Hyperexpression of the High-Affinity IgE Receptor-β Chain in Chronic Allergic Keratoconjunctivitis

Akira Matsuda, Yoshimichi Okayama, Nobuyuki Ebihara, Noribiko Yokoi, Junji Hamuro, Andrew F. Walls, Csíbié Ra, Julian M. Hopkin, and Shigeru Kinoshiba

PURPOSE. Although the existence of FcεRIαβγ2 and FcεRIαγ2 receptor subtypes was reported, there has been no direct evidence of these two subtypes of FcεRI in vivo. To investigate the existence of these two subtypes of FcεRI in vivo, the authors evaluated the expression of FcεRI-β in the giant papillae of chronic allergic conjunctivitis and compared the expression level of FcεRI-β with control conjunctivae using the anti-human FcεRI-β antibody.

METHODS. FcεRI-β expression in giant papillae obtained from patients with atopic keratoconjunctivitis and vernal keratoconjunctivitis in control conjunctivae was evaluated by immunohistochemistry using anti-FcεRI-β, -α, -γ, and anti-human mast cell tryptase, anti-chymase, anti-basophil, and anti-CD1a antibodies.

RESULTS. Statistical analyses revealed that the densities of FcεRI-β- cells, FcεRI-α- cells, tryptase- cells, and FcεRI-β+/tryptase- cells were significantly increased in giant papillae compared with controls. There were two types of FcεRI (αβγ2 and αγγ2) on the mast cells of the giant papillae. The ratio of the FcεRI-β+ cell number/FcεRI-α- cell number in the giant papillae (0.69 ± 0.08 [mean ± SD]) was significantly higher than that of the controls (0.07 ± 0.16). FcεRI-β/tryptase double immunostaining revealed that 81% ± 13% of tryptase- cells expressed FcεRI-β. FcεRI-β- cells were preferentially localized within and around epithelial tissue. The authors also found that FcεRI-β was expressed by basophils but not by FcεRI-αγ2-positive Langerhans cells in the giant papillae samples.

CONCLUSIONS. Preferential FcεRI-β expression observed in the mast cells and basophils of giant papillae suggests important roles of FcεRI-β in the pathophysiology of atopic keratoconjunctivitis and vernal keratoconjunctivitis. (Invest Ophthalmol Vis Sci. 2009;50:2871–2877) DOI:10.1167/iovs.08-3022

Human high-affinity IgE receptor (FcεRI) exists in two isoforms, a tetramer containing the β chain FcεRI-αβγ2 and a trimmer lacking the β chain FcεRI-αγγ2, depending on cell type. The FcεRI-β gene (MS4A2) has been recognized as an atopy-related gene, initially discovered from a genetic linkage study and also from a genetic association study by our group, and the functional roles of FcεRI-β protein have been investigated extensively. For example, the FcεRI-β chain mediates intracellular signaling through the immunoreceptor tyrosine-based activation motif and is phosphorylated in response to antigen cross-linking of the receptor-bound IgE. The human FcεRI-β chain acts as an amplifier for mast cell activation and cell surface expression of FcεRI. Although the existence of the FcεRI-αβγ2 and FcεRI-αγγ2 receptor subtypes has been reported, there has been no direct evidence of these two subtypes of FcεRI in vivo at the protein level. Furthermore, the precise pathophysiological roles of the FcεRI-β chain in human atopic diseases remain unclear. Recently, we raised an antibody against human FcεRI-β that was useful for the in situ detection of the FcεRI-β protein.

Atopic keratoconjunctivitis (AKC)^8,9 and vernal keratoconjunctivitis (VKC)^10 are the most severe forms of chronic allergic conjunctivitis. Massive infiltration of mast cells occurs, and serum and tear IgE levels are significantly higher than in healthy controls. In addition, AKC and VKC tend to form giant papillae at the upper tarsal conjunctiva. We rejected giant papillae for therapeutic purposes and carried out histopathologic analysis with the resected tissues using our newly generated anti-FcεRI-β-specific antibody. We reported previously that IgE-bearing FcεRI-α cells were increased in the giant papillae of patients with VKC, however, the expression of FcεRI-β has yet to be evaluated. We found preferential FcεRI-β expression in the mast cells of giant papillae samples compared with those of the control conjunctivae.

