Inverse Correlation between HLA-DR Antigen Expression and CD4 Positive Lymphocytic Populations in Normal Mucosa, Tubulovillous Adenoma, and Invasive Carcinoma of the Colon

Tamiolakis, D.[1], Venizelos, I.[2]

[1] Department of Cytology, General Hospital of Alexandroupolis, Greece
[2] Department of Pathology, Ippokration Hospital of Salonica, Greece

Summary

Background: HLA-A,B,C and HLA-D molecules present antigenic peptides to the antigen-specific receptor of autologous T lymphocytes. T-cell-mediated host-versus-tumor response might therefore depend on the presence of these molecules on tumor cells, although the absence of HLA-A,B,C determinants on a cell has been shown to increase its susceptibility to lysis by natural killer cells. The prognostic role of tumor stage and grade is well- established in colorectal cancer. In this study we used immunohistochemistry to analyse the expression of HLA-DR on epithelial cells of normal colonic mucosa, tubulovillous adenoma, and invasive carcinoma, as well as the magnitude of the stromal T lymphocytes at the relevant sites. HLA-DR expression was correlated to histological grade and Dukes stage in the cases of invasive cancer. Yet, we investigated the association of HLA-DR plus DQ genes and adenoma or carcinoma by PCR.
Materials and methods: 31 cases of normal colonic mucosa, 12 cases of tubulovillous adenoma, and 39 cases of invasive carcinoma were surveyed for the detection of HLA-DR monoclonal antigen, and the T helper (TH) marker (CD4) in the stroma (lamina propria) of the relevant cases.
Results: HLA-DR was expressed in 20 of 31 normal colonic mucosas (64.5%), 4 of 12 adenomas (33.3%), and in 10 of 39 invasive carcinomas (25.6%). A strong relation of HLA-DR expression and histological grade was found ($p<0.001$), but no association with Dukes stage ($p=0.141$). No significant correlation between HLA-DR plus DQ genes and adenoma or cancer of the colon was found. CD4 positive cells were found in 9 of 31 normal colonic mucosas (29%), 5 of 12 adenomas (42%), and in 26 of 39 invasive carcinomas (67%).
Conclusions: The results showed an inverse correlation between the expression of HLA-DR and the number of CD4 positive cells as the lesion progressed to malignancy. HLA-DR was significantly associated with tumor grade but not with Dukes stage in colonic cancer hosts. HLA-DR and DQ genes do not contribute to a susceptibility to adenoma or carcinoma.
Key words: HLA-DR – CD4 – normal colonic mucosa – colonic adenomas – invasive carcinoma of the colon

Souhrn

Inverzní vztah mezi expresí antigenu HLA-DR a pozitivitou lymfocytární populace v normální sliznici, tubovilózním adenomu a invazivním karcinomu tlustého střeva

Molekuly HLA – A, B, C a D předkládají antigenní peptidy antigen-specifickým receptorům autologních T-lymfocytů. Host-versus-tumor odpověď zprostředkovaná T-buňkami může tudíž záviset na přítomnosti těchto molekul na nádorových buňkách, i když bylo prokázáno, že nepřítomnost HLA – A, B, C determinant na buňce zvyšuje její náchylnost k lýze přirozenými zabíječi. U kolorektálních nádorů je dobře prokázán prognostický význam nádorového stádia (stage) a stupně diferenciace (grade). V této studii jsme pomocí imunohistochemie zjišťovali expresi HLA-DR epiteliálními buňkami normální sliznice, tubovilózního adenomu a invazivního karcinomu tlustého střeva a dále množství stromálních T-lymfocytů v těchto místech. U invazivních karcinomů byla exprese HLA-DR korelována s histologickým stupněm a stadiem dle Dukese. Dále jsme pomocí PCR zjišťovali geny HLA-DR a DQ u adenomů a karcinomů.
Bylo vyšetřeno 31 případů normální střevní sliznice, 12 případů tubovilózního adenomu a 39 případů invazivního karcinomu na přítomnost HLA-DR monoklonálního antigenu a T-helper (TH) markeru (CD4) ve stromatu lamina propria.
HLA-DR exprese byla prokázána ve 20 z 31 normálních sliznic (64,5 %), ve 4 ze 12 adenomů (33,3 %) a v 10 z 39 invazivních karcinomů (25,6 %). Byl zjištěn výrazný vztah mezi expresí HLA-DR a grade

