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A Novel Hypothesis on Excessive Activation of Residual B Lymphocytes in Common Variable Immunodeficiency Concurrent with Aseptic, Erosive Polyarthritits

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to report aseptic, erosive polyarthritits in a patient with common variable immunodeficiency (CVID), which is quite different from the vastly more common nonerosive form.





Material/Methods: Peripheral blood mononuclear cells of the patient were isolated. Flow cytometry was used to analyze the proportion and function of lymphocytes. A Parker-Pearson needle biopsy was performed on the right knee. Four of her unaffected family members were enrolled as controls.

Results: A 21-year-old woman was admitted for recurrent polyarthritits of 3-year duration. The right knee, hip, wrist, proximal interphalangeal joints, and left elbow were involved, with progressive joint destruction. She was diagnosed as having CVID based on her recurrent infections, poor response to vaccines, and marked hypogammaglobulinemia. No bacterium or mycobacterium was detected in synovium or synovial fluid. The synovium was infiltrated by lymphocytes rather than neutrophils. Polyarthritits did not resolve by adequate intravenous immunoglobulin substitution and empirical antibiotic treatment, but resolved gradually after treatment with methylprednisolone and tacrolimus, supporting the diagnosis of aseptic polyarthritits. Further analyses showed that although only 0.5% of residual B lymphocytes were existent in peripheral blood of the patient, expressions of activation marker CD69 and production of IL-1 β , IL-6, and TNF- α were high. Marked infiltration with CD19+B lymphocytes (as well as CD4+ or CD8+ T lymphocytes) was detected in the synovium. The proportion of IL21+CD4+Th cells from peripheral blood of the patient was high. CD4+ Th cells from the patient secreted nearly 3 times more IL-21 than the same cell type analyzed from unaffected family members, perhaps due to excessive compensation to assist the function of residual B lymphocytes.

Conclusions: A novel hypothesis in CVID concurrent with aseptic, erosive polyarthritits is that excessive activation of residual B lymphocytes infiltrate into the synovium of the involved joints and lead to polyarthritits and joint destruction.

MeSH Keywords: **Arthritis • B-Lymphocytes • Common Variable Immunodeficiency**

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Background

Common variable immunodeficiency (CVID) is not “common” as its name implies, with an estimated prevalence of 1: 50 000–1: 20 000. It is considered “common” because it is the primary immunodeficiency most commonly encountered in clinical practice. CVID is a heterogeneous syndrome with an autosomal recessive or dominant pattern or as sporadic cases. Some genetic defects of B lymphocytes associated with CVID have been identified, such as genes encoding inducible T cell co-stimulator (ICOS), tumor necrosis factor receptor superfamily, member 13B (TNFRSF13B, also known as TACI), TNFRSF13C (also known as BAFFR), or CD19 [1]. CVID is characterized by defects in B lymphocyte differentiation into memory B lymphocytes and plasma cells, causing varying degrees of hypogammaglobulinemia [2]. Unable to produce antigen-specific antibodies against vaccination or infections, CVID patients have an increased incidence of infections, malignancy, and autoimmune disorders [3].

Arthritis is a prominent feature driving CVID patients to see rheumatologists. However, many rheumatologists are not familiar with this morbidity and should learn more about it. For CVID concurrent with arthritis, septic arthritis should be considered first [4]; it is usually caused by *Mycoplasma* species. Aseptic polyarthritis is also reported in 2–3.2% of CVID patients [5–7], but the more characteristic form is symmetric polyarthritis of the large joints and is overwhelmingly nonerosive [7]. We report the case of a woman with CVID who had aseptic, erosive polyarthritis resulting in progressive joint destruction. Further, we illustrate the histological finding of synovium and present a novel hypothesis regarding this morbidity through analyzing the proportion and function of lymphocytes in peripheral blood.

Material and Methods

Patient

Our patient was a woman who had CVID with recurrent polyarthritis of 3-year duration. Her unaffected family members (2 parents, 1 sister, and 1 brother) were enrolled as controls. They all signed informed consent. The study was approved by the Medical Ethics Committee of Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University.

Peripheral blood mononuclear cells (PBMCs) and the function of lymphocytes

PBMCs were isolated by Ficoll-Hypaque gradient centrifugation and washed twice in Hank's balanced salt solution. All monoclonal antibodies were purchased from BD Bioscience

PharMingen™ (San Carlos, CA, USA) and used for different subsets of PBMCs staining.

