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Epithelial HLA-DR expression and lymphocyte subsets in gastric mucosa in type B chronic gastritis

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Summary. Lymphocyte subpopulations (B, T_4, T_8), monocytes/macrophages ($LeuM_5$, KiM5) and HLA-DR antigen expression were studied immunohistochemically in frozen sections from 32 antral and 37 fundal biopsies of type-B chronic gastritis. Aberrant HLA-DR antigen expression in epithelial cells of gastric mucosa was found in all cases closely related to mononuclear infiltrates. Epithelial HLA-DR expression and mononuclear infiltrates were practically absent in foci of intestinal metaplasia. These findings suggest that an immunopathologic mechanism probably plays a role in initiation or perpetuation of type-B chronic gastritis.

Key words: Type B chronic gastritis – HLA-DR – Lymphocyte subsets – Pathogenesis

Introduction

Two histologically identical types of chronic gastritis are recognized. Type A is relatively infrequent, involves the gastric body and accompanies pernicious anemia and other autoimmune disorders (Doniach et al. 1963; Buchanan et al. 1966). Type B involves the gastric antrum in most cases and is not associated with autoimmune disorders (Morson and Dawson 1979). Humoral immune mechanisms seem to play a central role in the pathogenesis of type A gastritis. Organ specific autoantibodies have been demonstrated by many investigators (Ardeman and Chanarin 1965; Jeffries and Sleisenger 1965; Hoedemaker and Ito 1970; Duchateau and Zeitoun 1983; Uibo et al. 1984).

In type B chronic gastritis irritants and/or infectious agents have been implicated in the pathogenesis (Edwards and Conghill 1966; Wyatt et al. 1986). In some cases, however, antibodies against gastric mucosal cell antigens have been demonstrated (Uibo and Krohn 1984; Vandelli et al. 1979).

In both types of chronic gastritis cellular immune mechanisms have been less extensively studied. In the majority of studies an in vitro cell response to gastric antigens has been sought (Salupere et al. 1972; Finlayson et al. 1972; Uibo and Salupere 1976; Ito et al. 1978).

Studies on the immunocompetent cell composition of the gastric lesion are very limited (Tsutsumi et al. 1984; Vecchi et al. 1985).

Presently, aberrant HLA-DR antigen expression on epithelial cells from organs involved by an autoimmune process, such as the salivary glands in Sjögren's syndrome (Lindahl et al. 1985; Moutsopoulos et al. 1986) thyroid follicles in Hashimoto and Grave's disease (Hanafusa et al. 1983; Jansson et al. 1984; Aichiger et al. 1985) bile ducts in primary biliary cirrhosis (Ballardini et al. 1984) have been demonstrated. Their role in presenting organ specific antigens and initiating or perpetuating autoimmune reactions has been emphasized (Bottazo et al. 1983; Todd et al. 1985).

The present study examined aberrant HLA-DR antigen expression in mucosal epithelial cells of type B chronic gastritis and its relationship to the lymphocytic subpopulations in order to elucidate the possible role of immune mechanisms.

Materials and methods

Multiple tissue specimens from 32 antral and 37 fundal gastric biopsies were taken through an Olympus GIF-1T flexible gastroscope from 55 patients. Tissue from both antrum and body

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in 14 cases from antrum only in 18 cases and from body only in 23 cases was sufficiently for immunohistochemistry. None of these patients had any clinical or serological evidence of an autoimmune disorder. A history of gastroduodenal ulcer was documented in 12 cases. A history of dyspepsia was the indication for gastroscopy in the remaining of the patients. In most of the cases the mucosa of the fundus and/or antrum appeared pathological with variable degrees of erythema, oedema and/or atrophy. Part of the biopsy specimens were routinely processed for light microscopy; the rest were immediately snap-frozen in isopentane-liquid nitrogen using as embedding medium OCT (Tissue Tek). The frozen specimens were stored at −70° C until processing.