nádoru (p < 0,001), nikoli ale vztah ke stadiu dle Dukese (p = 0,141). Nebyl zjištěn signifikantní vztah mezi geny HLA-DR a-DQ a adenomem či karcinomem. CD4 pozitivní buňky byly nalezeny v 9 z 31 normálních sliznic (29 %), v 5 z 12 adenomů (42 %) a ve 26 z 39 invazivních karcinomů (67 %).
Tyto výsledky ukazují na inverzní vztah mezi expresí HLA-DR a počtem CD4 – pozitivních buněk v průběhu malignizace léze. HLA-DR má významný vztah ke grade, ne však k Dukesovu stadiu. Geny HLA-DR a -DQ nepřispívají k predispozici k adenomu či ke karcinomu.

Klíčová slova: HLA-DR – CD4 – normální sliznice tlustého střeva – adenomy tlustého střeva – invazivní karcinom tlustého střeva

Čes.-slov. Patol., 42, 2006, No. 2, p. 52–58

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 (DR, DQ, and DP) at least three class II antigens, each consisting of glycoprotein 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 components (8) that neutralize and inactivate microorganisms 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), keratinocytes (12) and endothelial cells (13) have been shown to function in a limited context as antigen presenting cells, which are characteristically less efficient at antigen processing and presentation and 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 functional MHC class I related IgG receptor (FcRn) on intestinal epithelial cells (15, 16). Since both the female reproductive tract (FRT) 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 intestinal epithelial cells could present keyhole limpet hemocyanin (KLH) to a CD4+ T cell hybridoma and that an anti-class II mAb 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-workers (9) 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 positive lymphocytic populations in the lamina propria and muscular layer of the colon, during the progression from adenoma to invasive carcinoma. HLA-DR expression was related to histological grade but not to Dukes stage in invasive carcinomas. We also report no contribution of HLA-DR or –DQ to the susceptibility of patients with adenoma or carcinoma.

Materials and Methods

We studied 31 cases of normal colonic mucosa (control group, all healthy male blood donors), 12 cases of tubulovillous adenoma (7 males and 5 females), and 39 cases of invasive carcinoma of the colon (31 males and 8 females). 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 DRBI 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). Ten millilitres of venous blood with EDTA as anticoagulant, were collected from each subject. Genomic DNA extraction was done according to a modified Graham and Miller method (22, 23). Briefly, red cells were lysed using RBC lysis buffer-I containing 0.144 M NH4Cl& 1Mm NaHCO3 and RBC lysis buffer-II containing 0.3 M sucrose, 10Mm Tris-HCl (Ph 7.5), 5 Mm MgCl2 and 1% Triton-X-100. For destruction of WBCs a lysis buffer containing 0.075 Mm NaCl & 0.024 Mm Na-EDTA was used. Subsequently, 125 µl of 10% SDS and 1 ml of 5M NaClO4 were added. For salting out of proteins, 6 M NaCl was used. DNA was precipitated with isopropanol and washed twice with 70% ethanol. HLA typing was performed by PCR-SSP according to the Olerup and Zetterquist method (24). DNA was amplified using 18 PCR reactions for each individual. Each reaction was performed in a total volume of 20 µl containing 17µl PCR mixture (50 mM KCl, 10 mM MgCl2, 10 mM Tris-HCl; Ph 8.3, 0.001(w/v) gelatin, 200 mM of each dNTPs, 1 mM of specific primers and 0.2 mM of the internal control primers), 1 µl templates DNA and 2µl of Taq DNA polymerase (0.5 U/µl). DNA samples were amplified for 30 cycles. Each cycle consisted of denaturation at 94 centigrades Celcius for 30 seconds, annealing at 55 centigrades for 1 minute and extension at 72 centigrades for 1 minute. The extension was continued for a further 10 minutes at 72 centigrades. PCR products were electrophoresed on 1.5% agarose gel and the presence of specific DNA bands were analysed under UV light. The patients were also divided into different groups according to the age and and presence of cancer relatives, and compared with the controls.

