

Major histocompatibility complex class II antigen (HLA-DR) expression by ileal epithelial cells in patients with seronegative spondylarthropathy

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Abstract

Major histocompatibility complex molecules act as non-specific receptors for antigenic proteins and present them to T-cells. Presented antigen together with class II molecules activates antigen specific T-helper cells and may trigger a cellular immune response. The expression of HLA-DR antigens by epithelial cells was examined with an indirect peroxidase technique in ileal biopsies from 38 patients with seronegative spondylarthropathy and features of acute or chronic gut inflammation on biopsy, 14 patients with chronic inflammatory bowel disease, 10 rheumatic and 10 non-rheumatic controls. In acute ileitis, there was more HLA-DR expression in villous and crypt epithelial cells than in non-inflamed controls ($p < 0.01$). In chronic inflammation and in chronic inflammatory bowel disease, class II antigens were more expressed in villus ($p < 0.02$) and crypt epithelium ($p < 0.01$). Strong HLA-DR expression in crypt epithelial cells was connected with active inflammation ($p < 0.02$). These findings suggest binding of unknown enterobacterial or nutritional luminal antigens to HLA-DR antigens normally present in enterocytes. The enterocytes act as antigen presenting cells causing a local increase of targets for activated T-cells and trigger the gut inflammation responsible for the clinical symptoms of the seronegative spondylarthropathy.

The major histocompatibility complex is a gene sequence encoding two classes of immunoregulatory cell surface glycoprotein antigens. Class I molecules are found on all nucleated cells whereas class II antigens are found on a limited number of cells including B-lymphocytes, macrophages, Langerhans cells, dendritic cells, vascular endothelial cells, and epithelial cells including differentiated absorptive cells (enterocytes).¹⁻⁶ In man class II molecules consist of α and β glycoprotein chains encoded by the HLA-D region of chromosome 6.⁷ At least three subregions (HLA-DR, DP, and DQ) are now defined. Antigen specific helper T-cells are activated by presented antigen together with class II molecules.^{8,9}

Normal ileal enterocytes express HLA-DR antigens within the brush border of the villous epithelium but there is no expression in crypt epithelial cells.¹⁻⁵ The epithelial class II expression is increased in jejunal epithelium of patients with untreated coeliac disease,¹⁰⁻¹² in ileum affected with Crohn's disease,¹³ and in colonic

epithelium in idiopathic inflammatory bowel disease.¹⁴⁻¹⁶

In previous studies we have shown the existence of mostly asymptomatic acute or chronic gut inflammation in 62% of patients with seronegative spondylarthropathy (reactive arthritis and peripheral arthritis in ankylosing spondylitis).^{17,18} The intestinal inflammation is often localised in the terminal ileum and has histological and immunohistochemical features of enterobacterial induced enterocolitis and early or subclinical Crohn's disease.¹⁸⁻²⁰ The clinical and endoscopic features of the disease vary paralleling the histological type of gut inflammation.^{20,21} Although it is known that non-steroidal anti-inflammatory drugs (NSAIDs) may cause small intestinal inflammation, there is no evidence that the gut inflammation in seronegative spondylarthropathy is related to the use of NSAIDs.²⁰ In a combined endoscopic and histologic study in out patients and in patients with rheumatoid arthritis we found no relation between NSAIDs intake and the histologic appearances of ileocolonic biopsies (submitted data). An hypothesis is that intestinal inflammation and increased permeability of the gut wall caused by enterobacterial or nutritional factors initiate immune reactions responsible for the clinical picture of seronegative spondylarthropathy.

As the binding of antigen to a class II molecule is an essential step in T-cell activation, we aimed to study the HLA-DR expression in ileal epithelial cells of patients featuring acute and chronic arthritis related gut inflammation. It was also the purpose of this study to compare HLA-DR expression in arthritis related gut inflammation and in chronic inflammatory bowel disease.

Methods

PATIENTS

Ileal biopsies from five patient groups were used. Approval of the Central Ethical Committee was obtained for the study as well as the patient's verbal informed consent.

Control patients

Biopsies from 10 patients who had ileocolonoscopy because of spastic colon, chronic constipation, large bowel adenocarcinoma, or colonic polyps were studied. The patients (four women, six men, mean age of 48.8 years (range 38-68)) had no evidence of chronic inflammatory bowel disease or rheumatic diseases and had normal histology on ileal biopsies.

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Rheumatic patients without gut inflammation

Ten biopsies were also taken from this group. The patients had rheumatoid arthritis, psoriatic arthropathy or lumbar back ache. None had clinical or histological signs of inflammatory bowel disease. Seven of these patients were treated with the NSAIDs Piroxicam or Indomethacin. There were four women and six men, mean age of 32 years (range 21–47).