Frozen cryostat sections 5 μm thick were air-dried and fixed in absolute acetone. They were stained with monoclonal antibodies against B-lymphocytes (Pan-B) T-helper/inducer cells (Leu 3a, b, T4), T-suppressor/cytotoxic cells (T8), monocytes/histiocytes (LeuM5, KiM5) and class II major histocompatibility complexes antigens (anti-HLA-DR) using a sensitive two step indirect immunoperoxidase technique as previously described.

The monoclonal antibodies against Pan-B, T4, T8 and HLA-DR antigens were purchased from Dakopatts (Denmark). Anti-Leu 3a, b and LeuM5 monoclonal antibodies were obtained from Becton and Dickinson (CA, USA). Anti-KiM5 monoclonal serum was kindly provided by Prof. K. Lennert (University of Kiel, FRG). Rabbit antimouse peroxidase conjugated and swine anti-rabbit peroxidase congugated serums were purchased from Dakopatts (Denmark). The monoclonal antibody DAKO-UCHL1 which stains satisfactorily T-cells in paraffin sections (Norton et al. 1986) has been used in control gastric tissue from 15 consecutive autopsies of children.

Frozen sections where the specific monoclonal antibody were omitted served as negative controls.

The distribution and relative number of B-lymphocytes, helper/inducer T-cells, suppressor/cytotoxic T-cells and monocytes/histiocytes were estimated. Intraepithelial (IEL) and extraepithelial (EEL) cell infiltrations were evaluated. The density of EEL cells was expressed in numbers per high power magnification field. In every case the whole section was examined and a final mean number was estimated. The cases finally separated into four groups by using the following grading system of cell infiltrate density: + = 0–30, + + = 30–65, + + + = 65–100, + + + + = > 100 positive cells per high power field. Areas with germinal centers were evaluated separately.

The IEL cell density was expressed in mean cell number per gland units. As gland unit was considered the sum up to 10 cryptal or glandular cells.

Staining results for HLA-DR antigens were expressed as percentages of positive epithelial mucosal cells and four groups of cases were developed: 0–25, 25–50, 50–75 and 75–100% positive cells.

Light microscopy sections were examined blindly by two independent observers. There was agreement in all but 3 fundal biopsies being categorized as either active or quiescent superficial gastritis. After reexamination a final agreement was achieved. Histological classification was made according to Morson and Dawson (1979). Presence of polymorphonuclear leukocytes was taken as a sign of active inflammation.

Results

From the 32 antral biopsies 2 showed active superficial, 25 showed active atrophic and 5 quiescent atrophic gastritis. From the 37 fundal biopsies 8 showed active superficial, 8 quiescent superficial, 19 active atrophic and 2 quiescent atrophic gastritis.

In 1/4 of the cases, areas of intestinal metaplasia were found in paraffin and/or frozen sections. Seven antral and twelve fundal biopsies with areas of intestinal metaplasia were studied immunohistochemically.

Mononuclear cell infiltrates were mainly found in lamina propria of both antral and fundal gastric biopsies. Their topographical distribution was closely related to the histological subtypes of the gastritis. In all cases mononuclear cells were infiltrating the lamina propria densely around the crypts. In cases of superficial chronic gastritis the mononuclear infiltrates were located almost exclussively around the mucosal crypts (Fig. 1). The degree of their extension to the deeper parts of the mucosa depended on the severity of atrophic gastritis. In severe cases the mononuclear cells infiltrated densely the whole gastric mucosa (Figs. 2 and 3). In less severe cases of atrophic gastritis the mononuclear cell infiltrates were less prominent in deeper areas and focally distributed.

B-lymphocytes were less numerous than the other mononuclear cells and represented in most cases less than 50% of the total T-cells. In 22 antral and 20 body gastric biopsies well developed follicles with germinal centres were recognized. They were located either superficially or in the deeper mucosa. B-lymphocytes were practically absent in intraepithelial positions.