Source and preparation of tissues

The samples were obtained by colonoscopic biopsies from 82 patients (mean age 55 years, range 45 to 79 years). The local Hospital Ethics Committee approved all procedures. Written informed consent was obtained from all subjects (healthy and diseased). 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. Carcinomas were graded by histology as well (n=3), moderately (n=30), and poorly differentiated (n=6), and were classified according to Dukes as A (n=8), B (n=15), C (n=10), and D (n=6). Normal mucosa samples were not obtained from patients with carcinoma. Tissues were fixed in formalin and embedded in paraffin for immunohistochemical study.

Immunohistochemistry

Immunohistochemistry was performed with the various antibodies used on serial sections. An antigen retrieval method using a pressure cooker was performed before immunostaining. Tissue sections (5µm) were deparaffinized, rehydrated, and treated with 0.3 per cent hydrogen peroxide for 5 min to quench endogenous peroxidase activity. Non-specific binding was blocked with serum for 10 min. Slides were then incubated for 30 min with the monoclonal antibodies (1/40), namely mouse anti-human HLA class II (DR) (TAL.1B5) (DAKO), CD20 (L26) (DAKO), CD4(1F6) (DAKO) and CD8 (C8-144) (DAKO). Bound antibodies were visualised employing the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (25) and Fast Red for development. Negative controls were run in the assay by omission of primary antibody. Positive controls for anti-HLA II (DR) and anti-CD4 antibody were the staining of stromal cells and the tissue of tonsils respectively. We focused our attention on HLA class II (DR), and CD4 antibodies since the other antigens were beyond the scope of this study.

The immunostained sections were examined with a x 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 x 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.

$$\text{HLA-DR index} = \frac{\text{no of positive cells}}{\text{no total (positive+negative cells)}}$$

$$\text{CD4 index} = \frac{\text{no of positive cells}}{\text{no total (positive+negative cells)}}$$

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 (from18 to 50%), grade II; and high index (from 51 to 100%), grade III.

For all cases both the percentage and

intensity of HLA class II (DR) staining were numerically scored as in table 1. Particular emphasis was given on the total percentage of epithelium stained, the intensity of staining and uniformity. Intensity was derived by comparison of epithelial staining and stromal cell reactivity.

Results

The sections were examined independently by two observers, and positive cellular staining for HLA-DR and CD4 antigens were manifested as fine yellow-brown cytoplasmic expression (Fig.1, 3–5).

HLA-DR was expressed in 20 of 31 of normal mucosas (64.5%), in 4 of 12 adenomas (33.3%) (Fig. 1), and in 10 of 39 invasive carcinomas (25.6%) (Fig. 2, 3). CD4 was expressed in 9 of 31

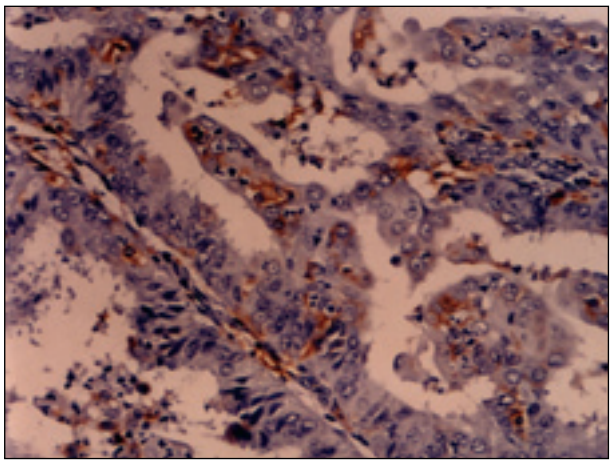

Fig.1. Tubulovillous colonic adenoma. Epithelial neoplastic cells strongly express the HLA-DR antigen. The HLA-DR-positive cells are predominantly observed in the superficial epithelial layer (Immunostaining with HLA-DR Mab, magnification x200)

