

## Letter to the Editor

**Lymphoid neogenesis in the giant papillae of patients with chronic allergic conjunctivitis**

To the Editor:

Lymphoid neogenesis (tertiary lymphoid organ [TLO]) resembles a secondary lymphoid organ (eg, the lymph node) and is observed in patients with chronic inflammatory diseases.<sup>1</sup> TLOs play roles in immune responses against persistent antigens; however, they can also induce severe tissue damage.<sup>1</sup> It has also been reported that the germinal centers and follicular dendritic cell (FDC) networks in TLOs relate to class-switch recombination.<sup>2</sup> In this study we examined the lymphoid neogenesis and lymphatic vessel formation in patients with atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC),<sup>3</sup> severe chronic forms of allergic conjunctivitis. AKC and VKC are often accompanied by giant papillae formation in tarsal conjunctivae and/or marked inflammatory cell infiltration in the limbal region, with prominent T<sub>H</sub>2 cytokine profiles.<sup>3</sup> We found various degrees of lymphoid neogenesis in the giant papillae with B-cell clusters, FDCs, and a marginal T-cell zone essential for TLO formation.<sup>1</sup>

The IgE class of immunoglobulin is produced by B cells and plasma cells through class-switch recombination in the germinal center of lymphoid tissues.<sup>2</sup> Preferential expression of the FcεRI β-chain protein, an amplifier of allergic responses, has been observed in the giant papillae of patients with AKC/VKC.<sup>4</sup> These findings suggested the role of an IgE–FcεRI β cascade in the pathophysiology of severe chronic allergic conjunctivitis. The IgE

might originate from tears<sup>5</sup> or from blood flow; however, local IgE class-switch recombination in the giant papillae itself, as well as in the nasal polyps,<sup>6</sup> might also be possible. Regional IgE expression at the mast cells, dendritic cells, and Langerhans cells of the giant papillae was reported previously.<sup>7</sup> Therefore it is important to clarify the origin of IgE production in patients with AKC/VKC because anti-IgE antibody therapy has recently been introduced for the treatment of severe atopic asthma, and it might also be effective for AKC/VKC.

Ten giant papillae obtained from patients with AKC/VKC were resected for a therapeutic purpose, as previously described.<sup>4</sup> The clinical information of the patients is summarized in Table I. All the patients had chronic allergic conjunctivitis and were treated with topical dexamethasone eye drops for at least 4 weeks before surgery. Total IgE concentrations and specific IgE titers against 26 common antigens were measured by SRL, Inc (Tokyo, Japan), using enzyme immunoassay methods. All procedures were approved by the ethics committee of Juntendo University School of Medicine and of Kyoto Prefectural University of Medicine. This study was conducted in accordance with the tenets of the Declaration of Helsinki, and written informed consent was obtained from all patients before their participation in the study. Immunohistochemical staining was performed according to the previously described methods,<sup>4</sup> and the list of antibodies is shown in Table II. To examine ε-chain mRNA expression and activation-induced cytidine deaminase (AID) mRNA expression in giant papillae, total RNA was isolated from biopsy specimens of the giant papillae

**TABLE I.** Clinical information of the patients and summary of the results

| Patient no. | Age (y) | Sex | Total IgE (IU/mL) | Specific IgE | Diagnosis* | CD20 <sup>+</sup> cell cluster† | FDC |
|-------------|---------|-----|-------------------|--------------|------------|---------------------------------|-----|
| 1           | 16      | F   | 509               | Positive     | VKC        | Large clusters                  | +   |
| 2           | 22      | M   | 89                | Positive     | VKC        | Diffuse                         | +   |
| 3           | 13      | M   | 2,319             | Positive     | VKC        | Large clusters                  | +   |
| 4           | 18      | M   | 375               | Positive     | AKC        | Diffuse and large clusters      | +   |
| 5           | 17      | M   | 17,260            | Positive     | AKC        | Large clusters                  | +   |
| 6           | 21      | M   | 1,904             | Positive     | AKC        | Small clusters                  | +   |
| 7           | 16      | M   | 3,763             | Positive     | AKC        | Large clusters                  | +   |
| 8           | 34      | M   | 22,800            | Positive     | AKC        | Large clusters                  | +   |
| 9           | 45      | F   | 28                | Negative     | AKC        | Small clusters                  | +   |
| 10          | 29      | M   | 56                | Positive     | VKC        | Small clusters                  | +   |

