

Prognostic significance of major histocompatibility complex class II antigens (HLA-DR) in normal colonic mucosa, tubulovillous adenoma, and invasive colonic carcinoma

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The major histocompatibility complex is a series of genes that participate in the regulation of the immune response. This complex encodes two classes of cell-surface glycoprotein antigens: class I, found in all nucleated cells; and class II antigens, normally found only on a limited number of cells (B lymphocytes, macrophages, Langerhans' cells, dendritic cells, vascular endothelial cells and some epithelial cells).¹⁻³ Class II antigens control cellular interactions between lymphocytes. In man at least three class II antigens (DR, DQ, and DP), each consisting of α and β glycoproteins chains, are encoded by the HLA-D region of chromosome 6.^{2,4,5}

The majority of pathogens gain access to the body at a mucosal site, and the epithelial cells lining the lumen of the mucosa provide the first barrier against their invasion. In addition to their barrier, absorption and transport functions, epithelial cells play an important role in both innate and adaptive immune responses. They secrete soluble molecules including defensins^{6,7} and complement components⁸ that neutralize and inactivate micro-organisms and their toxins. In addition, they can present foreign antigens to T cells affecting their proliferation, cytolytic activity and cytokine production. Typically, antigen-presenting cells are bone marrow derived cells such as dendritic cells, macrophages (MO) and monocytes (i.e. professional antigen presenting cells). However, certain other cell types, including intestinal epithelial cells,⁸⁻¹⁰ renal tubular epithelial cells,¹¹ keratinocytes¹² and endothelial cells¹³ have been shown to function in a limited context as antigen-presenting cells, which are characteristically less efficient at antigen processing and presentation and are thus referred to as non-professional antigen-presenting cells.

Epithelial cells can transport antigens from the lumen by a process of transcytosis for eventual processing and presentation by professional antigen-presenting cells found in the underlying sub-epithelial stroma. The transcellular transport of antigen by epithelial cells is generally a slow process, but may be enhanced by immunization.¹⁴ Studies by Blumberg and co-workers have demonstrated a functional MHC class I-related IgG receptor (FcRn) on intestinal epithelial cells.^{15,16} Since both the female reproductive tract and the gut have IgG, which increases in disease states, FcRn may facilitate transport of IgG-antigen complexes through epithelial cells into the basolateral sub-epithelium where antigen presenting cells and T cells reside.

Recent studies have established that intestinal epithelial cells can express MHC class II molecules and present antigen directly to CD4+ T cells. Kaiserlian et al.²⁰ demonstrated in a murine model that intesti-

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nal epithelial cells could present keyhole limpet hemocyanin (KLH) to a CD4+ T cell hybridoma and that an anti-class II monoclonal antibody blocked interleukin-2 production by T cells. Subsequent studies with other antigens confirmed these results, although most antigens were inefficiently presented.²¹ Hershberg and co-workers⁹ have demonstrated, using class II transfected human intestinal epithelial cell lines, both processing and presentation of antigen to CD4+ cells. There have been reported studies examining antigen presentation by isolated epithelial cells from the human colon.¹⁷⁻¹⁹

We report that there is a loss in the HLA-DR expression by the epithelial cells and a gain in the CD4 expression in the lamina propria and muscular layer of the colon during the progression from adenoma to invasive carcinoma.

Materials and Methods

We studied 31 cases of normal colonic mucosa, 12 cases of tubulovillous adenoma, and 39 cases of invasive carcinoma of the colon. The relationship between distribution of HLA alleles in patients with carcinoma and susceptibility to tumour was analysed to study the possible correlation between HLA class II DQA1, DQB1 and DRB1 genes and carcinoma in the study population. Genomic DNA from 51 patients with adenomas and carcinomas of the colon and 31 healthy controls, were typed by PCR-SSP (sequence specific primers). The patients were also divided into different groups according to the age and presence of cancer relatives, and compared with the controls. None of these HLA class II alleles showed significant positive or negative associations with either the overall population of patients with carcinoma or adenoma of the colon or the considered subgroups.

The samples were obtained by colonoscopic biopsies from 82 patients (mean age 55 years, range 45 to 79 years). All procedures were approved by the local Hospital Ethics Committee. Written, informed consent was obtained from all subjects. The polyps (12 cases, tubulovillous type) were located in the colon, and measured less than 1 cm in diameter (mean size 7 mm). Biopsies of endoscopically and histologically normal colonic mucosa were obtained from 31 patients with a functional bowel disease. In 39 cases the biopsy revealed invasive neoplastic changes and patients underwent surgery. Tissues were fixed in formalin and embedded in paraffin for immunohistochemical study.

