Human tonsil intraepithelial B cells: a marginal zone-related subpopulation

M Morente, M A Piris, J L Orradre, C Rivas, R Villuendas

Abstract

Aims: To determine if intraepithelial B cells in reactive human palatine tonsils were similar to the marginal zone cells of the spleen and Peyer’s patches.

Methods: Reactive human palatine tonsils were studied using conventional methods of light microscopy, electron microscopy, and a panel of monoclonal antibodies for lymphocyte common antigens.

Results: Clinically important numbers of marginal zone-related B cells around the mantle zone were absent in lymphoid follicles, but in the cryptal epithelium there were abundant lymphoid cells with centrocyte-like nuclei and clear cytoplasm, intermingled with macrophages and plasma cells. The immunophenotype of these intraepithelial B cells was distinctive and similar to that found in the splenic marginal zone cells (IgM+, IgD−, CD23−, CD10−, CD35+ or CD21+, bcl2+, OKB7+).

Conclusions: Intraepithelial B cells in human tonsil could represent the counterpart of the marginal zone described in Peyer’s patches. Their presence within the epithelium could reflect the destination for the malignant B cells in the lymphoepithelial lesion of mucosa associated lymphoid tissue (MALT) lymphomas.

Marginal zone B cells have been widely studied in splenic tissues. They are composed of perifollicular non-recirculating lymphocytes of clear medium sized cytoplasm and round or oval nuclei, which characteristically express IgM on their surface with little or no IgD. Cells of similar morphology and immunophenotype can also be found in lymph nodes and, mainly, in mucosa associated lymphoid tissue (MALT).

In the lymph node these cells occur in the periphery of the follicular mantle zone, but this does not form a distinct zone, except for occasional reactive mesenteric lymph nodes. In several instances, such as toxoplasmonic lymphadenitis and the lymphadenopathic period of HIV infections, marginal zone derived B cells form a distinct band at the periphery of the mantle zone and parasinusoid spaces. In these cases the cells take on a monocytoid appearance and are designated “monocytoid B cells”.

In MALT marginal zone is represented by the so-called “mixed cell zone.” These extrafollicular B cells are further distinguished by

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GC: Germainal centre.
and hairy cell leukaemia has been raised by Burke and Sheibani.20

The function of splenic marginal zone cells and equivalent areas of other secondary lymphoid organs is still not fully known. A primary function attributed to marginal zone cells is their role in the primary response to thymus independent antigens type 2.21

In the search for an analogy to the marginal zone we studied several reactive human palatine tonsils. The choice was based on the peculiarities of the organ: submucous situation, conspicuous development of lymphoid follicles without a distinct marginal zone, and notable presence of lymphoid cells within crypt epithelium. Because of the morphological similarities between tonsils and Peyer’s patches, we started to compare the morphology and immunophenotype of the intraepithelial B cell subset of tonsils with both benign and malignant marginal zone from MALT and spleen.

Methods

Fresh, human palatine tonsils were obtained from routine surgical specimens, corresponding to 10 cases of recurrent tonsillitis. Immediately after tonsillectomy one half of each specimen was processed for routine histopathological examination, fixed in B-5, embedded in paraffin wax, cut and stained with Giemsa and haematoxylin and eosin according to standard procedures. Part of each specimen was embedded on OCT compound (Miles Laboratory Inc, Naperville, Illinois, USA) and snap frozen in liquid nitrogen and stored at −70°C until use.

Samples of tissue fixed in 2-5% glutaraldehyde, cacodylate buffer (pH 7.4), postfixed in 2% osmium tetroxide, and embedded in Vestopal-W resin were studied ultrastructurally. Sections were stained with lead citrate and uranyl acetate and examined under a Hitachi HU-12A electron microscope.

Representative blocks of snap-frozen tissue from spleen, Peyer’s patches, and lymph node, some of them from toxoplastic lymphadenitis with abundant monocytoid B cells, were studied to characterise B cell compartments.

Paraffin wax sections and cryostat sections were stained with alkaline phosphatase-anti-alkaline phosphatase (APAAP).22 Monoclonal antibodies were incubated at appropriate dilutions that had been determined in preliminary experiments. A humidity chamber at room temperature was used. The monoclonal and polyclonal antibodies used are listed in table 1.

Briefly, 4 μm sections were thawed to room temperature and fixed for 10 minutes in acetone and 30 minutes in chloroform. Primary monoclonal antibodies were incubated for 30 minutes. Sections were overlaid with antimouse immunoglobulins (Dako, Denmark) and APAAP complex (Dako, Denmark) for 30 minutes each step. The colour reaction was developed with alkaline phosphatase substrate and counterstained with haematoxylin. Brief TRIS-buffered saline washes were carried out between each step.

