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The human gut contains a novel population of B lymphocytes which resemble marginal zone cells

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SUMMARY

B cells in normal human Peyer's patches and in primary B cell lymphomas of the stomach have been characterized in terms of their cellular morphology and their reactivity with a panel of monoclonal antibodies. A population of B cells is present in normal and malignant gut-associated lymphoid tissue which is composed of neither mantle zone cells nor germinal centre cells. In Peyer's patches these cells surround the follicles merging with the mantle zone and extending both into the dome region, infiltrating between the epithelial cells and also towards the serosa. They are intermediate in size with irregular nuclear outlines and they resemble the centrocytes in the follicle centre. They are quiescent, expressing C3b- and C3d-receptors and surface IgM but not surface IgD. These centrocyte-like cells which are not seen in the peripheral lymph nodes are identical to the B cells in the marginal zone of the spleen according to all of the criteria employed in this study.

Keywords B lymphocytes marginal zone cells human gut Peyer's patches

INTRODUCTION

B cell follicles in normal human lymphoid tissue are composed of germinal centre and mantle zone lymphocytes which are morphologically distinct B cell populations. The latter appears to correspond to circulating virgin B cells which express surface IgM and IgD, but the precise position of germinal centre cells in postulated schemes of B cell maturation and their developmental relationship to cells such as immunoblasts, plasma cells and memory cells is unclear. Previous studies have indicated the presence of extrafollicular B cells in human gut-associated lymphoid tissue (GALT). These cells are distinct from the mantle zone lymphocytes in that they morphologically resemble germinal centre cells (centrocytes), they lack surface IgD and they invade the epithelium over the dome regions of the lymphoid follicles (Spencer, Finn & Isaacson, 1985). Cells with these characteristics have also been observed in primary B cell lymphomas of the mucosae (Isaacson & Wright, 1984). We have studied and compared normal and neoplastic GALT using morphological and immunocytochemical methods aiming to define these cells phenotypically and then to consider them in the context of B cell ontogeny and their role in the immunological protection of mucosal surfaces.

MATERIALS AND METHODS

Tissue collection and processing. Terminal ileum was collected from four fresh right hemicolec-

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tomy specimens which were resected at laparotomy for carcinoma of the colon. Specimens of macroscopically normal ileum were fixed in 10% formalin containing 2% acetic acid (Curran & Gregory, 1980) and routinely processed and embedded for paraffin sections. Tissue was also snap frozen in liquid nitrogen for cryostat sections. Sections of terminal ileum were stained using haematoxylin and eosin for study of cellular morphology and frozen blocks that contained Peyer's patch follicles were selected for immunohistochemistry.

Eight cases of primary gastric lymphoma of the stomach were collected and specimens of macroscopically involved mucosa were fixed and processed as described above and also snap frozen in liquid nitrogen for cryostat sections. Suitable blocks for immunohistochemistry were selected following the examination of sections stained with haematoxylin and eosin.

Snap frozen specimens of normal tonsil, lymph-node and spleen were immunostained in parallel with the specimens of normal and malignant GALT.

Immunohistochemistry. The monoclonal antibodies used in this study, their source and reactivity are shown in Table 1. All immunohistochemistry was carried out on 6 μm cryostat sections. Precise details of the methods used have been published elsewhere (Isaacson & Wright, 1983). Briefly, sections were frozen overnight in a box containing silica gel then brought to room temperature and fixed in fresh acetone for 30 min. Following incubation with appropriately diluted monoclonal antibody for 1 hr, a rabbit anti-mouse secondary antibody conjugated to peroxidase (Dakopatts, High Wycombe, Buckinghamshire, UK) was applied to the sections. Peroxidase activity was visualized using the 3,3-diaminobenzidine reagent (Graham & Karnovsky, 1966). Sections were counterstained with haematoxylin.

Table 1. Sources and specificities of antibodies used in this study

Antibody	Source	Specificity
IgM	Coulter clone	μ-chains
IgD	Coulter clone	δ-chains
B_1	Coulter clone	B cells (anti-CD20) (Stashenko *et al.*, 1980)
KB61	*	B cells other than follicle centre cells (Pulford *et al.*, 1985)
MHM6	†	Activated B cells (Anti-CD23) (Rowe *et al.*, 1982)
E11	‡	C_3b-receptors (Hogg *et al.*, 1984)
B_2	Coulter clone	C_3d-receptors (Nadler *et al.*, 1981)
Ki67	§	Proliferating cells (Gerdes *et al.*, 1984)
leu-1	Beckton Dickinson	T cells and a small subset of normal B cells (anti-CD5)
HB61	¶	κ light chains
M15	¶	γ light chains

Antisera kindly provided by:

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† Professor A. J. McMichael, Department of Surgery, John Radcliffe Hospital, Oxford, UK.