To detect the function of B lymphocytes, we first gated on CD20⁺ B lymphocytes and detected the membrane immunoglobins (IgG, IgM, IgD) and activation markers (CD69, CD25). Then, PBMCs were stimulated with LPS for 6 h in the presence of brefeldin A (10 µg/ml) at 37°C with 5% CO₂. The cytokine production (IL-1β, IL-6, or TNF-α) of B lymphocytes was detected by flow cytometry. To detect the function of T lymphocytes, PBMCs were stimulated with PMA (1 µg/ml) plus ionomycin (1 µg/ml) for 6 h in the presence of brefeldin A at 37°C with 5% CO₂. The cytokine production from CD4⁺ T helper (Th) cells and CD8⁺ cytolytic T cells was detected by flow cytometry. IL-21 from Th cells in culture supernatant was detected by ELISA.

Synovial tissues and staining

A Parker-Pearson needle biopsy was performed on the right knee. Synovial samples were immediately fixed in 10% neutral formalin and embedded in paraffin. Sections (3-µm) were cut serially and mounted on adhesive glass slides. Serial sections of synovial tissues were stained with hematoxylin and eosin (H&E) and a 3-step immunoperoxidase method shown in detail in our previous study [8]. Nonspecific isotype IgG was used as a negative control in each staining run.

Results

Clinical characteristics

A 21-year-old woman with recurrent polyarthritis of 3-year duration presented to the Department of Rheumatology and Immunology in August 2011. Her medical history revealed poor response to vaccines and recurrent nasosinusitis and bronchitis since the age of 11. At age 18, she developed recurrent joint pain and swelling in the right knee, hip, wrist, proximal interphalangeal joints, and left elbow. The right hip pain became worse and caused her to have to walk with a crutch.

On admission, she presented with tenderness, swelling, and limited mobility in the right knee and right hip. Laboratory tests revealed C-reactive protein of 31.2 mg/L and hypogammaglobulinemia (IgA <0.254 g/L, IgG 2.03 g/L, IgM 0.22 g/L). Autoantibodies such as rheumatoid factor, anti-citrullinated peptide antibody, and antinuclear antibodies were all negative. HLA-B27 was negative. Radiograms showed widespread osteoporosis but no erosions in the hands. Articular spaces were markedly narrowed (right knee) or disappeared (right hip), with irregular bone sclerosis along the articular surface (Figure 1A). Ultrasound imaging of the right knee indicated synovial hyperplasia, joint

