. The expression of T_{FH}-specific markers showed only a non-specific scattered pattern in skin biopsy specimens. This is the first study to demonstrate that T_{FH}-like cells expanded in the peripheral blood of children with AD compared to adults. These results reinforce the importance of further investigations on T_{FH}-like cells in different phenotypes and endotypes of AD.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease. Clinically, it results in itchy, hyperaemic, swollen, excoriated plaques, which may also show lichenification. In about 80% of patients, the disease associates with elevated levels of serum immunoglobulin (Ig) E [1]. The prevalence of the disease has strongly increased in developed countries during the last few years. According to epidemiological studies, it affects approximately 2–10% of the adult and 15–30% of the paediatric population worldwide [2]. Genetic predisposition as well as environmental factors exerts a profound influence on the disruption of intact epidermal barrier and on the development of abnormal immune responses. The interaction of above mentioned elements contribute to the sensitization and the development of unique clinical features of the disease [3,4]. AD is known to be a complex heterogeneous disease with various phenotypes and endotypes which arises from age-related differences, acute or chronic phase of the disease and the presence or absence of allergen sensitizations may be associated with distinct branches of immune activation [5]. The pathomechanism of AD may be easier to understood as a biphasic system with the predominance of T helper (Th) 2 mediated responses with elevated interleukin (IL)-4 and IL-13 cytokines and Th22 cells in the acute phase followed by an expanded Th1 and Th17 guided responses in the chronic period [4,6,7]. Despite the complex interactions between keratinocytes, skin dendritic cells (DCs) and T cells, for the most part, generalized Th2 dysbalance and isotype switching of B cells to generate specific IgE is often associated with AD. However, the role of B cells and other participants in the regulation of humoral immunity in allergic or skin

Abbreviations: AD, atopic dermatitis; CSR, class-switch recombination; CXCR5, chemokine (C-X-C motif) receptor type 5; DCs, dendritic cells; DEHP, di-(2-ethylhexyl)phthalate; FFPE, formalin-fixed paraffin-embedded; GC, germinal center; ICOS, inducible T cell co-stimulator; Ig, immunoglobulin; IL, interleukin; PD-1, programmed cell death protein 1; PerCP-Cy5.5, Peridinin-chlorophyll protein-Cyanine dye 5.5; PMA, phorbol-12-myristate 13-acetate; SAP, signalling lymphocytic activation molecule (SLAM)-associated protein; SCORAD, SCORing Atopic Dermatitis; SHM, somatic hypermutation; STAT3, signal transducer and activator of transcription 3; T_{FH}, Follicular helper T; Th, T helper

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Table 1
Demographic data of AD patients and controls enrolled in the study.

Parameter	Healthy controls (total; n = 14)	Healthy children (n = 4)	Healthy adults (n = 10)	Patients with AD (total; n = 29)	Children with AD (n = 12)	Adults with AD (n = 17)
Age (years)						
range	4–37	4–15	23–37	4–42	4–16	18–42
mean ± SD	22.64 ± 10.16	8.50 ± 4.80	28.03 ± 4.14	18.97 ± 11.03	9.58 ± 3.82	26.58 ± 7.99
Sex (no.)						
female	11	3	8	19	10	9
male	3	1	2	10	2	8
SCORAD score						
range				24–72	28–68	24–72
mean ± SD				43.21 ± 13.04	43.67 ± 11.91	42.88 ± 13.82
IgE (kU/L)						
median (range)				1453 (22–10000)	637 (22–10000)	2537 (135–10000)

diseases has remained poorly examined. Although studies about the expansion of circulating B cells in patients with psoriasis and AD are reported, observations regarding B cell mediated immune responses are limited [8,9]. For that purpose, the profound examination of germinal center (GC) processes with a special emphasis on the regulation of IgE-producing plasma cell generation needs to be clarified in AD.