Patients with acute intestinal inflammation and seronegative spondylarthropathy

Twenty cases were studied (six women and 14 men, mean age 35.3 years (range 18–65)). Histological examination revealed acute gut inflammation in all biopsies as we described earlier.^{17,18} In summary, acute inflammation is characterised by ileal or colonic epithelial infiltration with granulocytes and crypt abscesses with preserved crypt and villus architecture.

Fourteen of these patients were treated with the NSAIDs Piroxicam or Indomethacin.

Patients with chronic intestinal inflammation and seronegative spondylarthropathy

Biopsies of 18 patients were examined (five women, 13 men, mean age of 37.2 years (range 23–65)). They showed a chronic type of inflammation as we previously reported.^{17,18} Briefly, the foremost changes were epithelial alterations: villous blunting and fusion, villous surface of colonic mucosa, crypt distortion and atrophy, increased mixed lamina propria cellularity, and basal lymphoid aggregates in the propria.

From this group 13 patients were treated with the NSAIDs Piroxicam or Indomethacin.

Patients with chronic idiopathic inflammatory bowel disease

Biopsies of 14 patients were used (five women, nine men, mean age of 38.9 years (range 23–72)). Thirteen of them had established Crohn's disease and one had active ulcerative colitis with backwash ileitis. Six of the patients with Crohn's disease and the patient with ulcerative colitis were treated with oral sulphasalazine.

TISSUE STAINING PROCEDURE

Fresh terminal ileal mucosal biopsies were used, snap frozen in isopentane at -120°C in liquid nitrogen and stored at -70°C until sectioning. Serial 5 μ cryostat sections were mounted on chrome gelatin coated slides for immunostaining with an indirect peroxidase technique. The sections were air dried and before immunostaining briefly (10 min) fixed in acetone. After washing in phosphate buffered saline at pH 7.6, the sections were incubated for 30 minutes at room temperature with the monoclonal antibody OKIa1 (HLA-DR; Ortho Pharmaceutical Corporation, Raritan, New Jersey). The dilution of the mouse monoclonal antibody was of 10 $\mu\text{g}/\text{ml}$. After incubation the sections were three times washed for five minutes in buffer. Then they were incubated for 30 minutes with 1:20 diluted peroxidase labelled rabbit anti-mouse

immunoglobulin (Dakopatts, Glostrup, Denmark). Again the sections were rinsed three times for five minutes in the buffer solution. Peroxidase activity was revealed with a solution of 2.5 mg of 3,3'-diaminobenzidine-tetrahydrochloride with 0.01% H_2O_2 . After counterstaining with haematoxylin the sections were dehydrated and mounted. Controls for the procedure included intrinsic positive controls of follicular mantle lymphocytes in gut associated lymphoid tissue indicating the reliability of the immunohistochemical reaction as well as the staining intensity of the individual reaction so that daily variations did not influence the evaluation. For negative controls, the primary antibody was not applied. The only staining observed was in granulocytes (eosinophils and neutrophils) whose endogenous peroxidase activity was not blocked by $\text{H}_2\text{O}_2/\text{methanol}$.

EVALUATION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION

Each slide contained highly stained macrophages, dendritic interstitial cells and B-lymphocytes serving as internal references for interpretation of the staining intensity. The HLA-DR expression in epithelial cells was estimated semiquantitatively. It was scored as strongly positive (++) when the staining was as intense as that of the reference cells and if it was diffusely present in the cytoplasm of the epithelial cells. It was considered to be weakly positive (+) when it was less pronounced than the staining of reference cells and negative (-) when only reference cells were stained in the same area. The DR antigen expression was also evaluated with regard to the degree of inflammation in the biopsy. The presence of active inflammatory infiltrates or ulcers was particularly considered.

STATISTICAL ANALYSIS

Pearson χ^2 tests were used so that the HLA-DR expression in enterocytes was related to gut inflammation and to the degree of inflammation. The rejection level was fixed at $p < 0.05$.

Results

The results are summarised in Tables I and II.

CONTROLS

From the 10 normal controls and 10 controls with rheumatic diseases without accompanying gut inflammation, nine showed weakly positive HLA-DR antigen expression within the brush border of villus epithelium (Fig 1a). In one case of both groups, however, there was strong positive staining for HLA-DR antigens at the brush border of the enterocytes but not in the cytoplasm. In one case there was weak DR antigen expression in crypt epithelial cells.