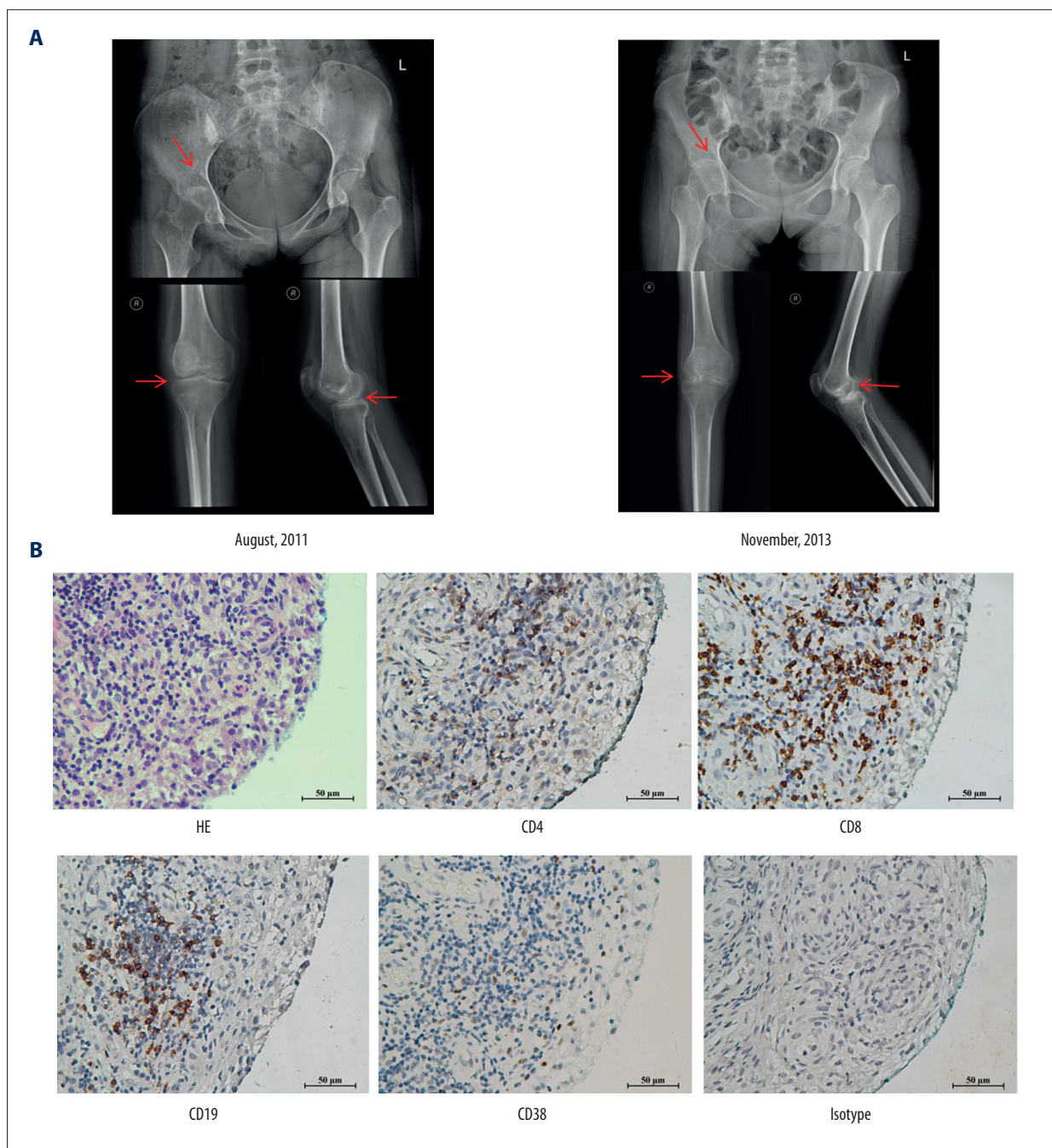


Figure 1. Radiograms and synovial histological finding of the involved joints of an adult CVID patient with aseptic and erosive polyarthritis. **(A)** Consecutive radiograms showed progressive joint space narrowing and joint destruction in right hip and right knee. **(B)** Serial paraffin sections were stained with mouse anti-human monoclonal antibody preparations (Invitrogen, Carlsbad, CA, USA): anti-CD4 (clone IgG2b) for T helper cells, anti-CD8 (clone SP16) for cytolytic T cells, anti-CD19 (clone MRQ-36) for B lymphocytes, and anti-CD38 (clone SPC32) for plasma cells according to standard staining protocols. Nonspecific isotype IgG was used as a negative control.

effusion, and local defect of bone cortex. Tuberculin skin test was negative. Microscopic inspection, DNA detection, and *in vitro* culture of synovial fluid were negative for *Mycobacterium tuberculosis*. Adequate intravenous immunoglobulin substitution

was used every 3~4 weeks to maintain IgG >5 g/L. Empirical antibiotics, including meropenem, ofloxacin, and teicoplanin, were used in succession, but polyarthritis still remained. At the time, PBMCs collection and synovial biopsy were performed.