Helper/inducer T-lymphocytes were the frequent cell type in extraepithelial areas of lamina propria. In the majority of the cases the relative numbers of helper/inducer to suppressor/cytotoxic T-lymphocytes ranged from 1,2:1 to 2:1. When areas with germinal centres were excluded, this ratio was lower (around 1:1) and in some cases suppressor/cytotoxic T-lymphocytes predominated. This was due to the relatively small numbers of suppressor/cytotoxic T-lymphocytes in the follicles. Both helper/inducer and suppressor/cytotoxic T-lymphocytic infiltrates were denser in the cryptal areas (Fig. 1 and 4). In severe cases of atrophic gastritis both T-cell subpopulations were observed in significant numbers in the deeper mucosa. Helper/inducer T-lymphocytes were infrequently seen within the epithelium (Fig. 2). The majority of IEL cells expressed the suppressor/cytotoxic phenotype (Fig. 4). Up to 10 suppressor/cytotoxic T-lymphocytes per glandular unit were found.

Most of IEL suppressor/cytotoxic T-lymphocytes were found in the crypts although their presence in glands of the body was not infrequent.

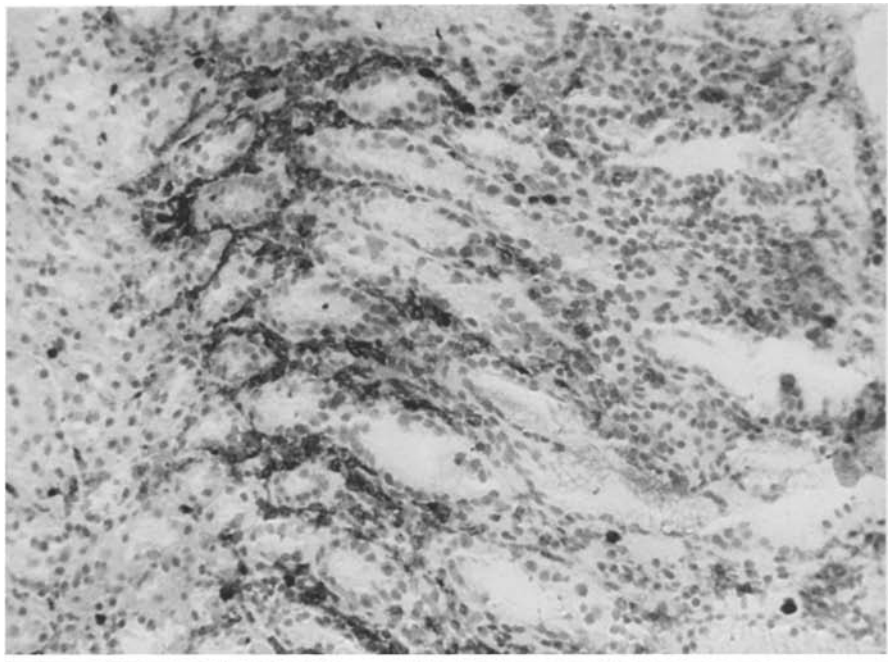

Fig. 1. Gastric body biopsy. T4-helper cells infiltrating the cryptal areas of mucosa predominantly. Immunoperoxidase × 120