Immunohistochemistry was performed with the

various antibodies used on serial sections. Tissue sections (5 µm) were deparaffinized, rehydrated, and treated with 0.3 per cent hydrogen peroxide for 5 minutes to quench endogenous peroxidase activity. Non-specific binding was blocked with serum for 10 minutes. Slides were then incubated for 30 minutes with the monoclonal antibodies (1/40), namely mouse anti-human HLA-DR, alpha-chain (DAKO) and CD4 (DAKO). Control slides were incubated for the same period with normal mouse serum. After several 10 minute washes in PBS, samples were developed with the peroxidase LSAB kit (labelled streptavidin-biotin method, DAKO), which allows the detection of the first antibody. The slides were briefly counterstained with Mayer's haematoxylin, mounted, and examined under an Olympus B×40 microscope.

The immunostained sections were examined with a ×40 objective and the distribution of HLA-DR and CD4 within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with HLA-DR and CD4 stainings, a 10×10 square calibrated grid was inserted into the eyepiece of an Olympus binocular microscope.

Five to ten fields were examined for each section, and at least 1000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the HLA-DR and CD4 indices. The HLA-DR index = number of positive cells/total number of cells (positive + negative) and the CD4 index = number of positive cells/total number of cells (positive + negative). The indices ranged from 0-100%, with a mean of 18%. The mean index was evaluated in three ranges: low index (under 18%), grade I; moderate index (from 18% to 50%), grade II; and high index (from 51% to 100%), grade III.

Results

The sections were examined independently by two observers. Positive cellular staining for HLA-DR and CD4 antigens were manifested as fine yellow-brown cytoplasmic expression. HLA-DR was expressed in 20 of 31 of normal mucosae (64.5%), in 4 of 12 adenomas (33.3%) (Figure 1), and in 10 of 39 invasive carcinomas (25.6%) (Figure 2, 3). Of 20 positive normal mucosae, 9 were scored as HLA-DR grade II and 11 as HLA-DR grade III. Of 4 positive adenomas 1 was scored as HLA-DR grade I, and 3 as HLA-DR grade II. Of 10 positive invasive carcinomas 1 was scored as HLA-DR grade I, 7 as grade II, and 2 as grade III.

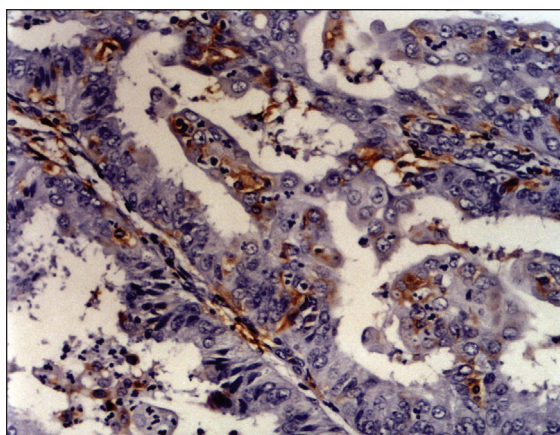


Figure 1. Tubulovillous colonic adenoma. Epithelial neoplastic cells strongly express the HLA-DR antigen. The HLA-DR-positive cells are predominantly observed in the superficial epithelial layer (Immunostaining with HLA-DR Mab, magnification $\times 200$).

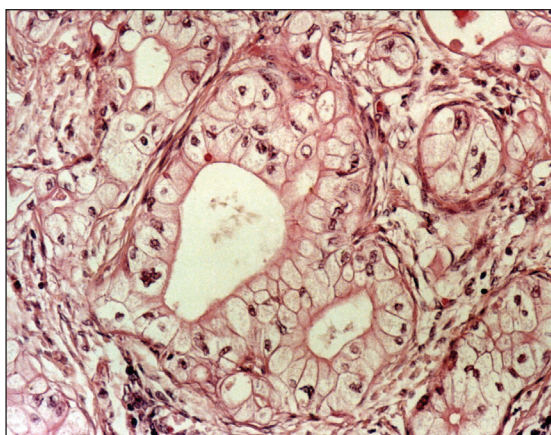


Figure 2. Well-differentiated adenocarcinoma of the colon composed of irregularly shaped glands and branching cords of tumor cells. The neoplastic glands are lined by tall columnar to cuboidal epithelium (Hematoxylin-Eosin stain, magnification $\times 200$).