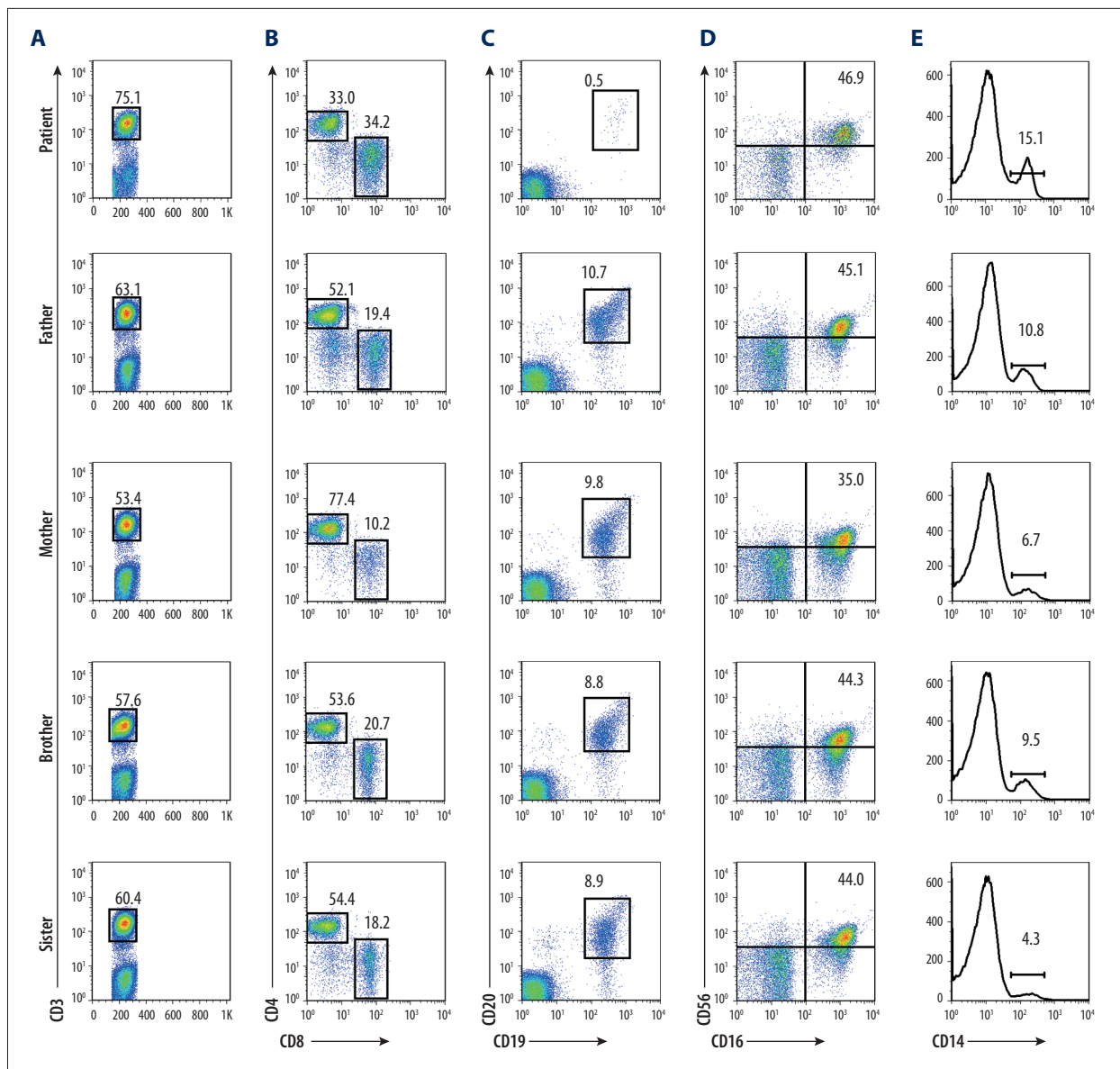


Figure 2. The proportion of different subsets of PBMCs. PBMCs were separated from peripheral blood of the patient and her unaffected family members. T lymphocytes (CD3⁺) (A) and the subsets (CD3⁺CD4⁺, CD3⁺CD8⁺) (B); B lymphocytes (CD19⁺CD20⁺) (C); NK cells (CD3⁺CD56⁺CD16⁺) (D) and the monocytes (CD14⁺) (E) were detected by flow cytometry.

Follow-up radiograms showed progressive joint space narrowing and joint destruction in the right hip and right knee (Figure 1A). Aseptic polyarthritis was suspected and antibiotics treatment was discontinued. Low-dose methylprednisolone (8 mg/d) and tacrolimus (2 mg/d) were used. The polyarthritis gradually resolved.

Histological finding of synovium

As show in Figure 1B, the synovial lining layer was composed of 1~2 layers of synovial cells, accompanied by mild hyperplasia of small vessels. There were many inflammatory cells

scattered on the sublining layer, with little inflammatory aggregation. Most of these inflammatory cells were lymphocytes, not neutrophils. There were no bacterium, mycobacterium, crystal, foreign body, or granuloma detected in the synovium. Immunohistochemistry staining verified that CD19⁺ B lymphocytes infiltrated into the synovium, CD8⁺ T cells were more common than CD4⁺ T cells, and CD38⁺ plasma cells were rare. Scattered CD68⁺ lining macrophage-like synoviocytes and sublining macrophages infiltrated in the synovium (Supplementary Figure 1).

