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

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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

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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 (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