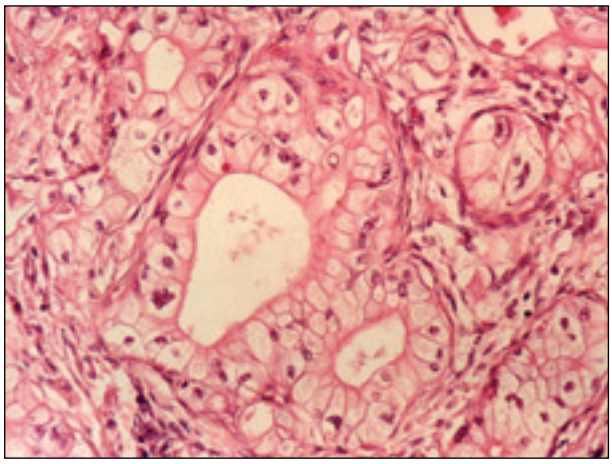

Fig. 2. Well-differentiated adenocarcinoma of the colon composed of irregularly shaped glands and branching cords of tumor cells. The neoplastic glands are lined by tall columnar to cuboidal epithelium (Eosin-Hematoxylin stain, magnification x200)

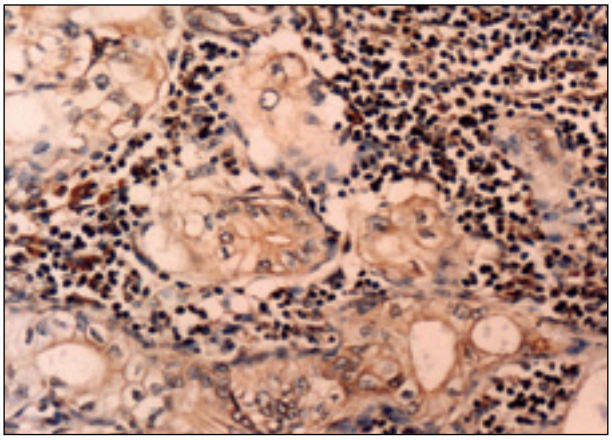

Fig. 3. Invasive adenocarcinoma of the colon with a dense stromal lymphocytic infiltrate amongst the neoplastic glands. A weak immunoreactivity of the epithelial neoplastic cells to HLA-DR antigen is detected (Immunostaining with HLA-DR Mab, magnification X200)

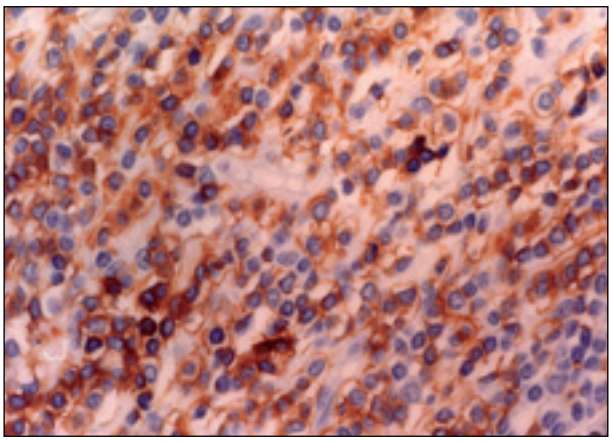

Fig. 4. A moderate cell infiltration by helper T-lymphocytes is detected in the stromal connective tissue in tubulovillous colonic adenoma (Immunostaining with CD4 Mab, magnification x200)

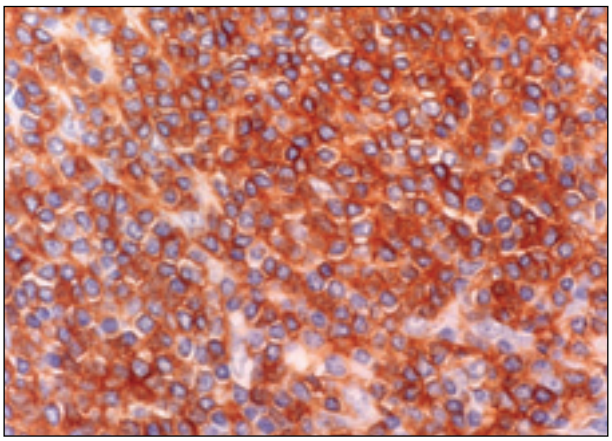

Fig. 5. A strong cell infiltration by helper T-lymphocytes is detected in the stromal connective tissue in colonic adenocarcinoma (Immunostaining with CD4 Mab, magnification x200)

normal mucosas (29%), in 5 of 12 adenomas (42%) (Fig. 4), and in 26 of 39 invasive carcinomas (67%) (Fig. 5). There was a variable reduction of the intensity and proportion of epithelial cells staining for HLA-DR from normal mucosas towards invasive carcinomas. On the contrary, there was a variable increase in the numbers of CD4 positive stromal infiltrates from normal mucosa to invasive carcinoma.

