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Expression of HLA-DR antigens on epithelium associated with lymphoid tissue in the human gastrointestinal tract

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SUMMARY Mucosa from human stomach, terminal ileum, appendix, and colon was studied for epithelial HLA-DR expression using an immunoperoxidase technique with a monoclonal antibody that detects HLA-DR antigens in paraffin embedded tissues. Expression of HLA-DR by epithelial cells was studied with particular reference to the effect of adjacent lymphoid tissue or surrounding chronic inflammation. In the stomach, epithelial HLA-DR appeared to be induced by chronic inflammation. Where lymphoid nodules were present only that epithelium directly adjacent to the lymphoid tissue expressed HLA-DR. Expression was independent of cell type. Epithelium adjacent to normal lymphoid tissue in the terminal ileum, appendix, and colon also expressed HLA-DR, the relationship between expression and proximity to lymphoid tissue being remarkably precise. Expression of HLA-DR by gastrointestinal epithelium appears to be an effect of adjacent lymphocytes, whether part of an inflammatory response or normal tissue. This must be taken into account when assessing HLA-DR expression by gastrointestinal epithelium.

Class II histocompatibility antigens (HLA-DR antigens in human tissue) are known to be present on many types of cells involved in immune responses, including B-cells, activated T-cells, some macrophages and interdigitating cells. Their presence on these cells is associated with antigen recognition and presentation to T-cells and the initiation of specific B and T-cell responses.[1 2] In addition to bone marrow derived cells it has been shown that other cells, including epithelial cells can also express HLA-DR antigens.[3–5]

Class II histocompatibility antigens on the epithelium in the small intestine of healthy rats have been shown so far to be confined to the epithelium of the villi,[4] whereas in man it has been observed that in addition to the villi the dome epithelium over the lymphoid follicles also expresses HLA-DR antigens.[6 7] In graft *versus* host disease in rats, the whole of the epithelium becomes HLA-DR positive, and this positivity has been shown to depend upon graft T-cells.[8] Because of the relationship between the expression of HLA-DR on epithelia and the presence of T-cells and also observations of HLA-DR positivity in the dome regions of lymphoid follicles in human tissue, we have studied the expression of HLA-DR antigens on epithelia in several regions of the gastrointestinal tract with special attention being given to the epithelium immediately adjacent to lymphoid follicles.

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Methods

TISSUE COLLECTION AND PROCESSING

Tissue was collected from fresh surgical specimens of stomach, ileum, appendix, and colon. The reason for laparotomy varied from case to case but the tissue for our study was always taken distant from the site of obvious disease. There was no macroscopic evidence of disease in any of the tissues used. Specimens were fixed in 10% formalin containing 2% acetic acid[9] and then routinely processed and embedded for paraffin sections. Sections stained with haematoxylin and eosin were examined and those tissues with lymphoid follicles were selected for immunohistochemical investigation.

IMMUNOCYTOCHEMISTRY

A monoclonal antibody, TAL-IB5 (mouse IgG1), which detects HLA-D region (HLA-DR) α-chains

in paraffin sections was used throughout this study.[10] Control sections were immunostained in parallel using the antibody Hle-1 to replace TAL-1B5. This antibody is specific for a leucocyte common antigen and is also a mouse IgGl. Antibodies were kindly provided by Drs T Adams and P Beverley of the Imperial Cancer Research Fund.

Paraffin sections were dewaxed, rehydrated, and stained using immunoperoxidase techniques. A peroxidase conjugated rabbit anti-mouse secondary antibody was used with the monoclonal primary antibodies.[11] Peroxidase activity was visualised using the 3,3-diaminobenzidine reagent as described by Graham and Karnovsky, 1966.[12] Nuclei were counterstained with haematoxylin.

Results

STOMACH

Sections of gastric mucosa from three of five gastrectomy specimens showed expression of HLA-DR by epithelium adjacent to lymphoid tissue, while the rest of the gastric mucosa in these cases was HLA-DR negative. On occasions only the epithelial cells in the part of the gland in immediate contact with the lymphoid tissue expressed HLA-DR antigens. Epithelial cells of many types and at different stages of maturity were seen to express HLA-DR antigens when in association with lymphoid tissue including the mucus neck, foveolar epithelium, parietal and chief cells and epithelium showing features of intestinal metaplasia (Fig. 1).