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RESULTS

Peyer's patches

Morphology. The germinal centres of the Peyer's patch follicles were surrounded by a mantle zone of small lymphocytes. The mantle was continuous with a population of slightly larger cells with more cytoplasm and irregular nuclear outlines, resembling the centrocytes in the germinal centre. These cells have been described in detail in appendix tissue (Spencer, Finn & Isaacson, 1985) but they were much more numerous in the Peyer's patches from terminal ileum. They surrounded the follicle extending into the dome region and infiltrating between the epithelial cells. On the serosal aspect of the follicle they merged with the zone of cells containing the high endothelial venules.

Immunohistochemistry. The result of immunostaining serial cryostat sections of Peyer's patches is shown in Table 2. The anti-B cell antibody B1 stained cells in the germinal centres and also an abundance of cells extending from the germinal centres both into the dome region of the Peyer's patches, between the epithelial cells and towards the serosa. These cells outside the germinal centre were also recognized by KB61. The mantle zone of cells expressing surface IgD was diminutive in comparison to the total number of B cells surrounding the germinal centre (Fig. 1). Surface IgD negative B cells which surrounded the mantle zone had the same distribution as the centrocyte-like cells observed in paraffin sections. Most of these cells appeared to express surface IgM (Fig. 1), C3b- and C3d-receptors. They lacked the antigens associated with proliferating cells recognized by MHM6 and Ki67.

Gastric lymphoma

Morphology. The histopathology of the cases of gastric lymphoma studied was consistent with that described by Isaacson and Wright (1984), characteristic of primary lymphomas of the mucosa-associated lymphoid tissue. Neoplastic germinal centres were accompanied by a diffuse infiltrate of cells with the appearance of centrocytes, which extended towards the mucosal surface and invaded the glandular epithelium, producing characteristic lymphoepithelial lesions (Issacson & Wright, 1984). The centrocyte-like cells differed from the centrocytes in the follicle centre in that they did not form follicular structures and did not contain a population of centroblasts

Immunohistochemistry. In individual cases the neoplastic germinal centre cells and the population of centrocyte-like cells expressed the same light chain type. The phenotypes of the malignant germinal centre cells and the centrocyte-like cells are shown in Table 2. The latter have very similar phenotype to the centrocyte-like cells in the Peyer's patches.

Table 2. Immunostaining characteristics of B cell populations studied

Antibody	Normal and malignant germinal centres	Mantle zone	Centrocyte-like cells in normal Peyer's patches	Malignant centrocyte-like cells	Splenic marginal zones
IgM	+/−	+	+	+	+
IgD	−	+	−	−	−
B_1	+	+	+	+	+
KB61	−	+	+	+	+
MHM6	+	−	−	−	−
E_{II}	+/−*	+	+	2/8	+
B_2	+/−*	+	+	1/8	+
Ki67	+	−	−	−	−
Leu-1	−	−	−	−	−

* Observed positivity may be due to the C_3b- and C_3d-receptors on dendritic reticulum cells.

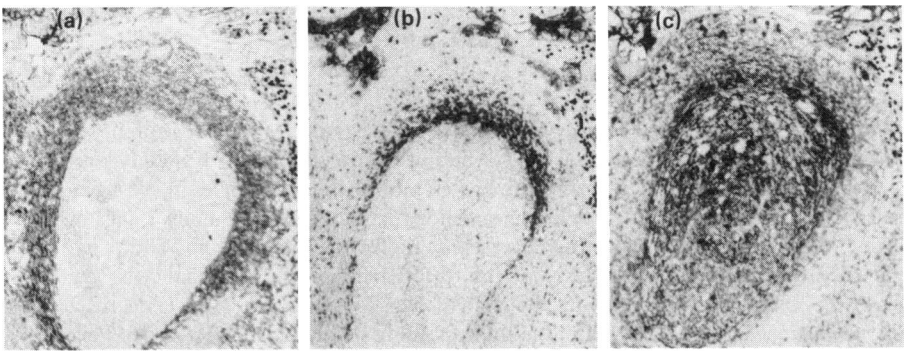

Fig. 1. Serial frozen sections of a lymphoid follicle from terminal ileum immunostained using monoclonal antibodies KB61 (a), anti-IgD (b) and anti-IgM (c). B cells identified byKB61, some of which express IgM, surround the mantle zone of IgD bearing cells. Immunoperoxidase, × 100.

Tonsil, Spleen and Lymph node

We have analysed samples of normal lymphoid tissue from other sites in the human body for cells possessing similar characteristics to those of the centrocyte-like population identified in normal and neoplastic gut-associated lymphoid tissue. An essentially identical morphological and phenotypic profile is shown by the marginal zone cells in the human spleen (Table 2). Some cells with this phenotype were also seen immediately beneath the sub-capsular sinus in the mesenteric lymph nodes.