HLA class II (DR) expression by malignant epithelium showed the greatest change from that seen in normal mucosa. In 10 cases of carcinoma, 3 cases exhibited epithelial HLA class II (DR) expression in a manner similar to that seen in normal mucosa, and 7 cases showed variable reduction of the intensity and numbers of epithelial cells stained. From the 4 cases of adenoma 2 cases showed HLA class II (DR) expression as in normal mucosa and 2 exhibited variable expression.

HLA-DR expression was related to histological grade; that is, tumors with strong HLA-DR expression were significantly more often poorly differentiated ($p < 0.001$). There was no association with HLA-DR expression according to Dukes stages ($p=0.141$) (Table 2).

None of HLA class II alleles showed significant positive or negative associations with either the overall population of patients with carcinoma or adenoma or the considered subgroups.

Lymphocytes: Stromal cells identified with CD20, CD4, and CD8 were morphologically lymphocytes. Both B and T lymphocytes were identified in a ratio of approximately 1 : 3.5, and with slightly more CD4 than CD8 positive cells. Intraepithelial cells were also present, the vast majority of which were T lymphocytes, mainly CD4. In adenoma and carcinoma, lymphocytes were predominantly in stroma around tumour cell islands, with only occasional single cells adjacent to tumour cells. B and T lymphocytes were present in a ratio of approximately 1:2, again with a predominance of CD4 cells being found.

Quantification: Stromal lymphocytes identified by each monoclonal antibody were present in far greater numbers in adenoma and carcinoma as compared with normal mucosa ($p<0.001$, ANOVA test).

No contribution of HLA-DR or-DQ genes in the susceptibility to adenoma or carcinoma was documented in our series, by PCR-SSP typing.

Tab. 1. Scoring system for HLA class II (DR) expression

Percentage of cell expression	score
0	0
1–17	1
18–50	2
51–100	3
Intensity of HLA class II(DR) expression	**score**
Negative	0
Very weak, just detectable	1
Readily detectable	2
Just less intense than stromal cells	3
Stromal intensity, equal to stromal cells	4

Tab. 2. Epithelial HLA-DR expression in relation to histological grade and Dukes stage in 39 invasive carcinomas

	HLA-DR expression				
	Total no	negative	weak	strong	p
Histological grade					
well-differentiated	3	2	1	0	
moderately-differ.	30	14	10	6	
poorly-differ.	6	4	1	1	<0.001
Dukes stage					
A	8	4	3	1	
B	15	9	4	2	
C	10	6	3	1	
D	6	3	2	1	0.141

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 clinically reported to be associated with the degree of differentiation and the prognosis regarding both class I (26–30) and class II antigens (27, 30–32). However, contradictory results have been also reported (33–37). Such controversy is probably not only due to the different tissue origins of various tumors but also due 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 most likely represent the whole population of tumor cells and are thus advantageous to the quantitative assessment of HLA expression.

Helper T Cells (CD4 phenotype). These are vital to cell proliferation and 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 antigen-presenting cells 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 preexistent adenomas, or areas of dysplasia. 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 developing.

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, tubulo-villous 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 (27, 30–33, 37, 38,), 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 (39, 40). In the present study, expression of HLA-DR by epithelial neoplastic cells was possibly mediated by stromal T helper lymphocytes as lymphoid cell infiltrates were observed in all biopsy specimens containing HLA-DR positive neoplastic cells**.** Lymphocytes that infiltrate tumor epithelium (intraepithelial lymphocytes- IELs), are specifically associated with improved survival and may be involved in an immune response. Immunohistochemical analyses have shown that the IELs infiltrating microsatellite instable colorectal cancers are predominantly cytotoxic, activated, and release mediators of target cell death. Follow-up analyses confirm improved survival in patients with these tumors. Increased apoptosis has also been demonstrated in these cancers but the link between increased lymphocytic infiltrate and apoptotic cell death has not yet been proven. Some argue that these infiltrates are secondary phenomena with no biological relevance and it has been suggested that IELs in microsatellite cancers simply represent proliferation of resident lamina propria lymphocytes with no immunological activation or role (41). 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.