Sections of gastric mucosa from the remaining two specimens showed more generalised HLA-DR positivity. In these tissues there was evidence of superficial chronic gastritis characterised by the presence of an infiltrate of plasma cells and lymphocytes. In each case the foveolar epithelium and mucus neck cells expressed HLA-DR antigens, and in one of these cases in which the lymphocytic infiltrate extended between the deeper glands, the parietal and chief cells also expressed HLA-DR antigens (Fig. 2).

PEYER'S PATCHES IN TERMINAL ILEUM

Four specimens of mucosa from terminal ileum which contained Peyer's patches were immunostained using the monoclonal antibody TAL-IB5. In mucosa between Peyer's patches HLA-DR was expressed on the epithelium of the villi but not the crypts. In relation to Peyer's patches, however, both the dome epithelium and the crypt epithelium surrounding the Peyer's patch follicles were seen to express HLA-DR antigens in each case studied. Sometimes the HLA-DR positivity in the crypts was confined to the side of the crypt which impinged directly on the lymphoid tissue (Fig. 3). This positivity was accompanied by a reduction in the number of goblet cells.

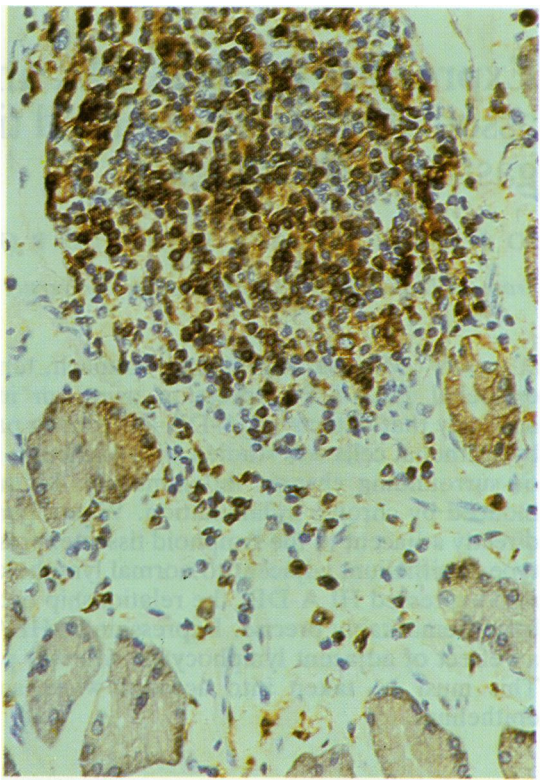

Fig. 1 *Paraffin section of gastric mucosa immunostained using the monoclonal antibody to HLA-DR. Only the parietal and chief cells in the gastric glands adjacent to the lymphoid tissue express HLA-DR antigens. Immunoperoxidase, original magnification ×80.*

APPENDIX

In the appendix, epithelium bearing HLA-DR antigen was found to be present only on some of the cells in the dome epithelium of the lymphoid follicles and was present in five of the 10 appendices studied. The presence of HLA-DR antigens on the dome epithelium could not be correlated with either age or the primary reason for appendicectomy.

COLON

In sections from four of the nine colons studied, HLA-DR antigens were present on the epithelial cells which were immediately adjacent to lymphoid tissue whereas the rest of the mucosa in the same blocks was HLA-DR negative. In two specimens of

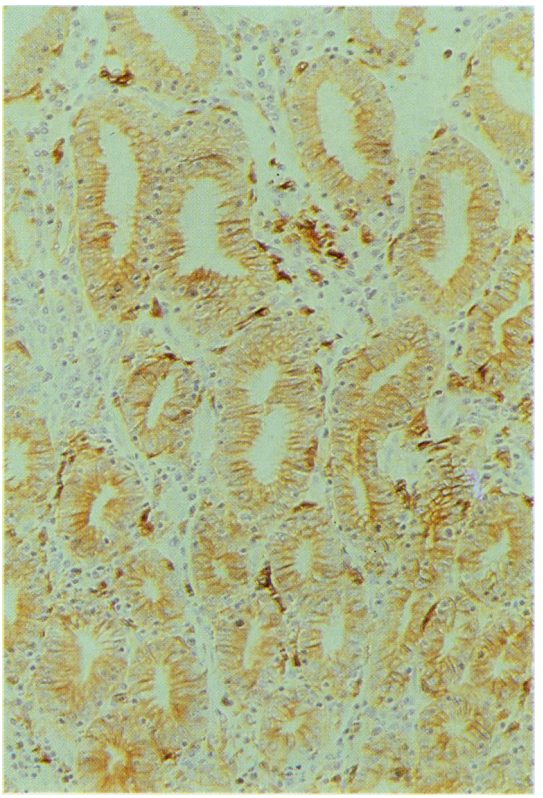

Fig. 2 *Paraffin section of gastric mucosa immunostained using the monoclonal antibody to HLA-DR. This specimen shows features of chronic gastritis. All of the epithelial cells can be seen to express HLA-DR antigens. Immunoperoxidase, original magnification ×80.*

