Immunohistological Expression of Interleukin 16 in Human Tonsils

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Background: Interleukin 16 (IL-16) acts highly chemotactic on CD4-bearing cells. Besides chemotaxis, IL-16 has numerous immunomodulatory effects, and not only on T cells.

Objective: To determine IL-16 expression in human tonsils.

Methods: Tonsillar follicles were immunohistologically characterized to elicit a possible cellular source of IL-16 expression.

Results: The mantle zone of immature and mature B cells was CD22 immunoreactive (ir), whereas the germinal center of activated B cells was CD23-ir. Plasma cells that were CD38-ir were observed extrafollicularly beneath the epithelium and within the germinal center. T cells were found most frequently in the extrafollicular space, with a majority of CD4 cells. CD68-ir macrophages were predominantly found within the germinal center. Immunostaining of anti–IL-16 revealed strong cytoplasmatic reactivity of extrafollicular cells and of cells at the outer rim of the mantle zone. Numerous cells adherent to the stratified squamous epithelium were IL-16-ir as well. Double immunostaining identified CD4+ T cells as the major cellular source of IL-16 expression. Furthermore, a population of CD22+ B cells at the outer rim of the mantle zone expressed IL-16 as well.

Conclusions: Interleukin 16 was mainly expressed in a typical CD4-like pattern in human tonsils. Our data strongly suggest that CD4+ lymphocytes constitute the major cellular source for IL-16. We hypothesize that the double-immunostained CD4+ and IL-16-ir cells represent activated T cells. Because CD22+ B cells at the outer rim of the mantle zone expressed IL-16 as well, we conclude that this area might constitute the locus of IL-16-mediated B-cell differentiation.


INTERLEUKIN 16 (IL-16) is synthesized as a precursor molecule of 68 kd (pro–IL-16) that is processed by caspase-3. The cleavage results in a 13-kd carboxy terminal peptide, which constitutes the bioactive form of IL-16.1 Interleukin 16, a proinflammatory and immunomodulatory cytokine formerly known as lymphocyte chemotactrant factor, acts highly chemotactic on CD4-bearing cells. Effects of IL-16 are transmitted by its surface receptor CD4.2

Effects of IL-16 on CD4+ T cells include chemotaxis, cell adhesion, induction of HLA-DR, induction of cytokine synthesis, and induction of IL-2 receptor expression (CD25).2-4 Furthermore, IL-16 is known as a CD4+ T-cell growth factor because it is capable of inducing a G0 to G1 cell cycle change.5 The cumulative effects of these functions on CD4+ T cells are increased CD4+ cell recruitment, priming for IL-2–responsive proliferation, and protection against Fas-mediated apoptosis.2,5

Besides affecting T cells, IL-16 also acts on other inflammatory cells. It is chemotactic on eosinophils and monocytes and induces HLA-DR expression in the latter.5 Furthermore, IL-16 might constitute an important mediator in cell-cell interactions of various inflammatory cells such as dendritic, mast, or B cells.5,8

Most work to date has focused on the chemotactic activities of IL-16 and therefore on diseases characterized by tissue infiltration of CD4+ cells. In individuals with atopic asthma, IL-16 represents a major source of lymphocyte chemotactic activity early after antigen challenge in which the major cell of origin is the epithelium, although mast cells, CD8 and CD4 cells, and eosinophils were described as additional sources.9 Kaser and coworkers9 on the other hand, suggested that activated T cells are a cellular source of IL-16 expression. Dendritic cells were described recently as another source of IL-16.6

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MATERIALS AND METHODS

Tonsils were obtained from 5 patients (mean ± SD age, 6.4 ± 1.4 years) undergoing routine tonsillectomy for recurrent tonsillitis without acute exacerbation. An allergic condition was ruled out by in vivo (skin test) and in vitro (Sx1 [identical to Phadiatop]; Pharmacia & Upjohn, Freiburg, Germany) tests to exclude possible effects of an atopic disease.

Tonsillar follicles were immunohistologically characterized to elicit a possible cellular source of IL-16 expression. B-cell differentiation and activation occur during migration from the outer mantle zone toward the germinal center. As a marker for immature and mature B cells, we chose anti-CD22, as described previously. Expression of CD23 in B cells is associated with immunoglobulin isotype switching. Therefore, CD23+ B cells are generally accepted to constitute activated B cells. Anti-CD38 was taken for detection of plasma cells. T-cell populations were detected by anti-CD3 (pan T-cell marker), and T-cell subpopulations were identified by anti-CD4 and anti-CD8. For detection of macrophages we chose anti-CD68 (clone KP1), as described elsewhere.