The 14 controls featuring inflammatory bowel disease showed strong HLA-DR antigen expression in villus epithelium (12/14; $p < 0.02$). Also in crypt epithelium there was weak (eight of 14;

TABLE I Expression of HLA-DR antigen in terminal ileal epithelial cells in control groups and in arthritis related acute and chronic gut inflammation

HLA-DR in	Controls			Acute inflammation (n=20)	Chronic inflammation (n=18)
	Normal (n=10)	RD* (n=10)	IBD† (n=14)		
Villi +	9	9	2	2§	4
Villi ++	1	1	12§	18¶	14§
Crypts -	10	9	2‡	3‡	4
Crypts +	-	1	8¶	4	10¶
Crypts ++	-	-	4‡	13¶	4‡

*RD: other rheumatic diseases; ‡0.02 < p < 0.05 v controls; §0.01 < p < 0.02; ¶p < 0.01; †IBD: inflammatory bowel disease; ++: strong staining; +: weak staining; -: negative staining.

TABLE II Ileal epithelial MHC antigen expression in biopsies featuring active inflammation or chronic quiescent disease

HLA-DR in	Active inflammation (n=32)	Inactive quiescent disease (n=20)	p Value
Villi +	2	6	p < 0.02
Villi ++	30	14	NS
Crypts -	7	1	NS
Crypts +	6	17	p < 0.01
Crypts ++	19	2	p < 0.02

++: strong staining; +: weak staining; -: negative staining; NS: not significant.

p < 0.01) or strong (four of 14; p < 0.05) HLA-DR antigen expression in the cytoplasm (Fig 1b).

In all cases there were HLA-DR positive macrophages, mononuclear cells, arterial capillary, and venular endothelial cells present in the lamina propria. In several biopsies from inflamed and non-inflamed mucosa, lymphoid follicles were present. It was a constant finding, even in normal mucosa, that the epithelia overlying the lymphoid tissue had more diffuse cytoplasmic expression of class II molecules than the surrounding epithelial cells (Fig 1c).

ACUTE INTESTINAL INFLAMMATION AND SERONEGATIVE SPONDYLARTHROPATHY
HLA-DR antigens were strongly expressed in villus epithelial cells in 18/20 biopsies (p < 0.01) and in crypt cells of 17/20 biopsies (p < 0.01) (Fig 2a). The HLA-DR antigens were present in the villus brush border and in the entire cytoplasm of the enterocytes. In the crypt epithelial cells the HLA-DR antigens were present in the cytoplasm.

CHRONIC INTESTINAL INFLAMMATION AND SERONEGATIVE SPONDYLARTHROPATHY

There was strong HLA-DR antigen expression in the villus epithelium of 14/18 biopsies (p < 0.02) (Fig 2b). The staining reaction in crypt epithelial cells was weak in 10/18 biopsies (p < 0.01) and strong in four of 18 cases (p < 0.05).

It also appeared that the DR antigen expression was related to the local degree of inflammatory cell infiltration - that is, presence of ulcers, fissures or active inflammatory cell infiltrates (Table II). In 32 biopsies there was acute or active inflammation whereas 20 biopsies featured chronic quiescent disease. The villous DR

expression in chronic inflamed mucosa was weaker than in active inflammation (p < 0.02).

Crypt epithelial HLA-DR expression was stronger in acute and active ileal inflammation than in quiescent inflammation (p < 0.02).

Discussion

In this study we have shown an increased HLA-DR antigen expression in ileal villus and crypt epithelial cells of patients with seronegative spondylarthropathy and asymptomatic intestinal inflammation. DR expression was more intense in crypts of actively inflamed mucosa, regardless of the histological type of inflammation. This was a consistent finding in differentiating non-inflamed or chronically inflamed mucosa from actively inflamed gut mucosa indicating a correlation between class II molecule expression and degree of inflammation. These results are in agreement with findings of others in normal and diseased small intestinal mucosa.^{1 2 4-6 10-13 22}

Like others we found HLA-DR expression in the cytoplasm of the epithelium overlying lymphoid tissue.^{23 24} In inflamed mucosa the DR expression is diffuse in the cytoplasm of villus and crypt cells giving evidence of endosomal DR handling and release at the basolateral cell membrane as suggested by Bland.⁴ These observations confirm those reported in human and rat small intestine.²⁵⁻²⁸

Class II antigens, which are normally not expressed in crypt epithelial cells, can be induced in inflamed mucosa by gamma-interferon.⁴ This is true in certain autoimmune diseases, in idiopathic inflammatory bowel disease and in coeliac disease.^{3 12 29} In untreated coeliac disease the pattern of HLA-DR expression is related to the degree of disease activity and an increase of CD8+ intra-epithelial lymphocytes.¹⁰⁻¹² This implies that once HLA-DR expression is induced, antigen can be presented to T lymphocytes which leads then to activation of B and T lymphocytes.²⁷⁻³⁰ As the intensity of immune responses is related to the density of class II antigens,³¹ it might be expected that in our patients there would be an increase of intraepithelial T cells. Although the number of lamina propria lymphocytes was augmented, however, there was no increase of intraepithelial lymphocytes (submitted data).