\*AKC was defined as a bilateral chronic inflammation of the conjunctiva and lids associated with atopic dermatitis. VKC was defined as a bilateral, chronic, conjunctival inflammatory condition of the conjunctiva found in subjects predisposed by their atopic background. Patients who had atopic dermatitis or corneal stromal neovascularization were excluded from the VKC diagnosis.

†Large lymphocyte clusters were defined as those having more than 10 lymphocytes in length/diameter.

**TABLE II.** List of antibodies used in this study

| Antigen                   | Class                | Dilution | Supplier                              |
|---------------------------|----------------------|----------|---------------------------------------|
| Anti-LYVE-1               | Rabbit polyclonal    | 1:400    | RELIAtech, Wolfenbüttel, Germany      |
| Anti-CD3                  | Murine monoclonal    | 1:100    | DAKO, Glostrup, Denmark               |
| Anti-CD20                 | Rabbit monoclonal    | 1:200    | Epitomics, South San Francisco, Calif |
| Anti-CD35                 | Murine monoclonal    | 1:50     | Dako                                  |
| Anti-PNAd                 | Rat monoclonal (IgM) | 1:25     | Becton Dickinson, Franklin Lakes, NJ  |
| Alexa 488–anti-mouse IgG  | Donkey polyclonal    | 1:1,000  | Invitrogen, Carlsbad, Calif           |
| Alexa 594–anti-rabbit IgG | Donkey polyclonal    | 1:1,000  | Invitrogen                            |
| Alexa 488–anti-rabbit IgG | Goat polyclonal      | 1:1,000  | Invitrogen                            |
| Alexa 594–anti-rat IgM    | Goat polyclonal      | 1:1,000  | Invitrogen                            |

**TABLE III.** Oligonucleotide primers used to amplify  $\epsilon$ -chain mRNA and AID mRNA

| Primer name | Sequence                           |
|-------------|------------------------------------|
| VF2         | 5'-AGTCTGGAGCAGAGGTG-3'            |
| CeR1        | 5'-CAGGACGACTGTAAGATCTTCACG-3'     |
| CeR2        | 5'-GGGGTGAAGTCCCTGGAGC-3'          |
| AIDF1       | 5'-GAGGCAAGAAGACACTCTGG-3'         |
| AIDR1       | 5'-GTGACATTCTGGAAGTTGC-3'          |
| AIDF2       | 5'-TAGACCCTGGCCGCTGCTACC-3'        |
| AIDR2       | 5'-CAAAAGGATGCGCCGAAGCTGTCTGGAG-3' |

obtained from 5 patients with VKC (see patients 1, 3, 5, 8, and 10 in Table I). cDNA was prepared according to a previously described method.<sup>8</sup>  $\epsilon$ -Chain mRNA and AID mRNA were amplified by using nested PCR methods, as previously described, with modification for the second-round PCR.<sup>6</sup> Touchdown PCR methods were used for the second-round PCR. The primers used for PCR reactions are shown in Table III. The PCR products were verified by means of direct sequencing with VF2 and CeR2 primers (for  $\epsilon$ -chain mRNA) and AID2F and AID2R primers (for AID mRNA),

respectively. The sequence of the VH region of immunoglobulin was verified by information obtained at the IgBLAST immunoglobulin analysis Web site (<http://www.ncbi.nlm.nih.gov/igblast/>).

