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 leucocyte 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+, CD21+, bcl2+, KB61+).

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 lympho-epithelial lesion of mucosa associated lymphoid tissue (MALT) lymphomas.

Human palatine tonsil lymphoid tissue has morphological, immunophenotypic, and pathological features similar to those of MALT.

Marginal zone B cells have been widely studied in splenic tissues.¹⁻³ They are composed of perfollicular 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^{3,4} and, mainly, in mucosa associated lymphoid tissue (MALT).^{5,6}

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 toxoplasmic 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 parasinusoidal spaces. In these cases the cells take on a monocytoid appearance and are designated "monocytoid B cells".^{7,8}

In MALT marginal zone is represented by the so-called "mixed cell zone."⁵ These extra-follicular B cells are further distinguished by

Table 1 Polyclonal and monoclonal antibodies used

CD	Antibody	Specificity	Source
	Anti-Ig	B lymphocytes, monoclonal	Dako
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	GRB1	HLA-DR	F Garrido (Granada, Spain)
19	Leu12a	Pan-B	Becton-Dickinson
21	OKB7	B subsets	Ortho
22	Leu14	B subsets	Becton-Dickinson
23	Tu1	B subsets	Clonab
38	GR7A4	Plasma cells, GC B cells	F Garrido (Granada, Spain)
	Leu8	Peripheral T and B cells	Becton Dickinson
10	CALLA	GC B cells	Ortho
	bcl.2	B cells other than GC cells and small T cells	DY Mason (Oxford)
w32	KB61	B cell subsets, macrophages other than GC cells	DY Mason (Oxford)
35	CRI	C3bR, monocytes, B cells	Serotec
25	IL2r	Activated T and B cells	T-cell sciences
30	BERH2	Activated T and B cells. RS cells.	Dako
	Ki67	Proliferating cells	Dako
	HML-1	Intraepithelial T cells	Immunotech
11c	p150/95	Monocytes	Dako
	DRC	Follicular dendritic cells	Dako
1a	T6	IDC, Langerhans' cells	Ortho
5	T1	T cells	Dako
2	Leu5	T cells	Becton Dickinson
3	Leu4	T cells	Becton Dickinson
	TCR-delta	T cell subsets	T-cell sciences
45	ALC	Leucocytes	Dako
	L26	B subsets	Dako
45RO	UCHL1	T subsets	Dako
45RA	Leu18	B and T subsets	Becton Dickinson
43	FT1	T subsets	DY Mason (Oxford)
68	KP1	Macrophages	DY Mason (Oxford)
	PKK1	Keratins	Labssystem

GC: Germinal centre.

Department of Pathology, Hospital General de Guadalajara, Universidad de Alcalá de Henares, Spain
M Morente

Department of Pathology, Hospital Virgen de la Salud, Toledo, Spain
M A Piris
J L Orradre
R Villuendas

Department of Pathology, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Spain
C Rivas

Correspondence to:
Dr M Morente, Servicio de Anatomía Patológica, Hospital General de Guadalajara, C/Donante de sangre s/n 19002 Guadalajara, Spain

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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,^{6,9} thyroid,¹⁰ salivary gland,¹¹ lung,^{12,13} thymus,¹⁴ and probably breast.¹⁵ Monocytoid B cell lymphomas have been reported in lymph nodes,¹⁶⁻¹⁹ but in some cases they could derive from MALT. The possible association between marginal zone

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 toxoplasmic 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 anti-mouse 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 2 Electron micrograph showing cryptal 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 crypts 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 tingible 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 (PKK1 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 CIGa positive. The comparative immunphenotype of B cells is summarised in table 2.

Some intraepithelial cells have antigenic determinants recognisable by monoclonal antibodies directed against T cells (CD2, CD3,

Table 2 Reactivity of tonsilar intraepithelial B lymphocytes, compared with other B cell subpopulations

Monoclonal antibody	Marginal zone	Monocytoid B lymphocytes	Follicular mantle zone	Germinal centre	Plasma cells	Tonsilar intraepithelial B lymphocytes
SigM	+	+	+	+	–	+
SigD	–	–	+	–	–	–/+
CD19	+	+	+	+	–	+
CD21	+	–	+	+	–	+/-
CD22	+	+	+	-/+	–	+
CD23	–	–	+	–	–	–
CD38	–	-/+	–	+	+	-/+
BCL2	+	-/+	+	–	+	+
LEU18	–	–	+	–	–	+/-
CD10	–	–	–	+	–	–
CD35	+	–	+	-/+	–	+/-
HLADR	+	+	+	+	–	+
CDw32	+	+	+	–	+	+

Figure 3 Electron micrograph showing intraepithelial centroyte-like cells (A) (arrows) and plasma cells (B) (arrowheads).

CD5, TCR δ (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-

sue (MALT) may be an essential component in some organs—for example, in the mucosa of bronchi and, mainly, intestine—or acquired as a result of an autoimmune disorder as in the stomach, thyroid, or salivary glands.²³

Morphologically, the most distinctive feature of MALT is the presence of a perifollicular rim of B cells with local epithelial tropism and noticeable immunophenotypic similarities with the B cells of the splenic marginal zone. These extrafollicular B cells are further distinguished by their size and nuclear morphology. They are larger than mantle zone cells and contain an irregular nuclear outline resembling that of centroytes.²³ Their similar lineage to monocytoid B cells has been pointed out by several authors,^{17 19 24} mainly in connection with “marginal-zone related B cell lymphomas.”²⁵

The organisation of human tonsil lymphoid tissue is distinctive and differs from that of peripheral lymph nodes, resembling those of MALT structures in the following ways: (1) absence of sinuses; (2) presence of intraepithelial macrophages presumably involved in antigen transport, like the mucosal cells of the intestinal epithelium; (3) intraepithelial lymphoid infiltrate, mainly B cells, contiguous with the marginal zone.

Characterisation of the intraepithelial B cells within the crypts of the human tonsils, shows a well defined morphological and immunophenotypical association with marginal zone. Thus follicle centre B cells (which are bcl.2–, CD10+, KB61–) and follicle mantle B cells (which are IgD+, CD23+) differ from the tonsillar intraepithelial B lymphocytes.

Partial loss of CD21 and CD35 antigens in some intraepithelial B cells may indicate the presence of activated cells with or without monocytoid morphology.

Tonsillar intraepithelial B lymphocyte localisation is reminiscent of the B cells in the dome epithelium overlying follicle centres of Peyer’s patches,⁵ intestinal epithelium in cases of florid reactive lymphoid hyperplasia of the terminal ileum,²⁶ and of epithermotropism of the B cells in low grade B-MALT lymphomas.^{23 24} Furthermore, lymphomas of Waldeyer’s ring seem to share some characteristics of MALT lymphomas and tend to invade the epithelium.²⁷ The classic lymphoepithelial lesions were not seen, however, perhaps reflecting the lack of glands in the tonsil. An association between the lymphoid tissue of Waldeyer’s ring and gastrointestinal MALT is also strengthened by the increased risk of secondary disease in the tonsil in patients with gastrointestinal lymphoma,²⁸ and the frequent relapses of Waldeyer’s ring lymphomas in the gastrointestinal tract²⁹ or salivary glands.²⁷ The epithelial tropism of this subpopulation may help to explain the epithelial infiltration of the tumours derived from the marginal zone cells in MALT, such as the low grade B cell lymphoma in the stomach, salivary gland, and thyroid.

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Figure 4 Intraepithelial lymphoid cells are mainly B cells (A and C), with scattered T cells (B) and macrophages (D) (A L26; B CD3; C CD19; D CD68).

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

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