MATERIALS AND METHODS

Antibodies

Rabbit antiserum against unique C-terminal sequences of human FcεRI-β (CYSLEDPGEMSPPIDLU) was generated and the antiserum was purified on a protein-A column, as previously described. Because this antibody was raised against the C-terminal region of human FcεRI-β protein, it did not recognize the truncated form of FcεRI-β protein described previously. Other antibodies used in this study included Alexa 488-conjugated goat anti-rabbit-F(ab′)2 and Alexa 594-conjugated goat anti-mouse IgG-F(ab′)2 (In Vitrogen, Carlsbad, CA), Cy5-conjugated goat anti-mouse IgG1 antibody (Southern Biotechnology, Birmingham, AL), rabbit anti-FcεRI-γ polyclonal antibody (Upstate Biotechnology, Lake Placid, NY), phycoerythrin (PE)-conjugated goat anti-mouse FcεRI-α monoclonal antibody (clone CRA1; eBioscience, Tokyo, Japan), mouse anti-chymase monoclonal antibody (clone CC1; Lab Vision, Fremont, CA), mouse anti-CD1a monoclonal antibody.
TABLE 1. Clinical Characteristics of Patients with AKC/VKC and FcεRI-β+ Cell Numbers of Giant Papillae

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Total IgE</th>
<th>Specific IgE</th>
<th>Diagnosis</th>
<th>FcεRI-β+ Cells (mean ± SD)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>F</td>
<td>509</td>
<td>Positive</td>
<td>VKC</td>
<td>62.5 ± 20.0</td>
<td>Dex, CsA</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>M</td>
<td>89</td>
<td>Positive</td>
<td>VKC</td>
<td>53.9 ± 8.3</td>
<td>Dex</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>M</td>
<td>2319</td>
<td>Positive</td>
<td>VKC</td>
<td>20.2 ± 8.3</td>
<td>Dex</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>M</td>
<td>375</td>
<td>Positive</td>
<td>AKC</td>
<td>49.3 ± 22.0</td>
<td>Dex, CsA</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>M</td>
<td>17260</td>
<td>Positive</td>
<td>AKC</td>
<td>56.2 ± 21.2</td>
<td>Dex, CsA, oral steroid</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>M</td>
<td>1904</td>
<td>Positive</td>
<td>AKC</td>
<td>34.9 ± 18.0</td>
<td>Dex</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>M</td>
<td>5765</td>
<td>Positive</td>
<td>AKC</td>
<td>29.2 ± 17.0</td>
<td>Dex</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>F</td>
<td>124</td>
<td>Negative</td>
<td>VKC</td>
<td>49.4 ± 20.0</td>
<td>Dex</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>M</td>
<td>20328</td>
<td>Positive</td>
<td>AKC</td>
<td>78.3 ± 25.5</td>
<td>Dex</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>28</td>
<td>Negative</td>
<td>AKC</td>
<td>48.2 ± 12.0</td>
<td>Dex</td>
</tr>
</tbody>
</table>

Dex. 0.1% dexamethasone eyedrop; CsA. 0.1% cyclosporine eyedrop.

AKC/VKC Patient Selection and Giant Papillae Tissue Processing

Giant papillae were resected for therapeutic purposes14 from six patients with AKC and four patients with VKC after obtaining informed consent (Table 1). AKC was defined as bilateral, chronic inflammation of the conjunctiva and lids associated with atopic dermatitis, and VKC was defined as bilateral, chronic inflammation of the conjunctiva associated with predisposition to atopy.18 Patients who had atopic dermatitis or corneal stromal neovascularization were excluded from the VKC diagnosis. All patients with AKC or VKC were treated by topical or the FcεRI-β or with anti-FcεRIγ polyclonal antibodies for 1 hour and then with Alexa 488-conjugated anti-rabbit IgG antibody for 30 minutes. For double-staining with anti-tryptase, anti-FcεRI-α, anti-basophil, or anti-CD1a monoclonal antibodies, the slides were incubated simultaneously with FcεRI-β or FcεRIγ polyclonal antibodies and with one of the monoclonal antibodies. In the case of double immunostaining with the anti-chymase antibody, the slides were incubated with the anti-chymase antibody overnight at 4°C, and then the FcεRI-β polyclonal antibody was added and further incubated for 1 hour at room temperature. Alexa 488-conjugated anti-rabbit IgG antibody and Alexa 594 conjugated anti-mouse IgG antibody were mixed and applied simultaneously as second antibodies. For triple immunostaining using the CD1a (class, mouse IgG1), FcεRI-α (class, mouse IgG2a), and FcεRI-β antibodies, the slides were incubated with the CD1a antibody and the FcεRI-β antibody for 1 hour at room temperature. After PBS washes, Alexa 488-anti-rabbit IgG antibody, Cy5-anti-mouse IgG1 antibody, and PE-anti-FcεRI-α (class, mouse IgG2a) were applied simultaneously for another hour. As negative controls, normal rabbit IgG (Santa Cruz Biotechnology) was used instead of the FcεRI-β polyclonal antibody at the same concentration, or the FcεRI-β IgG antibodies were preabsorbed with a fivefold excess amount of the peptide used for immunization. These slides were then visualized with a confocal laser scanning microscope (FV1000; Olympus Corp., Tokyo, Japan).