Our findings are not in accordance with those reported by Lovig et al (42), who have found a strong HLA-DR expression in microsatellite stable carcinomas of the large bowel and suggest that HLA-DR expression may be an important part of the anti-tumor immune responses in colorectal carcinomas. In our settings, gradual loss of epithelial HLA class II (DR) expression might be a manifestation of cellular differentiation from normal mucosa versus the neoplastic

one, signalling simultaneously a selective effect on the response capacity of the immune system.

References

1. **Frelinger JA.** Tissue distribution and cellular expression of Ia antigens. In: Ferrone S, David CS, eds. Ia Antigens I. Mice. Florida: CRC Press, Boca Raton, 1983. –2**. Lafuse WP, David CS.** Ia antigens. Genes, molecules, and function. Transplantation 1984;38:443–453. – 3. **Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ.** The detailed distribution of MHC class II antigens in normal human organs. Transplantation 1984;38:293–298. – 4. **Klein J, Figuerou F, Nagy A.** Genetics of the major histocompatibility complex: the final act. Annual Review of Immunology 1983;1:119–142. – 5. **Steinmetz M, Hood L.** Genes of the major histocompatibility complex in mouse and man. Science 1983;222:727–733. – 6. **O'Neil DA, Porter EM, Elewaut D et al.** Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. J Immunol 163 (1999), 6718–6724. – 7. **Eisenhauer PB, Harwig SS, Lehrer RI.** Cryptdins: antimicrobial defensins of the murine small intestine. Infect Immun 60 (1992), 3556–3565. – 8. **Andoh A, Fujiyama Y, Bamba T, Hosoda S.** Differential cytokine regulation of complement C3, C4, and factor B synthesis in human intestinal epithelial cell line, Caco-2. J Immunol 151 (1993), 4239–4247. – 9. **Hershberg RM, Framson PE, Cho DH et al.** Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. J Clin Invest 100 (1997), 204–215. – 10. **Bland PW, Warren LG.** Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. Immunology 58 (1986), 1–7. – 11. **Kelley VR, Singer GG.** The antigen presentation function of renal tubular epithelial cells. Exp Nephrol 1 (1993), 102–111. – 12. **Nickoloff BJ, Turka LA.** Immunological functions of non-professional antigen-presenting cells: new insights from studies of T-cell interactions with keratinocytes. Immunol Today 15 (1994), 464–469. – 13. **Savage CO, Brooks CJ, Harcourt GC et al.** Human vascular endothelial cells process and present autoantigen to human T cell lines. Int Immunol 7 (1995), 471–479. – 14. **Berin MC, Kiliaan AJ, Yang PC, Groot JA, Taminiau JA, Perdue MH.** Rapid transepithelial antigen transport in rat jejunum: impact of sensitization and the hypersensitivity reaction. Gastroenterology 113 (1997), 856–864. – 15. **Dickinson BL, Badizadegan K, Wu Z et al.** Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. J Clin Invest 104 (1999), 903–911. – 16**. Israel EJ, Taylor S, Wu Z et al**. Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. Immunology 92 (1997), 69–74. 17. **Bedossa P, Poynard T, Bacci J et al.** Expression of histocompatibility antigens and characterization of the lymphocyte infiltrate in hyperplastic polyps of the large bowel. Hum Pathol 1990, 21:319–324. – 18. **Teh M, Wee A, Raju GC.** HLA-DR antigen expression in colonic adenomas and adenocarcinomas. Pathology 1994, 26(2):123–126. – 19. **Degener T, Momburg F, Moller P.** Differential expression of HLA-DR, HLA-DP, HLA-DQ and associated invariant chain (Ii) in normal colorectal mucosa, adenoma and carcinoma. Virchows Arch A Pathol Anat Histopathol 1988, 412(4):315–322. – 20. **Kaiserlian D, Vidal K, Revillard JP.** Murine enterocytes can present soluble antigen to specific class II-restricted CD4+ T cells. Eur J Immunol 19 (1989), 1513–1516. – 21. **Kaiserlian D.** Epithelial cells in antigen. Sampling and presentation in mucosal tissues. Curr Top Microbiol Immunol 236 (1999), 55–78. – 22. **Graham DE.