Lymphatic vessel endothelial hyaluronan receptor 1<sup>+</sup> (LYVE-1<sup>+</sup>) lymphatic vessels were observed in the substantia propria of the giant papillae (Fig 1, A), and CD3/CD20 double immunostaining of the adjacent section showed nearby localization of the lymphatic vessels and TLOs (Fig 1, A and B). The TLOs were accompanied by CD35<sup>+</sup> FDCs (Fig 1, C). Peripheral node addressin (PNA<sup>+</sup>) positive high endothelial venules existed near the LYVE-1<sup>+</sup> lymphatic endothelial cells (Fig 1, D). Various degrees of TLOs were observed in all of the AKC/VKC tissues (summarized in Table 1).  $\epsilon$ -Chain mRNA and AID mRNA were detected in the cDNA sample obtained from the giant papilla of a patient with VKC (see patient 1 in Table I) by means of nested RT-PCR (Fig 2), and the sequence of the PCR products was verified by means of PCR direct sequencing. No  $\epsilon$ -chain mRNA or AID mRNA transcripts were detected in 2 control conjunctivae cDNA samples (samples were obtained during cataract surgery, data not shown).

Ectopic lymphoid neogenesis has been observed in patients with many chronic inflammatory disorders,<sup>1</sup> including rheumatoid synovitis, Sjogren syndrome, and a murine model of asthma.