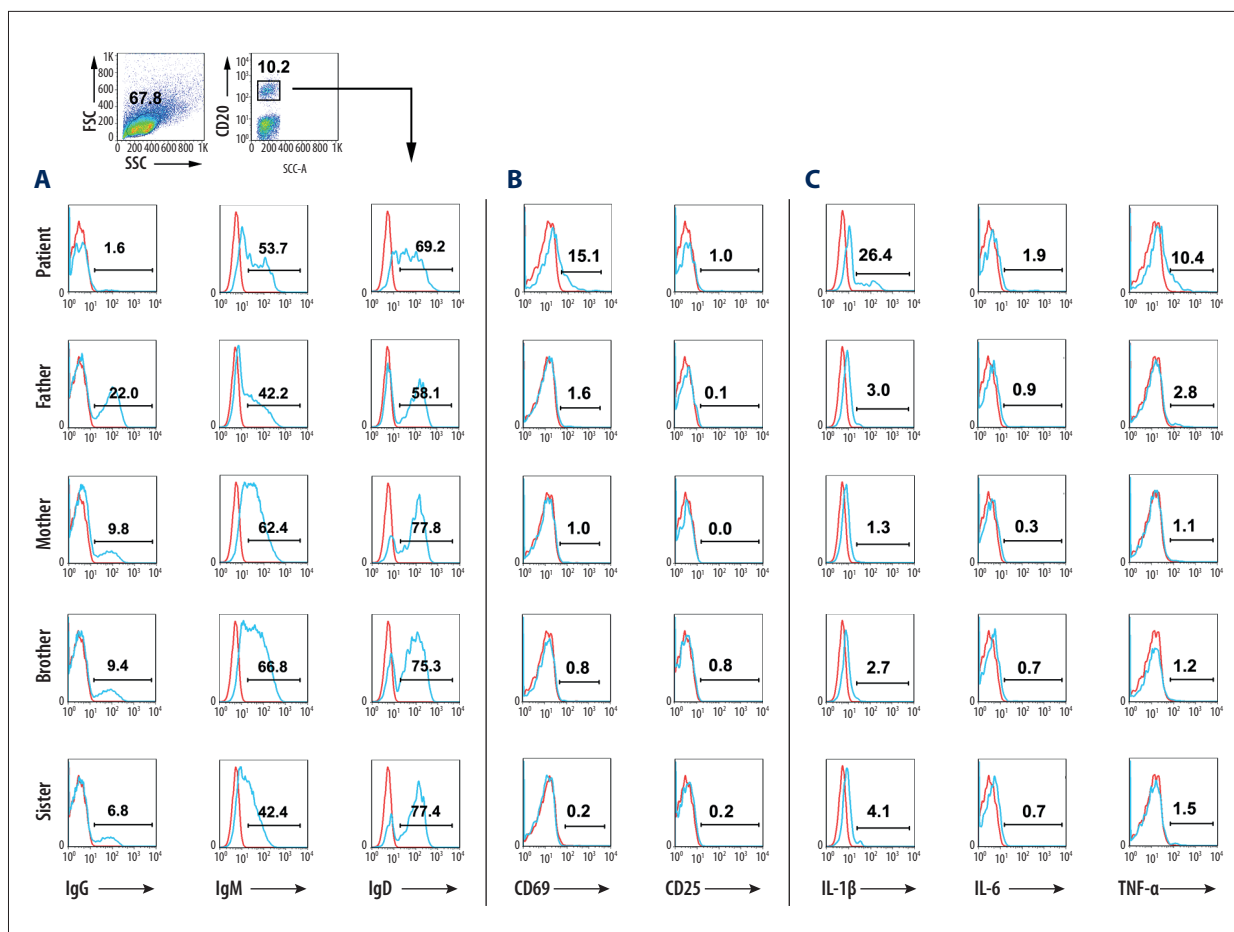


Figure 3. The function and activation of B lymphocytes detected by flow cytometry. PBMCs from the patient and her unaffected family members were gated on CD20⁺ B lymphocytes. (A) The membrane immunoglobulin IgG, IgM, and IgD. (B) The activation markers CD69 and CD25. (C) The expression of IL-1β, IL-6, and TNF-α in B lymphocytes after PBMCs were stimulated with LPS.

Excessive activation of residual B lymphocytes in peripheral blood

The proportion of B lymphocytes (CD19⁺CD20⁺) in the patient decreased to 0.5%, lower than that in unaffected family members (9.6%±0.7%) (Figure 2). The membrane IgG on CD20⁺ B lymphocytes from the patient was lower than that on CD20⁺ B lymphocytes analyzed from unaffected family members (Figure 3A). The expression of activation marker CD69 on CD20⁺ B lymphocytes from the patient was 15.1%, higher than that on CD20⁺ B lymphocytes analyzed from unaffected family members (0.9%±0.4%), while the expression of CD25 was similar (Figure 3B). Further analysis showed that the proportions of B lymphocytes that produced IL-1β, IL-6, or TNF-α in the patient were all higher than those of her unaffected family members (Figure 3C).

B lymphocytes proportions of another 2 CVID patients with-
out aseptic polyarthritis were zero (data not shown); thus,

no residual B lymphocyte could be isolated for function identification.

Excessive production of IL-21 from CD4⁺ Th cells

The proportion of T lymphocytes (CD3⁺) in the patient was higher than that in unaffected family members, while the ratio of CD4: CD8 decreased to less than 1 in the patient and was 4.0±1.8 in her unaffected family members (Figure 2). The proportion of IL21⁺CD4⁺Th cells essential for B lymphocytes activation was higher in the patient (15.2%) than in her unaffected family members (5.4%±1.6%, Figure 4A). IL-21 in culture supernatant of CD4⁺ Th cells from the patient was markedly higher (1806±213 pg/mL) than that of CD4⁺ Th cells from her unaffected family members (483±13 pg/mL for her sister; undetectable for her brother and parents, Figure 4B). The proportions of CD4⁺ Th cells that produced IFN-γ, TNF-α, IL-4, IL-5, IL-17, or IL-22 (data not shown) and the proportions of CD8⁺ cytolytic