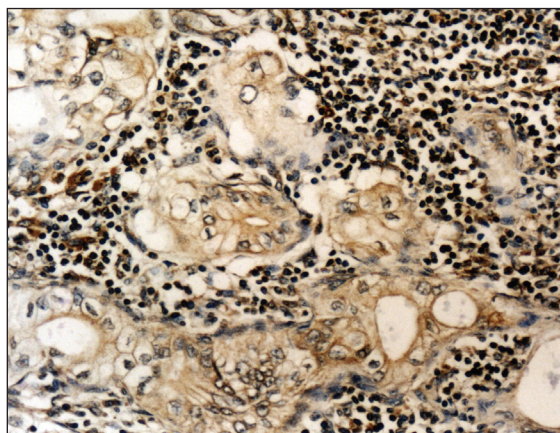


Figure 3. Invasive adenocarcinoma of the colon with a dense stromal lymphocytic infiltrate amongst the neoplastic glands. A weak immunoreactivity of the epithelial neoplastic cells to HLA-DR antigen is detected (Immunostaining with HLA-DR Mab, magnification $\times 200$).

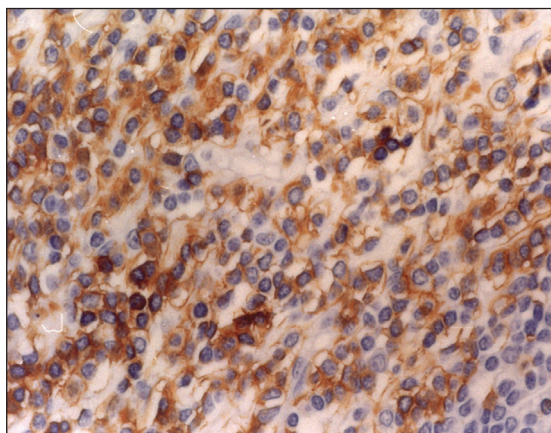


Figure 4. A moderate cell infiltration by helper T-lymphocytes in the stromal connective tissue in tubulovillous colonic adenoma (Immunostaining with CD4 Mab, magnification $\times 200$).

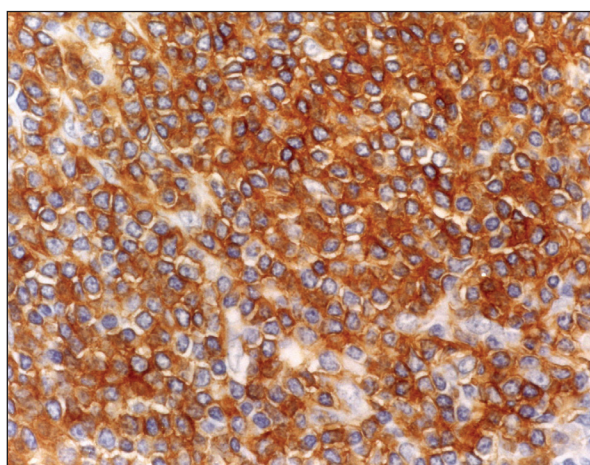


Figure 5. A strong cell infiltration by helper T-lymphocytes in the stromal connective tissue in colonic adenocarcinoma (Immunostaining with CD4 Mab, magnification $\times 200$).

CD4 was expressed in 9 of 31 normal mucosas (29%), in 5 of 12 adenomas (42%) (Figure 4), and in 26 of 39 invasive carcinomas (67%) (Figure 5). Of 9 positive normal mucosas, 4 were scored as CD4 grade II and 5 as CD4 grade III. Of 5 positive adenomas, 1 was scored as CD4 grade I, 3 as CD4 grade II and 1 as CD4 grade III. Of 26 positive invasive carcinomas, 4 were scored as CD4 grade I, 12 as CD4 grade II, and 10 as CD4 grade III.

Discussion

Major histocompatibility complex antigens (MHC), or human leukocyte antigens (HLA) in humans, are considered to be essential when tumor cells are recognized and attacked by host immune cells. Therefore, the tumor growth may be affected by the states of HLA expression. In various neoplasms, the grade of HLA expression has been reported clinically as being associated with the degree of differentiation and the prognosis regarding both class I^{22-26,36} and class II antigens.^{23,26-28,36} However, contradictory results have been also reported.²⁹⁻³³ Such controversy is probably not only due to the different tissue origins of various tumors, but also to the heterogeneous expression of individual tumor cells. It is difficult to quantitatively evaluate the heterogeneity of HLA expression using conventional tissue sections for a histologic examination. The dispersed cells of fresh tumor tissues mostly likely represent the whole population of tumor cells and are thus advantageous to the quantitative assessment of HLA expression.