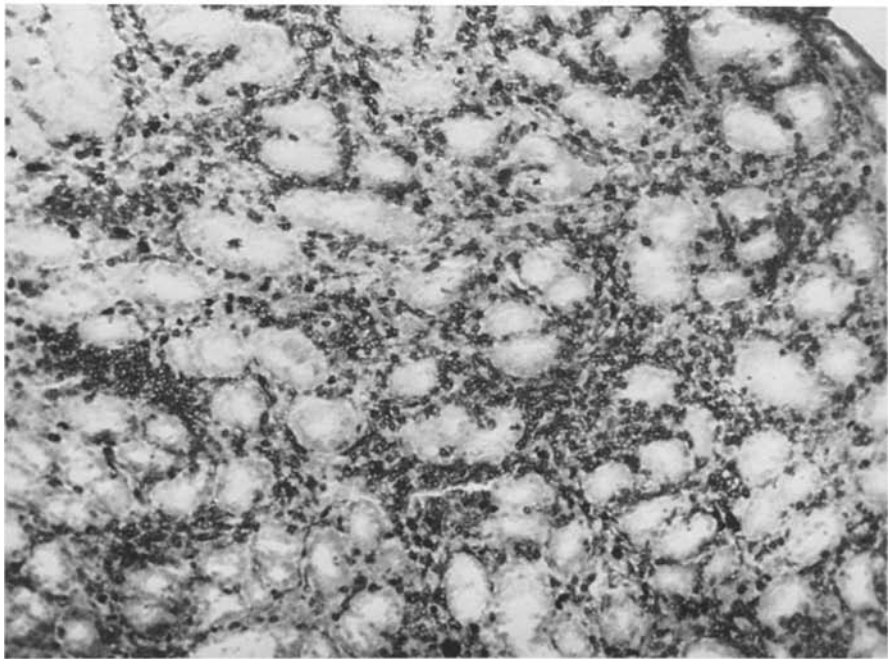

Fig. 2. Dense T4-cell infiltrates in deeper parts of gastric mucosa. Immunoperoxidase × 80

The distribution and number of monocytes/histiocytes (LeuM5 and KiM5 positive) paralleled those of T-lymphocytes and especially of the helper T-lymphocytes. In some areas they were densely located around crypts or glands like tight sleeves. However they were not found intraepithelially.

In areas of intestinal metaplasia B-lymphocytes, T-lymphocytes and monocytes/histiocytes were very rare (Fig. 5). In only two cases a mild to moderate mononuclear cell infiltration was observed especially at the periphery of the metaplastic foci. IEL suppressor/cytotoxic T-lymphocytes were also rare in metaplastic areas. They never exceeded 4 cells per glandular unit. Most of the cells seen among metaplastic glands were plasma cells.

In general HLA-DR positive epithelial cells were found close to lymphocytic infiltrations. In all cases the more steadily and intensely stained areas were those of the crypts. The percentages of HLA-DR positive epithelial cells ranged from 3 to 100 per cent (Fig. 6a , b) and paralleled the severity of the gastric inflammation. A relationship

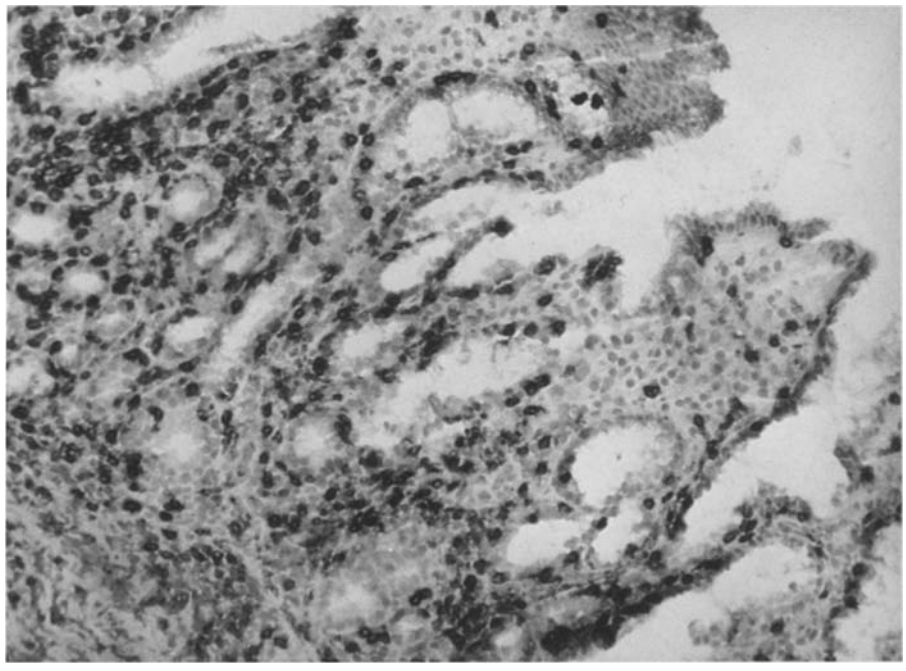

Fig. 3. Whole-depth T8-cell infiltration of the antral mucosa in a case of atrophic gastritis. Immunoperoxidase ×120

between percentages of HLA-DR positive epithelial cells and number of T-cells especially of the T_4 cell phenotype was observed in most cases (Table 1). Epithelial cells of gastric body glands were mostly negative, but in some cases small positive nests were found, especially in areas with lymphocytic infiltrations.