DISCUSSION

The B cells that we have described which surround the follicular mantle of IgD bearing cells in Peyer's patches have the same distribution as the intermediate sized cells with irregularly shaped heterochromatic nuclei observed in paraffin sections, and they are almost certainly equivalent. These cells can be distinguished phenotypically from mantle zone lymphocytes because they do not express IgD and germinal centre cells because they do not express the antigen recognized by MHM6. The centrocyte-like cells in primary B cell gastric lymphoma share phenotypic characteristics with centrocyte-like cells in normal GALT and also surround the mantle zone and disrupt the mucosal epithelium. Neoplastic centrocyte-like cells with the phenotypic features described have been observed only in lymphomas of mucosa-associated lymphoid tissue and not in tumours of peripheral lymph nodes. We feel that these cells are the malignant equivalent of the centrocyte-like cells which surround the lymphoid follicles in normal human GALT.

In the gastric lymphomas studied, the malignant germinal centre cells and the centrocyte-like cells show the same light chain restriction suggesting that despite their contrasting morphology and phenotype they are part of the same neoplastic clone. The pattern of reactivity of the tumour cells with the monoclonal antibody Ki67 which recognizes an antigen on proliferating cells suggests that the centrocyte-like cells have a low proliferation rate. This conforms with their lack of reactivity with the antibody MHM6 which again suggests that they are quiescent. The direction of cellular maturation within the tumours therefore appears to be from malignant germinal centre cell to centrocyte-like cell. This implies that the centrocyte-like cells in normal Peyer's patches are also the direct progeny of germinal centre cells. There is experimental support for this in the work of Faulk *et al.* (1971), who pulse labelled rabbit Peyer's patch cells with radioactive thymidine. Labelled cells were present within the follicles if the animals were killed after 1 h but had moved to sites in the dome region and between the epithelial cells when animals were killed at 24 h.

We have analysed samples of normal lymphoid tissue from other sites in the human body for cells possessing similar characteristics to those of the centrocyte-like population observed in normal and neoplastic GALT. An essentially identical morphological and phenotypic profile is shown by

the marginal zone cells in the spleen. Investigation of these cells in rats has shown them to be non-recirculating B cells which are able to respond to T-independent carbohydrate antigens, but their exact relationship to germinal centre cells, antibody producing cells and memory cells is as yet unclear (Kumaratratne, Bazin & MacLennan, 1981; MacLennan *et al.*, 1982). We were unable to detect an equivalent population of cells in the tonsil, though a few cells with the characteristics described could be detected in association with lymphoid follicles immediately beneath the subcapsular sinuses of mesenteric lymph nodes (Stein *et al.*, 1980).

Independent investigation of B cells by immunologists and histopathologists has resulted in schemes of B cell ontogeny on the one hand and the definition of populations of B cells in tissue sections on the other but it is not clear how these data correlate. It is not yet possible to describe with confidence either the route of activated B lymphocytes during their maturation to plasma cells within the structure of the lymph node or the changes in cellular morphology and phenotypic

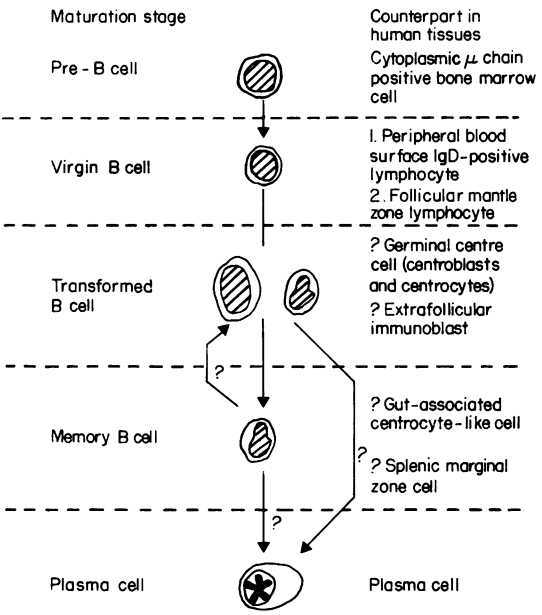

Fig. 2. Correlation between current schemes of B cell maturation and morphologically detectable lymphoid cell types.

determinants which undoubtedly occur during this process. If one attempts to place the centrocyte-like B cells that we have observed in human tissues within schemes of B cell development one may conclude that these cells correspond to memory B cells (Fig. 2). In the gut they appear to be produced locally in germinal centres following antigen induced stimulation, and then migrate towards the lumen of the gut where they await secondary antigenic challenge.

In conclusion this study shows the presence of a new population of B lymphocytes in human GALT which can be demonstrated using morphological and immunohistochemical methods. Further studies are required to establish whether the cells that constitute this population can differentiate into plasma cells and whether they bear any functional resemblance to splenic marginal zone B cells. Their role in the immunological protection of the mucosae, the significance of their invasion of the epithelium and any tendency to migrate between the gut and other organs also await investigation.

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