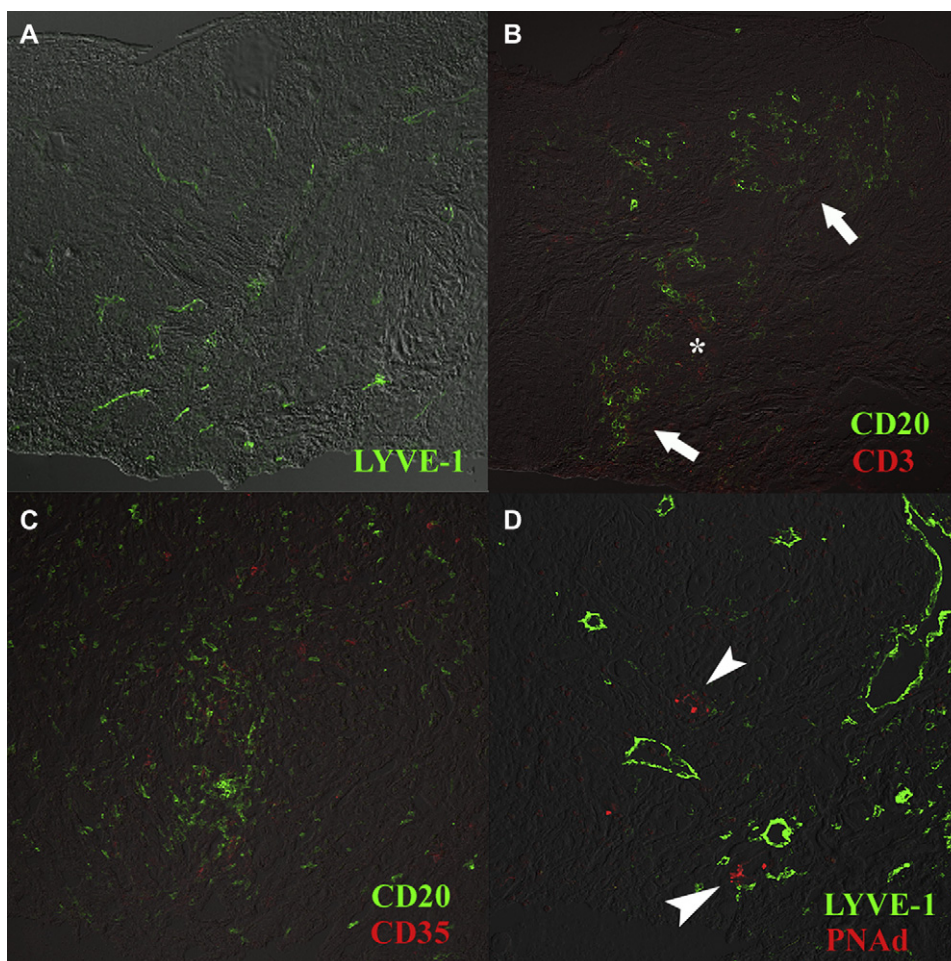


**FIG 1.** Lymphatic vessels and lymphoid neogenesis in the giant papillae. **A**, LYVE-1<sup>+</sup> lymphatic vessels were observed in the substantia propria of giant papillae. **B**, CD20<sup>+</sup> B-cell clusters (arrows) and T-cell zones (asterisk) were observed in the region corresponding to the lymphatic vessels. **C**, CD35<sup>+</sup> FDCs were observed around the B-cell cluster. **D**, PNA<sup>+</sup> high endothelial venules were observed around lymphatic vessels. Original magnification  $\times 200$  (Fig 1, A and B) and  $\times 400$  (Fig 1, C and D).

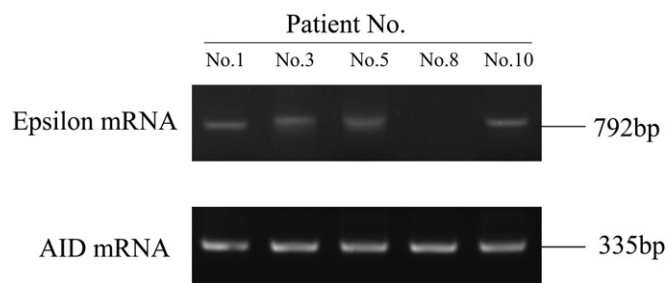


**FIG 2.** RT-PCR analysis of the  $\epsilon$ -chain mRNA and AID mRNA expression in the giant papillae tissues. Nested RT-PCR was carried out with cDNA specimens obtained from patients with AKC/VKC (patients 1, 3, 5, 8, and 10 in Table I). The 2% agarose gel electrophoresis showed positive bands (789 bp for  $\epsilon$ -chain mRNA and 355 bp for AID mRNA).

We found the essential structures for ectopic lymphoid neogenesis (clusters of B cells, the T-cell zone, and FDCs) in the giant papillae. PNAd<sup>+</sup> high endothelial venules around the TLOs, which are known to supply naive T cells, further suggested ongoing immune responses at the giant papillae of patients with chronic allergic conjunctivitis. The TLOs in the giant papillae might also contribute to regional immune responses against persistent antigens at the ocular surface and thus promote chronic inflammation.<sup>1</sup> Another function of lymphoid neogenesis is immunoglobulin class switching in the B-cell clusters.<sup>2</sup> In addition to  $\epsilon$ -chain mRNA expression in the giant papillae, we also detected AID mRNA, which encodes an essential enzyme that catalyzes the class-switch recombination process and is expressed only in germinal-center B cells. We also showed IgE plasma cells and IgE<sup>+</sup> B cells in the substantia propria of giant papillae (Fig E1). Although we could not exclude the possibility that class-switch recombination in B cells might occur in regional secondary lymphoid organs, our results suggested that direct class-switch recombination in the TLOs of the giant papillae is highly probable, as reported in cases involving nasal polyps.<sup>6</sup> It also should be noted that all of the giant papillae analyzed in this study were treated with topical glucocorticoid eye drops. A previous report<sup>9</sup> showed that glucocorticoid could induce IgE isotype switching in human B cells, and therefore further studies are essential to elucidate the effect of glucocorticoids in giant papillae.

In conclusion, we showed the formation of TLOs and some evidence of local IgE production in patients with severe chronic allergic conjunctivitis (AKC/VKC). Regional therapy, including anti-IgE therapy with eye drops, might be effective for the treatment of AKC/VKC.

Akira Matsuda, MD, PhD<sup>a</sup>  
Nobuyuki Ebihara, MD, PhD<sup>a</sup>  
Norihiro Yokoi, MD, PhD<sup>b</sup>  
Kazuichi Maruyama, MD, PhD<sup>b</sup>  
Junji Hamuro, PhD<sup>b</sup>  
Shigeru Kinoshita, MD, PhD<sup>b</sup>  
Akira Murakami, MD, PhD<sup>a</sup>

From <sup>a</sup>the Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan, and <sup>b</sup>the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan. E-mail: akimatsu@juntendo.ac.jp.