In the foci of intestinal metaplasia the epithelial cells were practically negative for HLA-DR antigens (Fig. 7). Only in three cases was mild HLA-DR expression noted in the cytoplasm of the metaplastic glandular cells, with a denser accentuation at the luminal surface.

A high percentage of EEL mononuclear cell infiltrates was HLA-DR positive, apparently corresponding to B-lymphocytes, histiocytes and activated T-lymphocytes (Fig. 7).

Difficulty in defining normal gastric mucosa is well recognized and it is impossible to perform gastric biopsies in clinically symptomless persons. For these reasons we examined immunohistochemically snap-frozen gastric tissues from two healthy children, taken shortly after their accidental death. In both cases antral and fundal mucosa showed only rare B- or T-lymphocytes. They never exceeded 3 cells per high power field. The epithelial cells were practically negative for HLA-DR antigen expression.

Additionally histological gastric sections from 15 consecutive autopsies of children under 12 years of age were searched for the presence of lymphoid follicles and mononuclear cell infiltrates. No follicles were found in the gastric body of any case. In 3 children (two with pyloric stenosis) there were some lymphoid follicles in antral mucosa but never exceeding one follicle per histological section.

Mononuclear cell infiltration in areas other than lymphoid follicles was rare. In 10 cases an attempt to reveal T-lymphocytes on paraffin sections was successful using DAKO-$UCHL_1$ monoclonal antibody and immunoperoxidase method.

Table 1. Relationship between the density of T4-cell infiltrates and the epithelial HLA-DR positivity

Number of T4-cells	% HLA-DR Positive epithelium				Total No
	0–25	25–50	50–75	75–100	
ANTRUM					
+		1	2		3
++		1	4	5	10
+++			1	8	9
++++				10	10
		2	7	23	32
BODY					
+	10	3	2		15
++	1	2	1	4	8
+++			2	8	10
++++				4	4
	11	5	5	16	37

+ = 0–30, ++ = 30–65, +++ = 65–100, ++++ = >100 cells/high power field

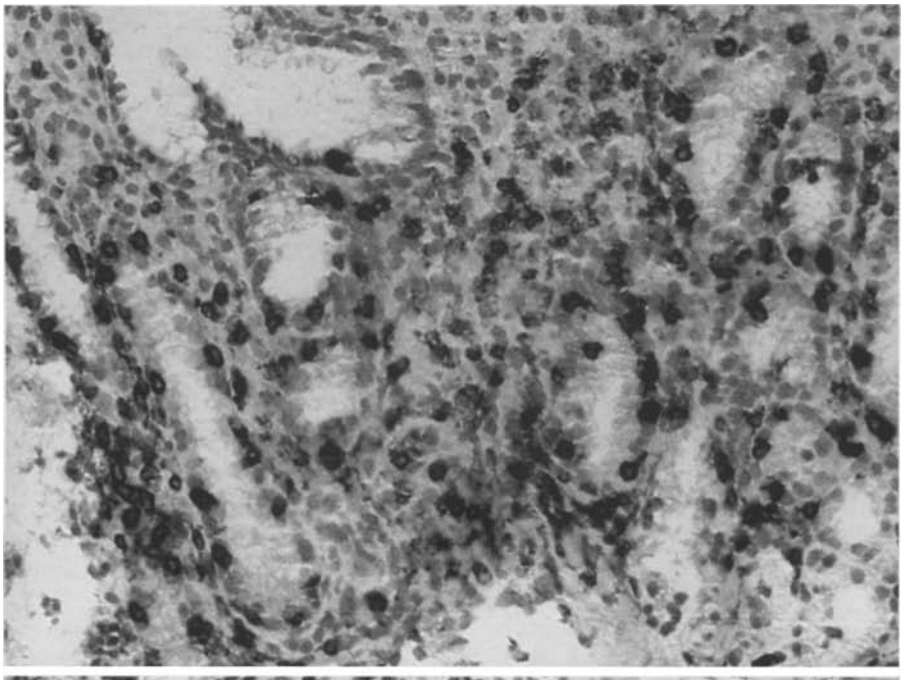

Fig. 4. Numerous intra-epithelial T8-cells in cryptal areas. Immunoperoxidase ×200