** The isolation of high molecular weight DNA from whole organisms or large tumor masses. Anal Biochem 1978;85:609–613. – 23. **Miller SA, Dykes DD, Polesky IF.** A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acid Res 1988:16:1215–1218. – 24. **Olerup O, Zetterquist H.** HLA-DR typing by PCR amplification with PCR-SSP in two hours. Tissue Antigens 1992;39:225–235. – 25. **Cordell JL, Falini B, Erber WN.** Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984;32:219–229. – 26. **Dammrich J, Muller-Hermelink HK, Mattner A, Buchwald J, Ziffer S.** Histocompatibillity antigen expression in pulmonary carcinomas as indication of differentiation and of special subtypes. Cancer 65 (1990), 1942–1954. – 27. **Redondo M, Concha A, Oldiviela R et al.** Expression of HLA class I and II antigens in bronchogenic carcinomas: its relationship to cellular DNA content and clinical-pathological parameters. Cancer Res 51 (1991), 4948–4954. – 28. **Pantel K, Schlimok G, Kutter D et al.** Frequent down-regulation of major histocompatibility class I antigen expression on individual micrometastatic carcinoma cells. Cancer Res 51 (1991), 4712–4715. – 29. **Goepel JR, Rees RC, Rogers K, Stoddard CJ, Thomas WEG, Shepherd L.** Loss of monomorphic and polymorphic HLA antigens in metastatic breast and colon carcinoma. Br J Cancer 64 (1991), 880–883. – 30. **van Duinen SG, Ruiter DJ, Broecker EB et al.** Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma. Cancer Res 48 (1988), 1019–1025. – 31. **Brunner CA, Gokel JM, Riethmullerand G, Johnson JP.** Expression of HLA-D subloci DR and DQ by breast carcinomas is correlated with distinct parameters of favourable prognosis. Eur J Cancer 27 (1991), 411–416. – 32. **Esteban F, Concha A, Huelin C et al.** Histocompatibility antigens in primary and metastatic squamous cell carcinoma of the larynx. Int J Cancer 43 (1989), 436–442. – 33. **Natali P, Bigotti A, Cavalieri R et al.** Gene products of the HLA-D region in normal and malignant tissues of nonlymphoid origin. Hum Immunol 15 (1986), 220–233. – 34. **Connor ME, Stern PL.** Loss of MHC class-I expression in cervical carcinomas. Int J Cancer 46 (1990), 1029–1034. – 35. **Stein B, Momburg F, Schwarz V, Schlag P,. Moldenhauer G, Muller P.** Reduction or loss of HLA-A, B, C antigens in colorectal carcinoma appears not to influence survival. Br J Cancer 57 (1988), 364–368. – 36. **Wintzer HO, Benzing M, von Kleist S.** Lacking prognostic significance of 2-microglobulin, MHC class I and class II antigen expression in breast carcinomas. Br J Cancer 62 (1990), 289–295. – 37. **Ghosh AK, Moore M, Street AJ, Howat JMT, Schofield PF**. Expression of HLA-D sub-region products on human colorectal carcinoma. Int J Cancer 38 (1986), pp. 459-–464. – 38. **Kamma H, Yazawa T, Ogata T, Horiguchi H, Iijima T.** Expression of MHC class II antigens in human lung cancer cells. Virchows Arch B Cell Pathol 60 (1991), 407–412. – 39. **Dammrich J, Buchwald J, Papadopoulos T, Muller-Hermelink HK.** Special subtypes of pulmonary adenocarcinomas indicated by different tumor cell HLA-expression and stromal infiltrates. Virchows Arch B Cell Pathol 61 (1991), 9–18. – 40. **Scupoli MT, Sartoris S, Tosi G et al.** Expression of MHC class I and II antigens in pancreatic adenocarcinomas. Tissue Antigens 1996;48(4 Pt 1):301–311. – 41. **Banerjea A, Ahmed S, Hands RE et al.** Colorectal cancers with microsatellite instability display m-RNA expression signatures characteristics of increased immunogenicity. Molecular Cancer 2004; 3(21): 1–11. – 42. **Lovig T, Andersen SN, Thorstensen L et al.** Strong HLA-DR expression in microsatellite stable carcinomas of the large bowel is associated with good prognosis. Br J Cancer 2002; 87:756–762.

Correspondence to:
Tamiolakis Demetrio, M.D.
e-mail:cyto@chaniahospital.gr