colonic mucosa, all of the epithelial cells expressed HLA-DR antigens and in two more specimens the whole of the epithelium was HLA-DR negative including that next to the lymphoid follicles. The epithelial cells in sections of one colon showed patchy positivity, with the luminal epithelium tending to be HLA-DR positive and the crypt epithelium tending to be HLA-DR negative.

The epithelium in control sections of each type of mucosa which were stained using the antibody Hle-1 instead of TAL-lB5 was negative in each case.

Discussion

Dendritic cells, macrophages, and B-cells which express HLA-DR antigens are involved in the processing and presentation of antigens. As yet HLA-DR has not been shown to have any function other than an immunological one. The presence of HLA-DR antigens on the villi but not in the crypts of healthy tissue in the small intestine has been well documented.[3] It has also been shown by Bjerke and Brandtzaeg[6] that the dome regions of the lymphoid follicles in the small intestine express HLA-DR antigens. We have confirmed these observations and have shown that glandular epithelium which would generally be expected to be HLA-DR negative may be HLA-DR positive when immediately adjacent to lymphoid tissue. This was not only true of the epithelium associated with lymphoid tissue in the small intestine but also in the stomach. The pattern of HLA-DR positivity in the appendix and colon was seen to be similar but more variable and the reason for this is unclear.

Epithelial cells at all stages of maturity in the stomach from the mucus neck cells to the foveolar epithelium and the parietal and chief cells could be seen to be HLA-DR positive in association with lymphoid cells. Ability to express HLA-DR antigens is not therefore an age related property of epithelial cells.

The frequent association of HLA-DR positivity on epithelium with lymphoid tissue suggests that there is a causal relationship between them. It is likely that a product of the lymphoid tissue is capable of inducing HLA-DR on adjacent epithelial cells. The T-cell product interferon γ has been shown to be able to induce HLA-DR antigens on a variety of cells *in vitro*[13–15] and it has been postulated by Rosa and Fellous[16] that this lymphokine may indeed have a role in recruiting cells for involvement in immune processes *in vivo*. Epithelial cells expressing HLA-DR antigens may be able to present antigens from the lumen of the gut to the underlying lymphocytes in the dome of the follicle. Both B- and T-cells have been shown to be in intimate contact with the epithelial cells in the dome regions of lymphoid follicles.[7 17] The epithelial cells in the crypts, however, which do not show the same tendency to be 'invaded' by lymphocytes,[7] can express HLA-DR antigens and appear to lack goblet cells. This may be an adaptation to enable the passage of antigens across the mucosa and perhaps then their subsequent presentation to underlying lymphoid tissue.

All of the specimens of gastric mucosa used in this study were selected because they contained lymphoid tissue and therefore they could all be said to show features of chronic gastritis. Two of the specimens studied showed generalised expression of HLA-DR on the epithelial cells, and the penetration of the lymphocytic infiltrate between the deeper glands was accompanied by even more extensive HLA-DR positivity. It is possible that the expression of HLA-DR antigens on these cells was induced