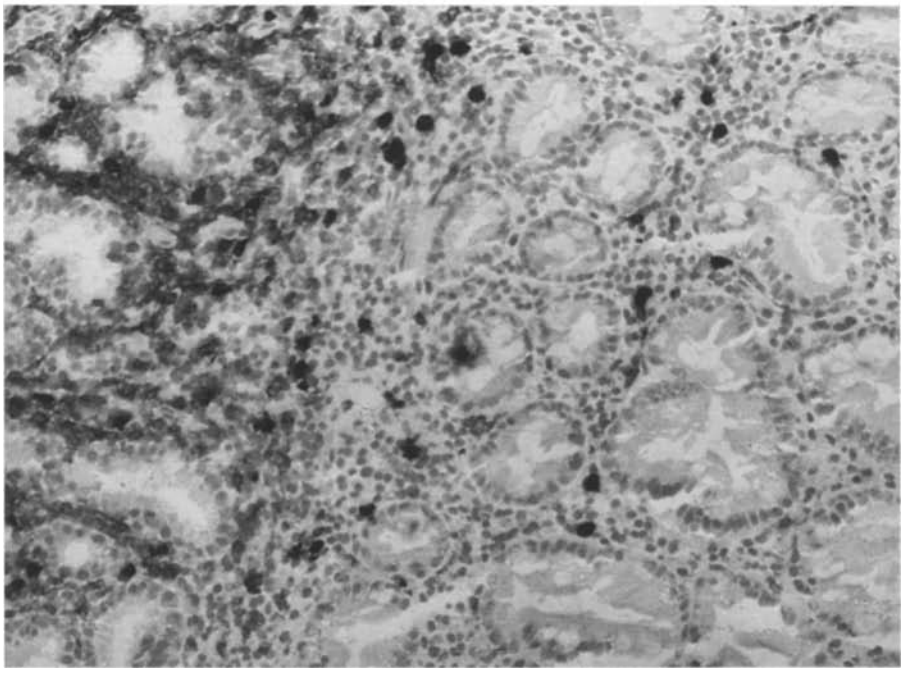

Fig. 5. T4-cells are absent in the area of intestinal metaplasia and numerous away from it. Immunoperoxidase ×200

Cell counts never exceeded 5 cells per high power field.

Discussion

In normal and inflamed mucosa of the small and large bowel the majority of IEL cells express the suppressor/cytotoxic T-lymphocyte phenotype, while in the EEL cells of the lamina propria the cells which predominate have the helper/inducer T-lymphocyte phenotype (Selby et al. 1981). Although such studies in gastric mucosa are less extensive, they seem to show a similar cell distribution, especially in cases of chronic gastritis (Vecchi et al. 1985; Tsutsumi et al. 1984). Aberrant HLA-DR expression by the epithelium of intestinal mucosa has been demonstrated in inflammatory conditions like ulcerative colitis and Crohn's disease (Selby et al. 1983).