IMMUNOHISTOCHEMISTRY

Specimens were shock frozen in liquid nitrogen and stored at −20°C. Cryostat sections (5 μm) were postfixed with acetone for 10 minutes. Thereafter, sections were rinsed in 0.05M phosphate-buffered saline solution (pH 7.4). Endogenous peroxidases were blocked by incubation in 0.3% hydrogen peroxide for 30 minutes. Nonspecific binding was minimized by incubation with fetal calf serum (1:20) for 30 minutes. The avidin-biotin-peroxidase complex method was used for detection of immunostaining, as described elsewhere. The avidin-biotin-peroxidase complex method was used for detection of plasma cells.19 T-cell populations were identified by anti-CD4 and anti-CD8. For detection of macrophages we chose anti-CD68 (clone KP1), as described elsewhere.

Interleukin 16 is released from CD4+ T cells in response to antigen, mitogen, histamine, or anti-CD3 stimulation. Interleukin 16 messenger RNA and pro–IL-16 are constitutively expressed by CD4+ and CD8+ T cells. But different from CD8+ T cells, processing and release of bioactive IL-16 by CD4+ T cells is activation dependent.1 An increased level of IL-16 after challenge has been described in asthma10 and in allergic rhinitis,11 in which IL-16 expression correlated with CD4 influx. Recently, Kramer et al12 identified IL-16 as a characteristic cytokine expressed in late-phase response after antigen challenge in allergic rhinitis. Increased expression of IL-16 was demonstrated in other chronic inflammatory processes characterized by CD4+ T-cell influx, such as atopic dermatitis, or granulomatous inflammation, such as sarcoidosis or tuberculosis,2,3 multiple sclerosis,4 and acquired immunodeficiency syndrome.5 Even autoimmune diseases such as systemic lupus erythematosus are characterized by increased levels of IL-16, which correlate with disease activity.6

To our knowledge, IL-16 expression by tonsillar tissue has not been studied to date. Tonsillar follicles belong to the organized mucosa-associated lymphoid tissue of the Waldeyer ring. Their B-cell and T-cell areas are the locus of lymphocyte differentiation and initiation of mucosal immune responses. Intense B-cell–T-cell interactions take place, suggesting a relevant role for IL-16 here.

Tonsils are locations of intense cell-cell interactions while promoting immune responses. As described in the previous paragraphs, IL-16 has far more immunomodulatory effects on various inflammatory cells than chemotaxis.

The aim of this study was to determine IL-16 expression in human tonsils. Tonsillar follicles were immunohistologically characterized to elicit a possible cellular source of IL-16 expression.

RESULTS

B-CELL AREA

The mantle zone of immature and mature B cells was CD22 immunoreactive (ir), whereas the germinal center of activated B cells was CD23-ir. CD38-ir plasma cells were observed extrafollicularly beneath the epithelium and within the germinal center (Figures 1, 2, and 3).
T-CELL AREA

T cells (CD3⁺) were found most frequently in the extrafollicular space, with a majority of CD4 cells. CD4-ir lymphocytes were observed in the mantle zone of the follicle too, whereas CD4-ir cells were only occasionally found in the germinal center (Figures 4, 5, and 6).
CD68-ir macrophages were inhomogeneously distributed within the tonsillar tissue, with a predominance in the germinal center (Figure 7).

EPITHELIUM

Invaginated stratified squamous epithelium formed tonsillar crypts. Diapedesis of the epithelium by CD4-ir and CD8-ir cells was observed. Cells adherent to the epithelium were CD4-ir and could be detected regularly.

IL-16 IMMUNOSTAINING

Immunostaining of anti–IL-16 revealed strong cytoplasmic reactivity of extrafollicular cells and of cells at the outer rim of the neighboring mantle zone. Only a few cells within the germinal center were IL-16-ir (Figure 8). Several cells adherent to the stratified squamous epithelium were IL-16-ir as well.

DOUBLE STAINING

Double immunostaining identified CD4+ T cells as the major cellular source of IL-16 expression. Most IL-16-ir cells were also CD4-ir, but not all CD4-immunoreactive T cells express IL-16. Furthermore, a population of CD22+ B cells at the outer rim of the mantle zone expressed IL-16 as well (Figures 9, 10, and 11).

COMMENT

Immunohistological characterization of human tonsillar follicles displayed the well-known structure of secondary lymph follicles: The mantle zone of immature and mature B cells was CD22-ir, whereas the germinal center of activated B cells was CD23-ir. The precursors of germinal center B cells are believed to be mantle B lymphocytes. Germinal centers represent antigen-dependent
B-cell compartments responsible for proliferative expansion of memory clones and differentiation to immunoglobulin-producing immunocytes. CD38-ir plasma cells were observed extrafollicularly beneath the epithelium and within the germinal center. T cells were found most frequently in the extrafollicular space. CD4+ cells were dominant. CD4-ir lymphocytes were observed in the mantle zone of the follicle too, whereas CD4-ir cells were only occasionally found in the germinal center. CD68-ir macrophages were inhomogeneously distributed within the tonsillar tissue, with a predominance in the germinal center. All this is in accordance with results of other researchers who described human tonsillar follicles using immunohistochemical analysis.