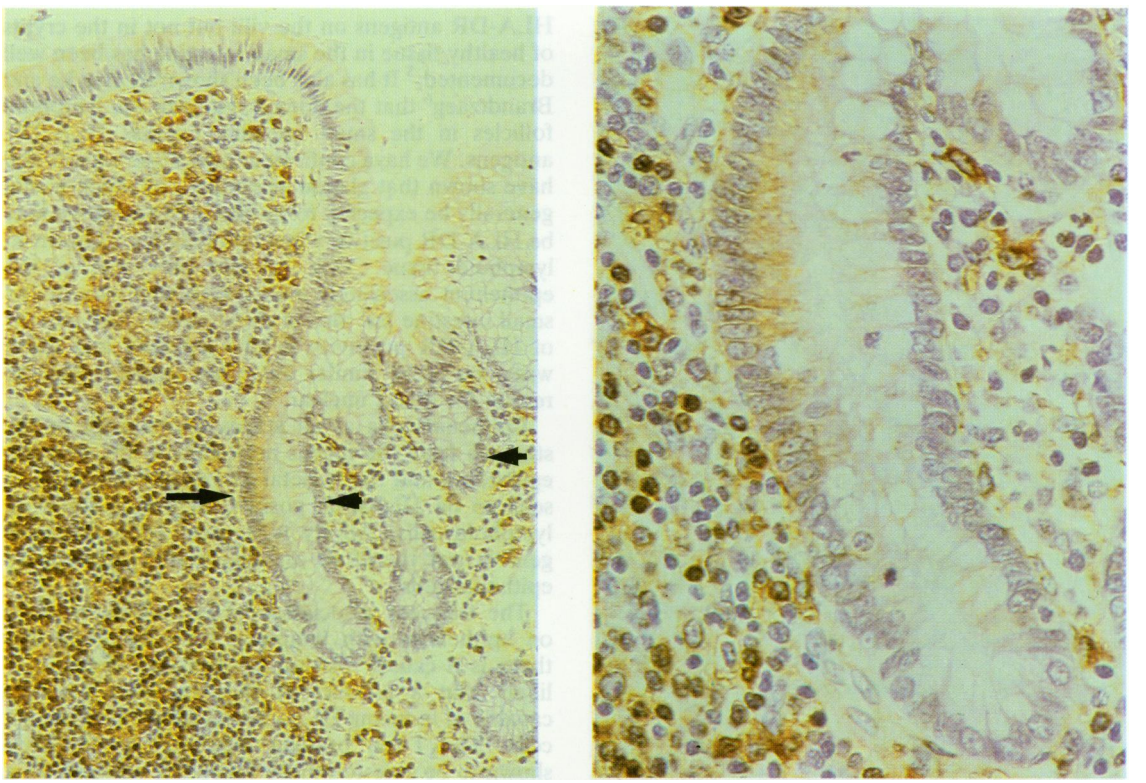

Fig. 3 *Paraffin section of a Peyer's patch from terminal ileum immunostained using the monoclonal antibody to HLA-DR. (a) Section showing expression of HLA-DR antigens by crypt epithelial cells adjacent to a lymphoid follicle (arrow), in comparison to the epithelial cells further from the lymphoid tissue wich are HLA-DR negative (arrow heads). (b) The crypt closest to the lymphoid tissue under higher magnification. The side of the crypt impinging directly on the lymphoid follicle expresses HLA-DR antigens and shows goblet cell depletion. Immunoperoxidase, original magnification (a) ×50, (b) ×150.*

by lymphocytes in the inflammatory cell infiltrate, perhaps by a mechanism similar to that implied for the induction of HLA-DR expression in normal tissue in the ileum. HLA-DR expression on epithelial cells has been shown by others to occur in diseases such as coeliac disease in which there is also an increase in the number of mucosal and intra-epithelial lymphocytes.[18] It has been suggested that the presence of HLA-DR antigens on epithelium signifies an immunological basis for the disease. Our findings in normal tissue suggest that the epithelial expression of HLA-DR antigens in coeliac disease may be induced by the lymphocytic infiltrate and therefore the expression of HLA-DR antigens is not the primary event in the pathogenesis of the disease.

The induction of HLA-DR expression on epithelial cells by lymphocytes may be relevant in the context of autoimmune reactions. It has been suggested[19 20] that HLA-DR positivity on thyroid epithelium is central to the pathogenesis of autoimmune thyroiditis. These authors suggested that HLA-DR expression on thyroid epithelium may be initiated by an interaction between T-cells and an external viral stimulus. The possibility that HLA-DR positivity on epithelium in our study was associated with viral infection could not be excluded in the two cases of chronic gastritis but it is unlikely in the epithelium which expressed HLA-DR antigens in association with normal lymphoid follicles. This suggests that the expression of HLA-DR antigens on thyroid epithelial cells could also be because of a factor released by cells in a lymphocytic infiltrate without the necessity for viral initiation. Expression of HLA-DR antigens by epithelial cells does not therefore necessarily imply autoimmune dysfunction.

Expression of HLA-DR antigens on epithelial cells appears to depend upon their proximity to lymphoid tissue, either to lymphoid follicles or else an inflammatory lymphocytic infiltrate. Whether the lymphoid cells and the epithelium are able to interact during immune responses to antigens at mucosal surfaces is not known but it is likely that the induced HLA-DR antigens have some immunological function.

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