In the present study of type-B chronic gastritis

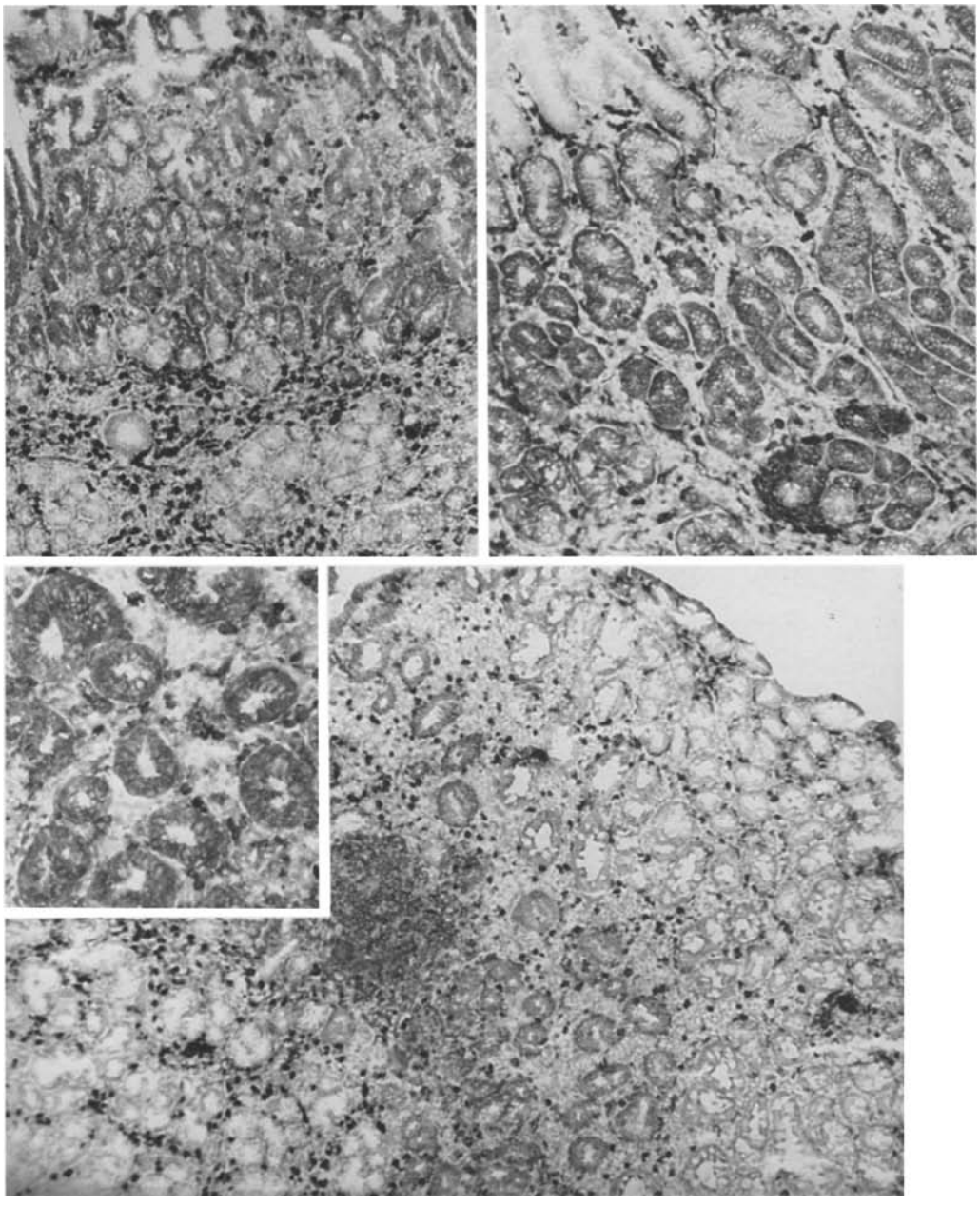

Fig. 6. HLA-DR positive epithelia located in cryptal (**a**) and in deeper parts of the mucosa. Immunoperoxidase ×60 and ×100 respectively

Fig. 7. HLA-DR positive epithelial cells and round cell infiltrations at the periphery of an area of intestinal metaplasia which lacks HLA-DR positivity. ×60 (Inset ×200)

in both antral or fundal biopsies the IEL cell infiltrates express almost exclusively the suppressor/cytotoxic T-lymphocyte phenotypes. In contrast, in the extraepithelial spaces the helper/inducer T-lymphocytes were more numerous than the suppressor/cytotoxic T-lymphocytes but in lower ratios than those reported in intestinal mucosa (Selby et al. 1981).