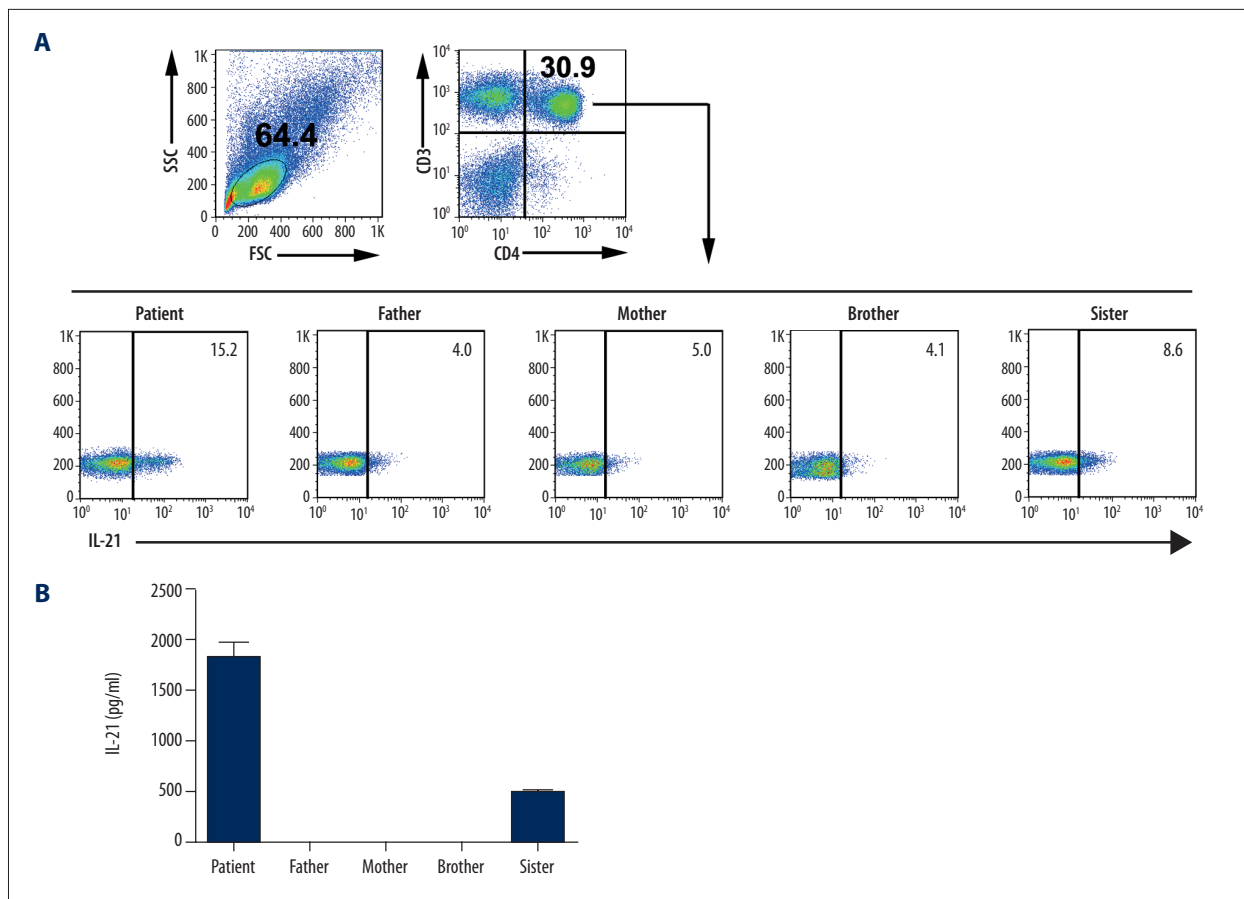


Figure 4. IL-21 production of CD4⁺ T helper cells. PBMCs from the patient and her unaffected family members were stimulated with PMA plus ionomycin. **(A)** The cells were gated on CD3⁺CD4⁺ T helper cells and IL-21 production was detected by flow cytometry. **(B)** IL-21 in culture supernatant was detected by ELISA.

T cells that produced IFN- γ , TNF- α , or IL-4 (Figure 5) were all similar between the patient and unaffected family members.

Discussion

This is the first detailed report on CVID concurrent with aseptic, erosive polyarthritis, which alters the traditional view that aseptic polyarthritis in CVID is overwhelmingly nonerosive. Our patient was diagnosed as having CVID based on recurrent infections, poor response to vaccines, and marked hypogammaglobulinemia [9]. There were no bacterium or mycobacterium detected in the synovium or synovial fluid. The synovium was infiltrated by lymphocytes but not by neutrophils. Polyarthritis did not resolve after adequate intravenous immunoglobulin substitution and empirical antibiotic treatment, but it did resolve gradually after treatment with methylprednisolone and tacrolimus, supporting the diagnosis of aseptic polyarthritis.