Immunohistochemical Analysis of Giant Papillae

Seven-micrometer cryostat sections were made from the specimens and air dried. Sections were then postfixed with 4% PFA-PBS. After blocking with 1% bovine serum albumin (BSA) in PBS, the slides were reacted with anti-FcεRI-β or with anti-FcεRIγ polyclonal antibodies for 1 hour and then with Alexa 488-conjugated anti-rabbit IgG antibody for 30 minutes. For double-staining with anti-tryptase, anti-FcεRI-α, anti-basophil, or anti-CD1a monoclonal antibodies, the slides were incubated simultaneously with FcεRI-β or FcεRIγ polyclonal antibodies and with one of the monoclonal antibodies. In the case of double immunostaining with the anti-chymase antibody, the slides were incubated with the anti-chymase antibody overnight at 4°C, and then the FcεRI-β polyclonal antibody was added and further incubated for 1 hour at room temperature. Alexa 488-conjugated anti-rabbit IgG antibody and Alexa 594 conjugated anti-mouse IgG antibody were mixed and applied simultaneously as second antibodies. For triple immunostaining using the CD1a (class, mouse IgG1), FcεRI-α (class, mouse IgG2a), and FcεRI-β antibodies, the slides were incubated with the CD1a antibody and the FcεRI-β antibody for 1 hour at room temperature. After PBS washes, Alexa 488-anti-rabbit IgG antibody, Cy5-anti-mouse IgG1 antibody, and PE-anti-FcεRI-α (class, mouse IgG2a) were applied simultaneously for another hour. As negative controls, normal rabbit IgG (Santa Cruz Biotechnology) was used instead of the FcεRI-β polyclonal antibody at the same concentration, or the FcεRI-β IgG antibodies were preabsorbed with a fivefold excess amount of the peptide used for immunization. These slides were then visualized with a confocal laser scanning microscope (FV1000; Olympus Corp., Tokyo, Japan).

Figure 1. Anti-FcεRI-β immunostaining of giant papillae: Immunohistochemical staining was carried out with giant papillae specimens from patients with AKC or VKC using the anti-FcεRI-β antibody (A) and control rabbit IgG (B) at the same concentration. Original magnification, 200×. This is representative data obtained from 1 of 10 patients (patient 5 in Table 1).
Cell Counting

Numbers of FcεRIβ⁺ and FcεRIα⁺ cells (membrane staining) and tryptase⁺ cells (intracellular staining) were counted manually using 200× magnification immunostaining images. Positive cells, both in the epithelium and in the substantia propria, were counted per 1-mm unit length of the conjunctival surface as total. We counted three sections per each patient for FcεRIβ immunostaining and two sections for the remaining staining. All immunostained sections were evaluated by an observer who was blinded to the clinical data of the patients. We carried out two independent series of cell staining and counting, and we showed one representative result.

RESULTS

FcεRIβ Immunostaining of Giant Papillae and Control Conjunctivae

All the tested giant papillae samples (n = 10; Table 1) showed focal-positive immunostaining in the epithelium and in the substantia propria with anti–FcεRIβ (Fig. 1A), and the negative-control slide stained by normal rabbit IgG (Fig. 1B) and by the preabsorbed FcεRIβ antibody (data not shown) did not show any positive staining. In addition to anti–FcεRIβ staining (Fig. 2A), all the tested giant papillae showed positive staining with anti–FcεRIα antibodies (Fig. 2B); there were FcεRIα/FcεRIβ double-positive and FcεRIα single-positive cells (Fig. 2C). Double-immunohistochemical staining of the nearby section shown in Figure 2A with anti–FcεRIγ antibodies (Fig. 2D) and with anti–FcεRIα antibodies (Fig. 2E) showed that all the FcεRIα⁻ cells were also FcεRIγ⁻ (Fig. 2F). Control upper bulbar conjunctivae from the conjunctivochalasis or SLK patients (n = 10; Table 2) showed a few FcεRIα⁺ cells (Figs. 3A, 3C) or a few tryptase-positive mast cells (Figs. 3B, 3D) in its substantia propria but a negligible number of FcεRIβ⁻ cells (Fig. 3D; Table 3).