Figure 1 Intraepithelial lymphoid cells: (A) Nests of lymphocytes within the epithelium. (Giemsa) (B) Lymphocytes (short arrows) and plasma cells (long arrow) situated above basal membrane (Giemsa.)

their size and irregular heterochromatic nuclei which resemble those of centrocytes. These centrocytic-like extrafollicular B cells are invariably present in the dome epithelium over the lymphoid follicle.

Low grade B cell lymphomas of MALT are derived from these extrafollicular subsets in the gastrointestinal tract,9 thyroid,10 salivary gland,11 lung,12 13 thymus,24 and probably breast.13 Monocytoid B cell lymphomas have been reported in lymph nodes,14,15 but in some cases they could derive from MALT. The possible association between marginal zone
Electron micrograph showing criptal epithelium (E) with interspersed lymphocytes.

Results

Light microscopic findings
Two main epithelial patterns were found: the surface epithelium exhibited a compact pattern with occasional infiltrating lymphocytes; on the other hand, epithelium in the neck and deep portions of the cript displayed a network pattern with abundant intraepithelial lymphocytes, granulocytes, and macrophages (fig 1). Intraepithelial lymphocytes were observed in nests, haphazardly arranged along the epithelium and surrounded by an empty halo caused by artefactual retraction. The lymphocytes had medium sized clear cytoplasm and irregularly outlined nuclei with fine chromatin and occasional small nucleoli, resembling centrocyte-like cells.

Electron microscopic findings
Epithelial cells appeared as large elements, containing electron dense mitochondria, peripherally situated to filament bundles, and some vacuoles (fig 2). Intraepithelial cells were recognised as lymphocytes, macrophages with tigible bodies, and granulocytes, mainly neutrophils. A spectrum of lymphoid cells was evident ultrastructurally (fig 3).

Centrocyte-like cells were medium sized lymphoid cells with oval and irregular nuclei with some indentations, marginal dense chromatin, and small single nucleoli. The cytoplasm contained few organelles, such as ribosomes, mitochondria and different degrees of development of rough endoplasmic reticulum.

There were some larger activated lymphoid cells with the same characteristics as those described above. The nuclei, however, were irregularly shaped and clearer because of diffuse chromatin distribution. Hyperplastic nucleoli and a well developed rough endoplasmic reticulum were also observed.

Plasma cells in different degrees of development were observed.

Immunohistological findings
In the neck and deep portions of the crypt interconnected epithelial cells (PKI positive) constitute a network of star-shaped cells which surround cavities filled with infiltrating lymphocytes. Immunostained intraepithelial lymphocytes in paraffin wax and frozen sections showed a similar pattern of reactivity in all the specimens studied. Surface epithelium contained T cells that were predominantly CD4 positive; in the crypt epithelium monocytes (1–5%), T cells (10–20%), and mainly B cells (80–90%) were present (fig 4).

A percentage of B cells seem to carry surface immunoglobulin, mainly IgM (70%) and IgD (20%). The immunophenotype of these intraepithelial B cells was HLA-DR, CD19, CD21, CD22, CDw32, CD35, L26, Leu18 and bcl-2 positive (fig 5), but negative for CD10 and CD23. Intraepithelial plasma cells contain mainly intracytoplasmatic IgG, but subepithelial plasma cells are mainly IgA positive. The comparative immunophenotype of B cells is summarised in table 2.

Some intraepithelial cells have antigenic determinants recognisable by monoclonal antibodies directed against T cells (CD2, CD3,
CD5, TCRδ (40%) and HML1 (20%) positive), with a CD4:CD8 ratio of 2:1. A small percentage of the total number of intraepithelial cells reacted against anti-IL2r monoclonal antibody, but were CD30 negative. Only occasional proliferating cells, defined by Ki67, were identified.

**Discussion**

A relatively independent mucosal immune system exists together with a systemic immune system. This mucosa-associated lymphoid tis-

**Figure 3** Electron micrograph showing intraepithelial centrocyte-like cells (A) (arrows) and plasma cells (B) (arrowheads).
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Figure 4 Intrathelial lymphoid cells are mainly B cells (A and C), with scattered T cells (B) and macrophages (D) (A L26; B CDS3; C GD19; D CDS68).

Figure 5 Intrathelial B cells express CD35 (A and CD21 (B). They are mainly IgM+ (D) but only scattered IgD+ cells are present (C). (A) CR1; B OKB7; C anti-IgD D anti-IgM.

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