This study also reports a novel hypothesis of aseptic, erosive polyarthritis in CVID (Figure 6). Microbial antigens are not

eradicated completely in CVID patients due to immunodeficiency. Massive antigen load after recurrent or persistent infections may affect tolerance or cause naivety via molecular mimicry or by providing superantigens [10]. The activation of T lymphocytes yields a variety of effector functions that are pivotal to combating infections. Persistent activation of T lymphocytes, without tight regulation, can also precipitate damage to host tissues and provoke autoimmune responses. In this study, the ratio of CD4: CD8 decreased to less than 1, and in the synovium there were more CD8⁺ cytolytic T cells than CD4⁺ Th cells, indicating CD8⁺ cytolytic T cells increased more than CD4⁺ Th cells.

T lymphocytes also promote autoimmunity through the augmentation of B lymphocyte responses. IL-21 is a cytokine produced by activated CD4⁺ Th cells, which is a key cytokine for regulating B lymphocyte immunity. Co-stimulation with IL-21 promotes differentiation of memory B lymphocytes into plasma cells, inducing class switch recombination and stimulating poorly responsive naive cord blood B lymphocytes to transform into IgG-secreting plasma cells in humans [11]. In this study,

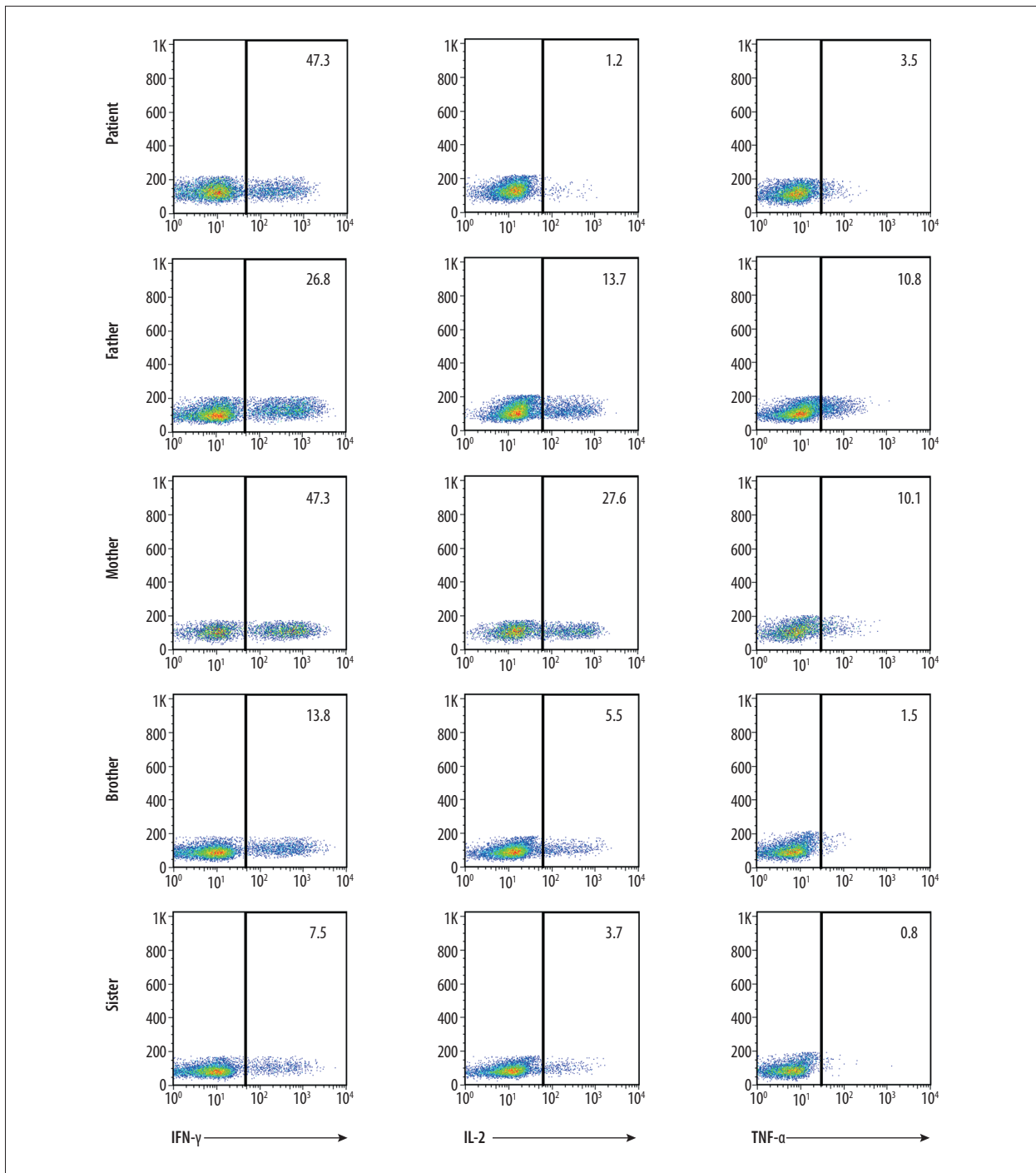


Figure 5. The proportions of CD8⁺ cytolytic T cells which produced IFN- γ , TNF- α , or IL-4. PBMCs of the patient and her unaffected family members were stimulated with PMA plus ionomycin. The cells were gated on CD3⁺CD8⁺. IFN- γ , TNF- α , and IL-4 were detected by flow cytometry.

