Abstract  Invariant chain (Ii) is a chaperone molecule that inhibits binding of endogenous antigens to class II molecules. High levels of Ii in cancer cells may prevent tumour antigen expression with class II and render the tumour less immunogenic. To correlate the expression of Ii and class II molecules in colon carcinomas with the density of tumour infiltrating lymphocytes (TILs), surgical specimens from a total of 48 patients with well-differentiated adenocarcinomas (WDAC), moderately (MDAC) and poorly differentiated adenocarcinomas (PDAC), adenoma with high-grade dysplasia (AdHGD) and adenomas were immunostained for Ii and class II antigen (HLA-DR). Aggregates of TILs were graded in H&E-stained sections. Normal colonic epithelium was negative for Ii and HLA-DR. Invasive carcinomas showed a linear increase in the expression of Ii in the progression from low- to high-grade tumours, while there was no significant difference in HLA-DR expression across the groups. Invasive carcinomas showed a disproportionate increase in Ii over HLA-DR. Frequency of TILs showed inverse correlation with expression of Ii and tumour grade. This is the first demonstration that expression of Ii increases in the progression from low- to high-grade colon neoplasms and is most marked in the poorly differentiated carcinomas. Ii expression in carcinomas is inversely related to the frequency of TILs. The findings suggest that increased Ii renders the tumour less immunogenic and less likely to stimulate a host immune response.

Key words  Invariant chain · HLA-DR · Colon neoplasms

Introduction

Tumour-host interactions influence the growth of neoplasms, and it has been suggested that patients with tumours containing more infiltrating lymphocytes (TILs) tend to have a better prognosis than those with fewer TILs [22].

One factor that might influence the host lymphocytic response to tumour is the expression of tumour antigens with class II molecules on the cell surface. Another factor that might influence the binding of peptides to class II molecules is the class II-associated invariant chain (Ii). It is a type-II membrane glycoprotein synthesized as different isoforms required for class II assembly in endoplasmic reticulum (ER). It plays a central part in the intracellular transport of class II molecules. When it remains associated with these molecules during their transport from the ER to the trans-golgi network, they are unable to bind endogenously derived peptides present in these compartments [2, 3, 6]. Increased expression of Ii in malignant neoplasms may thus prevent presentation of endogenous tumour antigens by class II molecules, and this in turn would prevent host immune response against the tumours.

Some human tumours, including colon carcinoma, express class II molecules (HLA-DR) [8, 13, 18]. However, class II expression in tumour cells does not always correlate with prognosis [5, 17, 18]. We aimed to correlate the expression of Ii and HLA-DR in colon neoplasms, including adenoma, adenoma with high-grade dysplasia and colon carcinoma, with the density of TILs. Our results showed that expression of Ii increased from well- to poorly differentiated carcinoma, and the carcinomas with high expression of Ii had fewer lymphocytic aggregates. These data support the speculation that expression of Ii prevents tumour cells from presenting their endogenous tumour antigens to T-lymphocytes and contributes to a
reduced host immune response to colon carcinomas. They also imply that Ii may be a potential marker for more aggressive behaviour of colon carcinoma.

Materials and methods

Colonic resections or biopsy specimens with well- (WDAC, \( n = 8 \)), moderately (MDAC, \( n = 12 \)) and poorly differentiated adenocarcinomas (PDAC, \( n = 5 \)), adenoma with high-grade dysplasia (AdH-GD, \( n = 8 \)), adenoma (\( n = 14 \)), and normal colon (NC, \( n = 8 \)) were identified in the surgical pathology files available at the University of Massachusetts Medical Center from 1991 to 1997. Cases with slides and blocks available were included in the study. Carcinomas included tumours in stage 0 (\( n = 8 \)); stage I (\( n = 3 \)); stage II (\( n = 10 \)); stage III (\( n = 8 \)); and stage IV (\( n = 4 \)). The 15 cases of adenoma included villous adenomas (VA, \( n = 5 \)), tubulovillous adenomas (TVA, \( n = 5 \)), and tubular adenomas (TA, \( n = 5 \)). Nine sections of normal bowel were obtained from the margins of the resection specimens or from the biopsies. All H&E-stained slides of the lesions were reviewed, and one representative section was selected from each case for immunostaining. Tumour grade was assessed according to standard criteria [15]. Tumour stage was obtained from surgical pathology reports.

The formalin-fixed, paraffin-embedded sections were cut at 4\( \mu \)m, heated at 60\(^\circ\)C for 30 min, then deparaffinized and hydrated through a series of xylenes and alcohols. Optimum pretreatment and dilutions were determined by testing with both known positive and negative material. Mouse monoclonal antibody against Ii (CD74) and HLA-DR (DAKO, Carpinteria, Calif.) at dilutions of 1:200 and 1:250, respectively, with antigen retrieval gave us the best signal-to-noise ratio in the positive test material and no staining in the negative test material. The slides were microwaved with a proprietary antigen retrieval solution (citrate buffer; BioTek Solutions, Santa Barbara, Calif.) for 5 min in an 800-W microwave oven. Following replenishment of this solution the slides were microwaved again for an additional 5 min and then allowed to cool for 20 min. The slides were stained on a BioTek Solutions TechMate 1000 automated immunostainer using an avidin-biotin complex (ABC) staining procedure (BioTek). Following a hydrogen peroxide block of endogenous peroxide and a serum-blocking step, the slides were incubated with the primary antibody for 45 min, followed by brief buffer washes and then incubation in a cocktail of biotinylated anti-mouse IgG/IgM and anti-rabbit IgG (BioTek) for 30 min. The sections were then washed, incubated in avidin-biotin complex (BioTek) for 30 min, washed, then reacted with diaminobenzidine and hydrogen peroxide to visualize the end-product. Sections were counterstained with haematoxylin. A duplicate set of slides was also stained in exactly the same manner except that normal mouse serum was substituted for the primary antibody, to serve as a negative control.

The expression of Ii and HLA-DR was graded as 0=no positive cells; 1+=<10% of tumour cells positive; 2+=10–30% of tumour cells positive; 3+=>30% of tumour cells positive. For data analysis, grade 2+ and 3+ staining was regarded as significant. TILs were evaluated around the periphery of the tumours and invasive nests of carcinoma cells, but not near areas of ulceration of tumour necrosis. Lymphoid aggregates were evaluated as follows: 0=no lymphoid aggregates or at most one single, small lymphoid aggregate in each tumour section; 1+=occasional, usually small lymphoid aggregates with rare or absent germinal centres; and 2+ =numerous, large lymphoid aggregates with frequent germinal centres [11].

![Fig. 1 Microscopic sections showing A invariant chain (Ii) and B class II antigen (HLA-DR) locally expressed in a well-differentiated adenocarcinoma of the colon, and C Ii diffusely positive and D HLA-DR positive in a few cells in a poorly differentiated adenocarcinoma of the colon](image-url)