Helper T cells (CD4 Phenotype) are vital to cell proliferation and the secretion of antibodies by mature B lymphocytes. These processes are initiated by a foreign antigen being phagocytosed and partially digested by an antigen-presenting cell (APC). The products of this antigen processing pass via the endosomal pathway of the cell to the APC surface where they are presented on Class II MHC molecules (a family of cell surface molecules found mainly on dendritic cells, macrophages, B lymphocytes and other APCs with similar functions) expressed on the cell membrane. This combination of antigen and MHC II molecule is then presented at the cell surface to a helper T cell, which recognizes the foreign peptide plus part of the Class II MHC molecule via its T-cell receptor. This interaction, together with secondary signals from cytokines released by the APC, and interactions with other cell adhesion molecules expressed on the two cells concerned, causes the activation and proliferation of the helper T cell. The T cell then activates B lymphocytes, which are

stimulated to differentiate into plasmacytes secreting antibody corresponding to the particular antigen involved in the APC-T-cell interaction. In this highly regulated way, clones of B cells that produce specific antibodies against an antigen can be stimulated to proliferate and secrete their products. Helper T cells are also required to supplement the activation of cytotoxic T cells, although a separate group of helper T cells is probably involved.

There is strong evidence to suggest that virtually all colonic adenocarcinomas arise within pre-existent adenomas, or areas of dysplasias. The risk of malignancy increases as an adenoma becomes larger, has a greater villous component, or has more high-grade dysplasia. However, exceptions exist, and some carcinomas probably develop in small and highly dysplastic flat adenomas. Carcinomas arising anew from normal mucosa have never been convincingly documented. Removal of adenomas endoscopically prevents colorectal cancer from developing.

Recent advances in molecular genetics have now begun to unravel events responsible for the adenoma-carcinoma sequence. The earliest chance may involve the acquired mutation of a suppressor gene at the adenomatous polyposis coli region of chromosome 5, or an adjacent region termed MCC (mutated in colon cancer). Subsequent events include point mutation of ras oncogenes and the deletion of suppressor genes on chromosome 18 (DCC, or deleted in colon cancer) and p53 on chromosome 17. In particular, the p53 gene has attracted considerable attention, since it appears to play a key role in the conversion of an adenoma to a carcinoma. p53 is involved in the normal regulation of transcription and arrests progress through the cell cycle in G1 when DNA damage is present. Mutations of p53 and specific site mutations of K-ras correlate with short survival in carcinoma of the large bowel.³⁷

To elucidate the clinico-biological significance of HLA expressed on neoplastic cells, we have quantitatively assessed the degrees of the class II expression using paraffin-embedded neoplastic cells, and also the grade of T helper lymphocytic infiltration in normal colonic mucosa, tubulovillous adenoma and invasive carcinoma of the colon. In the present study, we clearly demonstrated a loss of HLA-DR expression from adenoma towards invasive carcinoma of the colon. This suggests that the change in HLA-DR expression is not intrinsic to the neoplastic process but may merely be due to the fact that malignant cells, as they become less differentiated, tend to show alterations in their antigenic phenotype.

It is well known that HLA class II antigens are usually expressed on such immune cells as macrophages, B cells and activated T cells and that they are also involved in antigen presentation as well as in the regulation of the helper T cell function. A number of studies have also revealed the expression of class II antigens by both various non-immune normal and malignant cells^{23,26-29,32,33} although the biological significance of the class II expression of such cells remains unclear.

On the other hand, in view of immunological aspects, the class II expression of tumor cells has been reported to correlate with the local infiltration of lymphocytes.^{34,35} In the present study, expression of HLA-DR by epithelial neoplastic cells was pos-

sibly mediated by stromal T helper lymphocytes as lymphoid cell infiltrates were observed to all biopsy specimens containing HLA-DR positive neoplastic cells. The increased aberrant expression of HLA-DR in tumor cells has been viewed as an important feature to escape tumor recognition by immune cells, and correlates with high-grade malignancy and enhanced metastatic potential.

In our series of colon epithelial tumors, there was a decreased expression of HLA-DR as the neoplastic process progressed to malignancy and a subsequent increased immune response by activated helper T-lymphocytes, providing new insights for a better understanding of the tumor-host relationships in this form of neoplasia.

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