the proportion of IL21⁺CD4⁺Th cells from peripheral blood of the patient was high, and CD4⁺ Th cells from the patient secreted nearly 3 times more IL-21 than the same cell type analyzed from unaffected family members, perhaps as excessive compensation to assist the function of residual B lymphocytes.

Further analyses showed that although only 0.5% of residual B lymphocytes existed in peripheral blood of the patient, the expression of activation marker CD69 and production of IL-1 β , IL-6, and TNF- α were high, indicating excessive activation of residual B lymphocytes. We also verified that residual

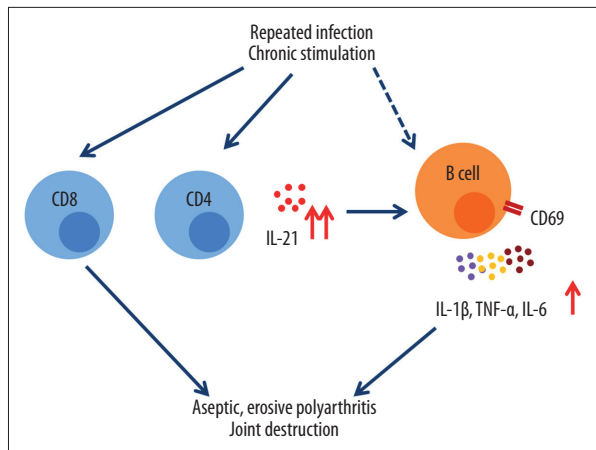


Figure 6. Diagram for a novel hypothesis of aseptic, erosive polyarthritis in CVID. Repeated and chronic infections stimulate the persistent activation of T lymphocytes. Activated CD4⁺ T helper cells produced increased IL-21 for excessive compensation to assist the function of residual B lymphocytes. The residual B lymphocytes exhibit high expression of activation marker CD69, with high ability to produce IL-1 β , TNF- α , and IL-6. Excessive activation of residual B lymphocytes infiltrate into synovium, leading to aseptic, erosive polyarthritis and joint destruction.

B lymphocytes infiltrated into the synovium. Synovial B lymphocytes were reported to be correlated with joint destruction in rheumatoid arthritis in our previous study [8]. It can be hypothesized that excessive activation of residual B lymphocytes in CVID causes aseptic, erosive polyarthritis.

The major limitation in this study is that it only included 1 case due to the extremely low incidence; thus, further case series are needed to verify this hypothesis. Another limitation is that it does not include a CVID patient without arthritis as a pathological control, because there was no residual B lymphocyte identified for function detection in the 2 CVID patients without aseptic polyarthritis.

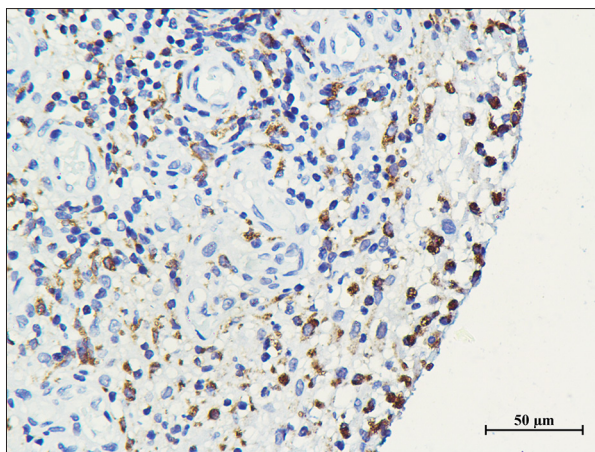
Conclusions

To conclude, this exploratory study illustrates a novel hypothesis of CVID concurrent with aseptic, erosive polyarthritis, indicating that excessive activation of residual B lymphocytes infiltrate into the synovium of the involved joints and lead to polyarthritis and joint destruction, while excessive production of IL-21 from CD4⁺ Th cells may contribute to this excessive activation.

Conflicts of interest

None.

Supplementary Figure



Supplementary Figure 1. Lining macrophage-like synoviocytes and sublining macrophages in synovium of the CVID patient. A paraffin section was stained with mouse anti-human monoclonal antibody preparation to CD68 (clone KP1, Invitrogen, Carlsbad, CA, USA) according to standard staining protocol.

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