Follicular helper T (T_{FH}) cell, a specialized subset of $CD4^+$ T cells provides fundamental help to antigen specific B-cells in proliferation, differentiation as well as antibody production, are one of the most intensively studied cells [10,11]. In the past few years, numerous research groups have described altered ratio of circulating T_{FH}-like cells in autoimmune and inflammatory diseases. Expansion of $CD4^+CXCR5^+$ T_{FH}-like cells was reported in autoimmune diseases, including systemic lupus erythematosus [12,13], Sjögren's syndrome [14,15], rheumatoid arthritis [16], juvenile dermatomyositis [17] and bullous pemphigoid [18]. However, in dermatology, investigations of T_{FH}-like cells are limited and occur mostly in cutaneous lymphomas [19] or inflammatory disorders such as psoriasis [20–22]. T_{FH} cells arise from activated T cells following the encounter with DCs, and migrate to the border of T and B cell areas in the lymph node under the guidance of Bcl-6 or c-Maf mediated chemokine receptors [23,24]. GC localized T_{FH} cells are distinguished from other effector T cells by the intense expression of chemokine (C-X-C motif) receptor type 5 (CXCR5), inducible T cell co-stimulator (ICOS), programmed cell death protein 1 (PD-1) and the production of IL-21. Additional features include the elevated expression of CD40L, OX40L, signalling lymphocytic activation molecule (SLAM)-associated protein (SAP) and SLAM family receptors such as CD84 [25–27]. The interplay between T_{FH} and activated B cells is essential not only for GC reactions, including somatic hypermutation (SHM) and immunoglobulin class-switch recombination (CSR), but for the generation of extrafollicular responses as well [28].

The special capability of T_{FH} cells to direct B cell responses and antibody production proposed the possibility that they may play an important role in the pathogenesis of AD. Based on that potential concept, we investigated the distribution of T_{FH} cells and their markers not only in the peripheral blood, but in skin biopsy samples of patients with AD as well.

2. Material and methods

2.1. Patients and healthy individuals

Peripheral blood was obtained from 29 patients with AD (19 female and 10 male; mean age 18.97 ± 11.03 years) for flow cytometric analysis. Fourteen healthy individuals (11 female and 3 male; mean age 22.64 ± 10.16 years) served as controls. Among control subjects, there were 4 individuals under 16 years (3 female and 1 male; mean age 8.50 ± 4.80 years). Since their cell ratios did not differ significantly, we included them into one control pool. AD patients were classified based on their age: one group comprised subjects with 12 children (10 female and 2 male; mean age 9.58 ± 3.82), while the other group consisted of subjects with 17 adults (9 female and 8 male; mean age 26.58 ± 7.99). Skin biopsy specimens were also collected from 5 patients at the Department of Dermatology, Clinical Center, University of Debrecen. All patients fulfilled the diagnostic criteria for AD established by Hanifin and Rajka [29]. The severity of the disease was determined by the SCORAD (SCORing Atopic Dermatitis) index. The mean SCORAD index of the patients was 43.21 ± 13.04 with the range of 24–72. In children, the mean SCORAD index was 43.67 ± 11.91 with the range of 28–68, while in adult it was 42.88 ± 13.82 with the range of 24–72. The median serum total IgE was 1453 kU/L with a range of 22–10000 kU/L. The titer of IgE was 637 kU/L (22–10000 kU/L) in children and 2537 kU/L (135–10000 kU/L) in adults. None of the patients received any antihistamines or topical corticosteroids for at least 5 days, or systemic immunosuppressive or immunomodulating medications for at least 4 weeks prior to the collection of blood samples. No patients or controls had ongoing or recent previous infections during the study. Data were obtained retrospectively from patients' records which contained detailed information about other possible allergic disease and sensitization. Informed written consent was obtained from the subjects, and the study was approved by the Ethics Committee of the University of Debrecen. All experiments carried out were in compliance with the Declaration of Helsinki. Demographic data were summarized in Table 1.

2.2. Determination of circulating T_{FH}-like cells by flow cytometry

For the assessment of circulating T_{FH} cells, cells from 100 µL of heparinized whole peripheral blood were stained with CXCR5-Alexa Fluor 488 (clone: RF8B2)/ICOS-PE (clone: DX29)/PD-1-Peridinin-chlorophyll protein-Cyanine dye 5.5 (PerCP-Cy5.5) (clone: EH12.1)/CD4-APC (clone: RPA-T4) (all from BD Biosciences, San Jose, CA, USA) fluorochrome-labeled monoclonal antibodies. After the incubation period, erythrocytes were haemolysed. Stained cells were assessed using a FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and data analysis was performed by FlowJo Software (Treestar, Ashland, OR, USA). At least 40.000 $CD4^+$ events of each sample were analysed within the entire lymphocyte population. The absolute number of T_{FH}-like cells was determined from the percentage of these cells calculated by flow cytometry and from the absolute number of lymphocytes ($\times 10^9$ L^{-1}) detected by a haematological cell counter.

2.3. Determination of single-cell cytokine production of circulating T_{FH}-like cells by intracellular cytokine analysis

For intracellular cytokine examination, diluted whole blood was

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