Supported by Grants-in-Aid from MEXT Japan (no. 19659454 to Dr Kinoshita and no. 18604009 to Dr Matsuda).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

## REFERENCES

1. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol* 2006;6:205-17.
2. Gould HJ, Takhar P, Harries HE, Durham SR, Corrigan CJ. Germinal-centre reactions in allergic inflammation. *Trends Immunol* 2006;27:446-52.
3. Barney NP. Vernal and atopic keratoconjunctivitis. In: Kratchmer JH, Mannis MJ, Holland EJ, editors. *Cornea*. 2nd ed. Philadelphia: Mosby; 2005. p. 667-74.
4. Matsuda A, Okayama Y, Ebihara N, Yokoi N, Hamuro J, Walls AF, et al. Hyperexpression of the high-affinity IgE receptor-beta chain in chronic allergic keratoconjunctivitis. *Invest Ophthalmol Vis Sci* 2009;50:2871-7.
5. Ebihara N, Okumura K, Nakayasu K, Kanai A, Ra C. High level of Fc epsilon receptor I-bindable immunoglobulin E in the tear fluid and increased immunoglobulin E-saturated cells in the giant papillae of vernal keratoconjunctivitis patients. *Jpn J Ophthalmol* 2002;46:357-63.
6. Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol* 2005;174:5024-32.
7. Montan PG, Biberfeld PJ, Scheynius A. IgE, IgE receptors, and other immunocytochemical markers in atopic and nonatopic patients with vernal keratoconjunctivitis. *Ophthalmology* 1995;102:725-32.
8. Matsuda A, Ebihara N, Yokoi N, Kawasaki S, Tanioka H, Inatomi T, et al. Functional roles of thymic stromal lymphopoietin for chronic allergic keratoconjunctivitis. *Invest Ophthalmol Vis Sci* 2010;51:151-5.
9. Jabara HH, Ahern DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J Immunol* 1991;147:1557-60.

doi:10.1016/j.jaci.2010.06.001

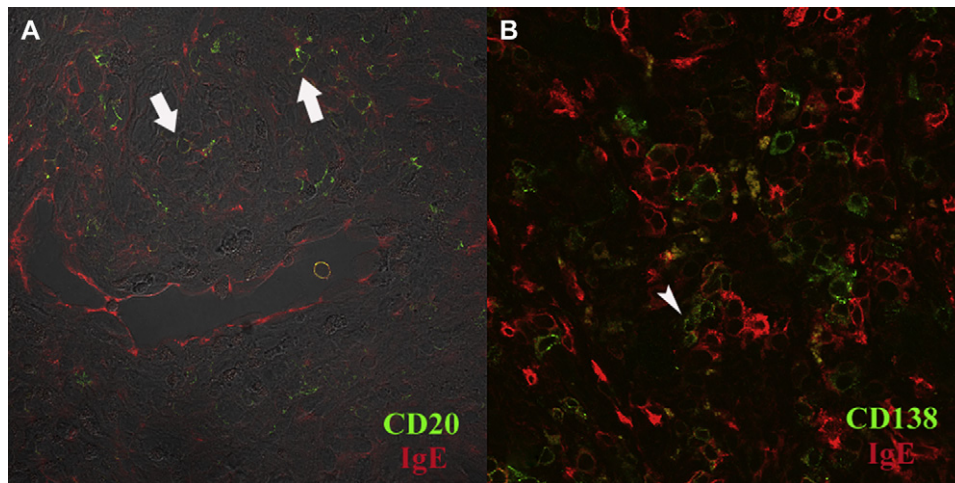


**FIG E1.** IgE plasma cells and IgE<sup>+</sup> B cells observed in the substantia propria. **A**, CD20<sup>+</sup> B cells were observed in the substantia propria of the giant papillae, and some of the plasma cells (*arrows*) were IgE<sup>+</sup>. **B**, CD138<sup>+</sup> plasma cells were observed in the substantia propria of the giant papillae, and some of the plasma cells (*arrowhead*) were IgE<sup>+</sup>.