Immunolocalization and Quantification of FcεRIβ⁺ Cells in Giant Papillae

The giant papillae were also double immunostained with anti-FcεRIβ and the antibodies to typical mast cell proteases (tryptase and chymase). FcεRIβ⁺ immunostaining was observed at the cell periphery of the tryptase⁺ cells (Figs. 4C, 4G). Some of the tryptase⁺ cells were FcεRIβ negative (Fig. 4C, 4G).
Control 1.1

luted epithelium22 and pseudotubules23 (Figs. 5A, 5B; asterisks).

tryptase

0.69

than the ratio of the control samples (0.07

TABLE 3. Comparison of the FcεRI- and Tryptase-Positive Cells between Giant Papillae and Control Samples

<table>
<thead>
<tr>
<th></th>
<th>FcεRI-β+ Cells (mean ± SD)</th>
<th>FcεRI-α+ Cells (mean ± SD)</th>
<th>Tryptase+ Cells (mean ± SD)</th>
<th>Ratio of FcεRI-β+ and FcεRI-α+ Cells (mean ± SD)</th>
<th>Ratio of FcεRI-β+ and Tryptase+ Cells (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant papillae</td>
<td>55.9 ± 24.6*</td>
<td>99.0 ± 33.2†</td>
<td>71.1 ± 24.1‡</td>
<td>0.69 ± 0.08§</td>
<td>0.81 ± 0.13</td>
</tr>
<tr>
<td>Control</td>
<td>1.1 ± 2.3*</td>
<td>19.6 ± 16.9‡</td>
<td>17.6 ± 9.8‡</td>
<td>0.07 ± 0.16‡</td>
<td>0.06 ± 0.11</td>
</tr>
</tbody>
</table>

Student’s t test: *P = 0.0000007; †P = 0.0001; ‡P = 0.000005; §P = 0.00000007; ||P = 3 × 10⁻⁹.

FcsRI-β staining (green). Double immunostaining with FcεRI-β and chymase (Figs. 5E, 5F) showed cytoplasmic chymase staining (red) at some of the FcεRI-β+ cells (green). The results of the FcεRI-β+ cell quantification are summarized in Table 3. Statistical analyses revealed that the densities of FcεRI-β+ cells, and FcεRI-β+tryptase+ mast cells were significantly increased in giant papillae compared with control conjunctivae. Although the average number of FcεRI-β+ cells was higher for SLK (1.7 ± 0.7) than for conjunctivochalasis (0.7 ± 1.1) samples, the difference was not statistically significant (P = 0.38, Student’s t-test), and the average number of FcεRI-β+ cells in SLK samples was still significantly lower than the number of FcεRI-β+ cells in giant papillae (P = 0.00001, Student’s t-test).

FcsRI-β Expression in the Basophils of Giant Papillae Tissues but Not in Langerhans Cells (LCs)

During immunohistochemical analysis we found some tryptase–FcsRI-β+ cells in the giant papillae sections, so we
speculated that the FceRI-β⁺ cells included basophils. Positive basophil staining was observed at the substantia propria of the giant papillae (Fig. 6A). Double immunostaining using anti-FceRI-β and anti-basophil antibodies showed FceRI-β immunostaining (green) at the periphery of the basophil (red) (Fig. 6C). To examine the FceRI-receptor subtype in LCs of the giant papillae, immunostaining with CD1a and FceRI-α/FceRI-β antibodies was carried out. Double-staining did not reveal any CD1a/FceRI-β⁺ cells (Figs. 7A, 7C, 7E). On the other hand, two CD1a⁺ cells were FceRI-α⁺ cells (Figs. 7B, 7C, 7D; arrows). By triple-immunohistochemical staining, we confirmed the existence of FceRI-α⁺/CD1a⁺/FceRI-β⁻ LCs (Fig. 7E, arrow) and of FceRI-α⁺/FceRI-β⁻/CD1a⁻ mast cells (Fig. 7E, arrowhead).