The DR expression in ileal epithelial cells of patients with seronegative spondylarthropathy is not indicative of the aetiology of the disease because it also occurs in different inflammatory conditions in the intestine^{3 12 13} and in other organs.³²⁻³⁵ The major known function of class II molecules is to present antigen to autologous T cells. Increased HLA-DR expression indicates augmented antigen handling with intracellular processing, transfer to the basolateral cell membrane, antigen presentation and recognition by the T cell receptor.^{27-30 36 37} The class II molecule antigen complex creates a structural determinant recognised by T-helper cells, the predominant lymphocyte subset in the lamina propria (submitted data). T cells circulate rapidly through the basement membrane and then return to the lamina propria, both in inflamed and non-inflamed mucosa.³⁸

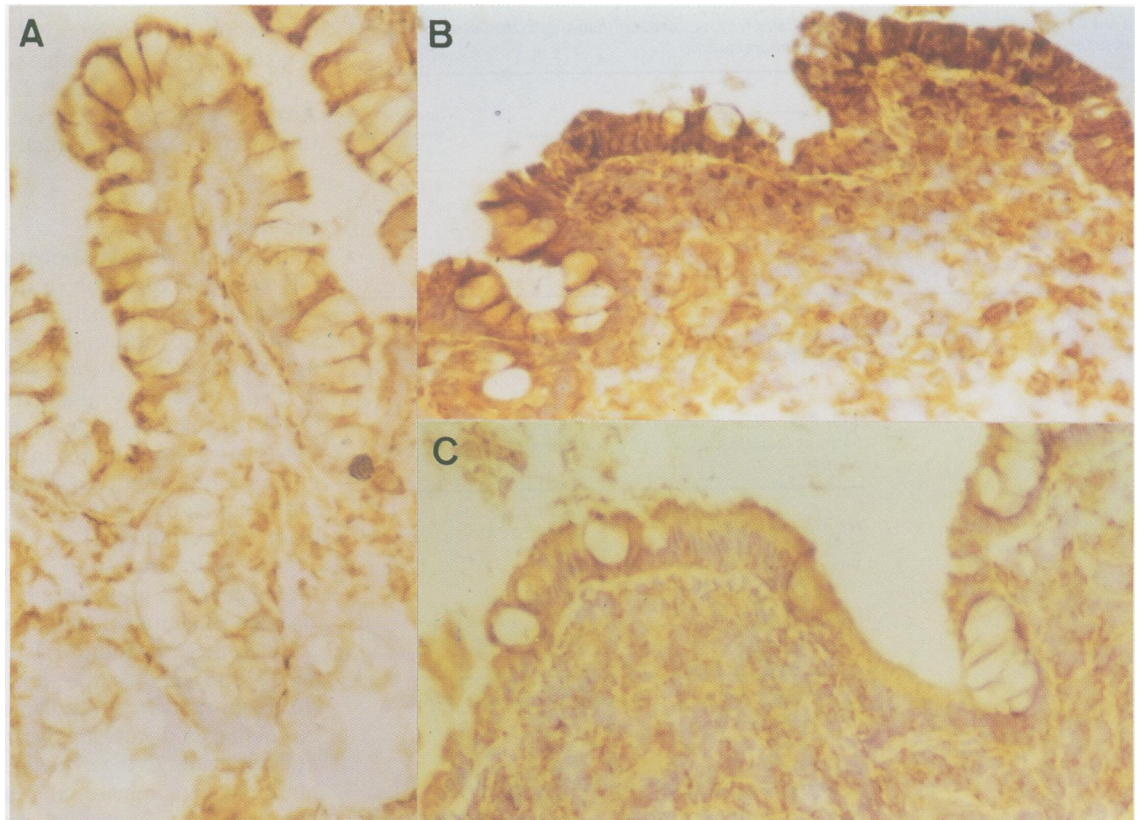


Figure 1: Immunostained frozen sections: (a) normal ileum with HLA-DR antigens on the brush border of enterocytes and on lamina propria cells; (b) strong HLA-DR expression in the cytoplasm of enterocytes and lamina propria cells in active Crohn's disease; (c) normal ileum with cytoplasmic HLA-DR expression in enterocytes overlying lymphoid tissue.