Immunostaining of anti-IL-16 revealed strong cytoplasmatic reactivity of extrafollicular cells and of cells at the outer rim of the neighboring mantle zone. Only a few cells within the germinal center were IL-16-ir. Several cells adherent to the stratified squamous epithelium were IL-16-ir too. Double immunostaining identified CD4+ T cells as the major cellular source of IL-16 expression. Most IL-16-ir cells were also CD4-ir, but not all CD4-ir T cells expressed IL-16. Furthermore, a population of CD22+ B cells at the outer rim of the mantle zone expressed IL-16 as well. This immunohistological distribution of IL-16-ir CD4+ T cells and IL-16-ir CD22+ B cells suggests that the outer rim of the mantle zone constitutes the location of IL-16-mediated B-cell differentiation.

Immunohistological examination of IL-16 expression in human lymphoid organs other than tonsils revealed similar results: examination of human lymph nodes showed that IL-16 is immunohistologically expressed in lymphoctic cytoplasm within T-cell zones extrafollicularly and only occasionally in lymphocytes of the germinal center. Interleukin 16 was mainly expressed in a typical CD4-like pattern in human tonsils. Unlike atopic airways, our data strongly suggest that CD4+ lymphocytes constitute the major cellular source of IL-16 in human tonsils. Furthermore, and unlike atopic airways, CD8+ T cells, the epithelium, or macrophages did not express IL-16, at least not by means of immunohistochemistry. In addition, we found IL-16-ir CD22+ B cells. These B cells might contribute to the observed IL-16 expression as well.

Double immunostaining clearly identified CD4+ cells as the major cellular source of IL-16 expression. Comparison with anti-CD3 staining led us to believe that most of these CD4+ cells are T cells. However, CD4-bearing cells other than lymphocytes under this IL-16-ir cell population cannot be excluded in the end but seem unlikely to be of relevant amounts.

Tonsillar T cells are suggested to enter the extrafollicular tonsillar area through so-called postcapillary or high-endothelial venules from the blood. Their extravasation and chemottractant are not understood. αβ-Leukointegrins are suggested to constitute selective adhesion receptors. Our data suggest chemotactic effects of IL-16 on these CD4+ T cells.

As described earlier in this article, IL-16 has far more immunomodulatory effects on various inflammatory cells than chemotaxis. Interleukin 16 further promotes cell adhesion, HLA-DR induction, and induction of cytokine synthesis on T cells and acts as a T-cell growth factor. Induction of IL-2 receptor expression (CD25) is mediated by IL-16 as well, leading to a synergistic activation of CD4+ T cells by IL-16 and IL-2. The cumulative effects of these functions on CD4+ T cells are increased CD4+ cell recruitment and priming for IL-2-responsive proliferation. Tonsillar T lymphocytes have striking peculiarities: most are activated, express IL-2 receptor (anti-Tac positive), and respond to IL-2 stimulation. Furthermore, most of these activated T lymphocytes are T-helper cells and are localized mainly extrafollicularly. Results of in vitro studies have indicated that these IL-2-activated T cells are involved in differentiation of tonsillar B cells to plasma cells, which is indirectly supported by our data. Furthermore, tonsillar B and T cells are characterized by more frequent DR expression compared with blood lymphocytes. The abundant intrafollicular DR expression (B cells, T cells, dendritic cells, etc) probably serves to modulate local interactions between antigen-presenting cells, T cells, and B cells. Taken together, our data point to IL-16 as a relevant factor in the immune response of human tonsils. Different from CD8+ T cells, processing and release of bioactive IL-16 by CD4+ T cells is activation dependent. Therefore, our data might be interpreted as showing that the observed IL-16-ir and CD4-ir cells constitute activated T cells. Triple staining with IL-2 receptor promises to be a challenging but interesting target for a future study.

Besides affecting T cells, IL-16 acts on other inflammatory cells too. It is chemotactic for eosinophils and monocytes and induces HLA-DR expression in the latter. Furthermore, IL-16 constitutes an important mediator in cell-cell interactions of various inflammatory cells: T cells and mast cells interact bidirectionally in various aspects of immune response, in which IL-16 plays an important role, and T-cell–dendritic cell interactions also depend on IL-16. Interleukin 16 is important
in B-cell–T-cell interactions as well. For instance, B-cell precursors need IL-16 for their maturation, which again is supported by our finding of IL-16-ir CD22+ B cells. All these interactions are pivotal parts in initiating and promoting an immune response and point to IL-16 as a relevant factor.

CONCLUSIONS

Interleukin 16 is mainly expressed in a typical CD4-like pattern in human tonsils. Our data strongly suggest that CD4+ lymphocytes constitute the major cellular source for IL-16. We hypothesize that the double-immuno-stained CD4+ and IL-16-ir cells represent activated T cells. CD22+ B cells at the outer rim of the mantle zone expressed IL-16 as well, leading us to conclude that this area might constitute a locus of IL-16–mediated B-cell differentiation.

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