**DISCUSSION**

To the best of our knowledge, this study is the first to demonstrate the existence of both FceRI-αβγ鲶 and FceRI-αγ鲶 receptor subtypes in the giant papillae of patients with chronic allergic conjunctivitis. FceRI-β staining (Figs. 1, 2) showed a membranous staining pattern at the giant papillae. This staining pattern is consistent with our previous in vitro study using cultured human mast cells. FceRI-α staining of the same slide showed a broader expression at the conjunctiva than that of FceRI-β (Fig. 2C), and all the FceRI-α⁺ cells were FceRI-γ鲶 (Fig. 2F). These findings showed the existence of FceRI-αβγ鲶 mast cells and FceRI-αγ鲶 mast cells in the giant papillae. In our previous study, we quantified FceRI-β protein expression in human mast cells using flow cytometry. We found monophasic rather than biphasic FceRI-β expression pattern in human mast cells, and Western blotting showed that the sensitivity of the FceRI-β antibody is approximately 100 pg in the recombinant FceRI-β protein (Matsuda A, unpublished data, 2008). These results suggested that there are various levels of FceRI-β expression in each mast cell, and we identified an area of the mast cell population that expresses the FceRI-β protein past the threshold as FceRI-β-positive cells. We could not deny the possibility that FceRI-αβγ鲶 and FceRI-αγ鲶 receptor subtypes could be coexpressed in one mast cell. Nevertheless, we could conclude that there were at least two types of mast cells dominated by FceRI-αβ鲶 or by FceRI-α鲶 receptors. We further examined FceRI-β expression in the conjunctivae of two nonatopic conjunctival diseases as controls. We selected patients with conjunctivochalasis because relatively large upper bulbar conjunctival samples could be obtained at the time of surgery. We examined patients with SLK as controls for two reasons:

**FIGURE 5.** Localization of FceRI-β⁺ cells in convoluted epithelium and pseudotubules. Giant papillae with convoluted epithelium were immunostained with the anti-FceRI-β antibody (A, green). Asterisks indicate convoluted epithelium and pseudotubules. The staining around convoluted epithelium (B, C, E) and pseudotubules (D, F) is shown at higher magnification. Some slides were double immunostained with anti–tryptase (C, D, red), and FceRI-β was merged with them. Arrows indicate FceRI-β⁺ tryptase⁺ cells. Adjacent sections were double immunostained with anti–chymase (E, F, red), and FceRI-β was merged with them. Arrows indicate FceRI-β⁺ chymase⁺ cells. Original magnifications, 100× (A); 200× (B–F). This is representative data obtained from 1 of 10 patients (patient 2 in Table 1).

**FIGURE 6.** Expression of FceRI-β in basophils of giant papillae. Giant papillae specimens from a patient with AKC (patient 6 in Table 1) were immunostained with the anti–basophil antibody (A, arrows indicate areas of positive staining). The same sections were stained with anti-FceRI-β antibody and are shown in higher magnification (B). Double immunostaining using anti-FceRI-β and anti–basophil antibodies is shown in (C). FceRI-β immunostaining (green) was observed at the periphery of the anti–basophil-positive (red) cells. Original magnifications, 200× (A); 1000× (B, C).
immunostaining with anti–FcεR1-α antibody (B), and anti–CD1a antibody (C). Double immunostaining with the anti–FcεR1-β antibody and CD1a antibody (D) showed two CD1a/FcεR1-α double-positive cells (D; arrows). Triple immunostaining using CD1a (silver), FcεR1-α (red), and FcεR1-β (green) antibodies showed no existence of FcεR1-β/CD1a double-positive cells. CD1a+/FcεR1-α+/FcεR1-β− LCs and FcεR1-β+/FcεεR1-α− mast cells (E, arrows and arrowhead, respectively). Merged image using a differential contrast microscope (F). Original magnification, 400×.

This is the first study to show the existence of FcεR1-αγε− basophils (Fig. 6) and FcεR1-αγε+ LC (Fig. 7) at the protein level in giant papillae. Recently, it was reported that basophils play major roles in delayed-phase allergic reaction, independently of T cells and mast cells.29 We are now investigating the role of FcεR1-β− basophils in the pathophysiology of AKC/VKC. The existence of FcεR1-αγε− LC is consistent with previous findings in mRNA levels.30 and FcεR1-αγε− LCs play important roles for enhanced antigen presentation in atopic disorders.

Recent studies have also indicated that the role of the FcεR1-β protein in mast cells depends on the amount of IgE-specific antigen, with the low concentration of antigen FcεR1-β chain working as an amplifier31 but with a supraoptimal amount of antigen FcεR1-β chain acting as an inhibitory molecule for degranulation and cytokine expression.32,33 In this study we showed preferential FcεR1-β expression in the giant papillae samples in AKC/VKC, suggesting the involvement of an FcεR1-β-mediated mechanism for amplifying reactions against chronic low concentration of antigens. Additional functional studies will be necessary for investigating the roles of the FcεR1-β chain; this antibody will be a useful tool for in situ analysis.

Acknowledgments

The authors thank Hisako Takeshita for excellent technical assistance and John Bush for reviewing the manuscript.

References