In patients with seronegative spondylarthropathy the pathogenesis of gut inflammation could be as follows: intestinal luminal antigen – enterobacterial or nutritional – binds to HLA-DR molecules, normally present at the brush

border of enterocytes, in genetically predisposed persons.^{39,40} This antigen class II molecule complex presents antigen via the endosomal pathway directly to T cells at the basolateral cell wall and triggers off local gut inflammation.³⁰

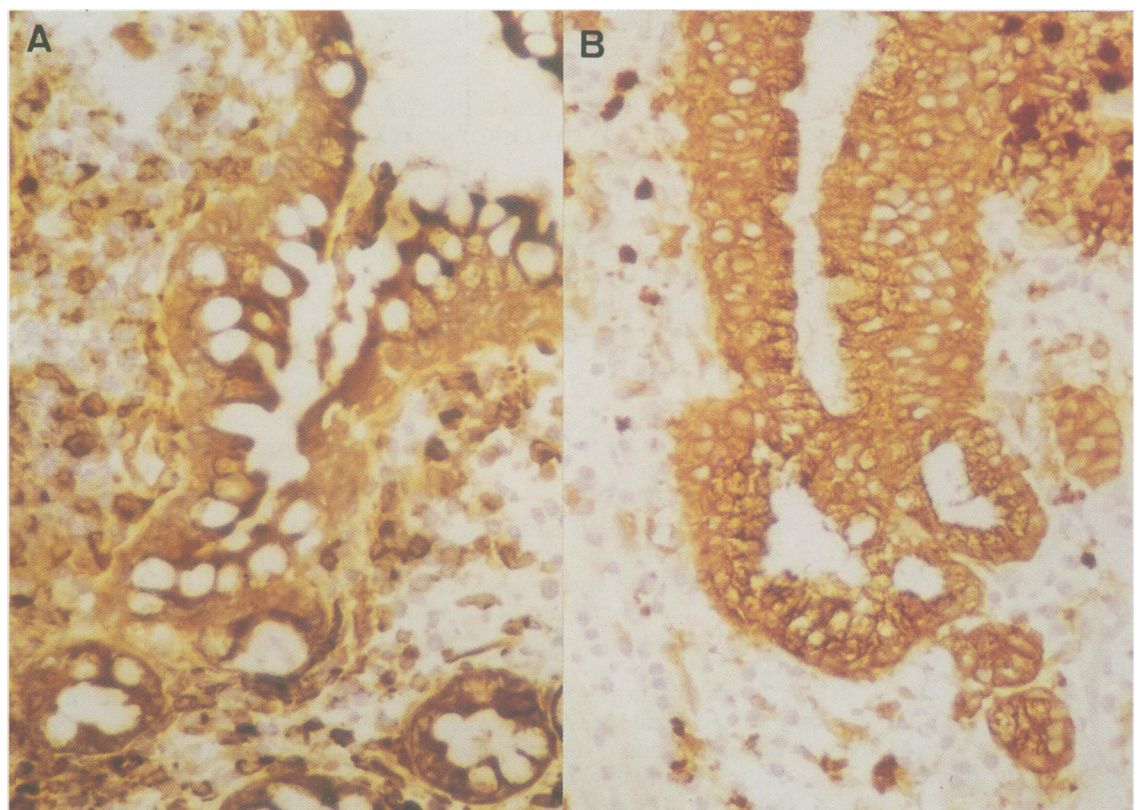


Figure 2: Immunostained frozen sections: (a) strong HLA-DR expression in enterocytes and crypt epithelial cells in acute ileitis; (b) chronic ileal inflammation with strong HLA-DR expression in the villus epithelium and weak staining in crypt cells.

Alternatively, DR molecules could act as an immunological molecular sieve, binding and giving access only to those antigens to which the individual can respond.⁴ This could explain why some subjects are genetically susceptible to develop ileal inflammation in the absence of enteropathogens. The so initiated intestinal inflammation could sustain the clinical picture of the seronegative spondylarthropathy.

As the same features of class II molecule expression in the presence of active inflammation are observed in chronic inflammatory bowel disease, including our cases of early or sub-clinical Crohn's disease, these are also possible mechanisms for initiating the inflammatory immunocascade responsible for the chronic gut inflammation of inflammatory bowel disease.

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