In all biopsies of our chronic gastritis cases a variable degree of aberrant epithelial HLA-DR expression was present. The extent of positivity was analogous to the severity of chronic gastritis and it was located in proximity to the mononuclear cell infiltrates. Constant HLA-DR antigen expression by the cryptal epithelial cells can easily be attributed to the fact that in all stages of chronic

gastritis this area represents the main site of inflammation. The fact that aberrant epithelial HLA-DR expression closely relates to the degree of lymphocytic infiltrate is supported by the observation that in areas with intestinal metaplasia, where lymphocytic infiltrates are rare, aberrant HLA-DR antigen expression is absent. The minimal HLA-DR antigen expression at the luminal surface of the metaplastic epithelium in 3 cases can be attributed to the tendency of these areas to mimic normal small intestinal epithelium (Scott et al. 1980; Selby et al. 1981). The tendency towards "normalization" of the intestinal metaplasia areas in chronic gastritis has been supported by the findings of Tsutsumi et al. (1984), who demonstrated that the predominant plasma cells in these areas are IgA positive cells and that the metaplastic epithelium contains the secretory component.

The epithelial HLA-DR expression in all cases of type-B chronic gastritis supports the notion that an autoimmune mechanism may play some role in either initiation or perpetuation of the chronic inflammation. This hypothesis is also supported by the observations of other investigators who showed autoantibodies to gastric cells (Uibo and Krohn 1984) and cell mediated hypersensitivity to gastric antigens (Ito et al. 1978) in some cases of type-B chronic gastritis.

The functional role of HLA-DR antigens is to mediate communication among immunocompetent cells. The induction of activated helper T-lymphocytes requires presentation of specific antigens by HLA-DR positive cells as are macrophages, B-lymphocytes and certain T-lymphocytes (Hämmerling 1976).

A similar spatial arrangement between helper T-lymphocytic infiltrates and aberrant epithelial HLA-DR expression has been recently reported in various autoimmune states (Hanafusa et al. 1983; Ballardini et al. 1984; Jansson et al. 1984; Lindahl et al. 1985; Moutsopoulos et al. 1986). It is proposed that such epithelial cells possibly interfere with the presentation of preexisting auto-antigens to the immunocompetent cells and thus an autoimmune state begins. Alternatively the inappropriate HLA-DR antigen expression can be the result of the activated helper T-lymphocytes. In vitro experiments revealed that γ-interferon (Bottazzo et al. 1983; Todd et al. 1985), as well as other stimuli such as lectins (Pujol-Borrell et al. 1983) and hormones (Klareskog et al. 1980) induce aberrant HLA-DR antigen expression. In the case of type-B chronic gastritis the initiating factor of the inflammation is unknown.

Recently a bacterium named Campylobacter pyloridis has been found in 90% of type-B chronic gastritis cases and has been proposed as the aetiological factor (Wyatt et al. 1986). Antibodies against C. pyloridis have been found to circulate (Rathbone et al. 1986) or absorbed by bacteria in chronic gastritis lesions (Wyatt et al. 1986). The classes of the absorbed anti-C pyloridis antibodies have been related to the activity of gastric inflammation. It is interesting that the distribution of epithelial HLA-DR expression in our material parallels the tissue distribution of C. pyloridis reported by Wyatt et al. (1986). More specifically, numerous bacteria were found by Wyatt et al. in the crypts where abundance of HLA-DR expression was noted by us. In contrast, these bacteria were absent in the intestinal metaplastic areas where we found lymphocytic infiltrates and aberrant HLA-DR expression to be minimal.

All the above suggest the possibility that C. pyloritis bacterial products may result in HLA-DR expression either directly, like lectins, or indirectly through γ-interferon production by activated T-lymphocytes after a local absorption of specific antibacterial antibodies.

References

Aichinger G, Fill H, Wick G (1985) In situ immune complexes, lymphocytes subpopulations, and HLA-DR-positive epithelial cells in Hashimoto thyroiditis. Lab Invest 52:132–140

Ardeman S, Chanarin I (1965) Intrinsic factor antibodies and intrinsic factor mediated vitamin B_{12} absorption in pernicious anaemia. Gut 65:436–440

Ballardini G, Biachi FB, Doniach D, Mirakian R, Pisi E, Bottazzo GF (1984) Aberrant expression of HLA-DR antigens on bileduct epithelium in primary biliary cirrhosis: Relevance to pathogenesis. Lancet II:1009–1013

Bottazzo GF, Pujol-Borrell R, Hanafusa T (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet II:1115–1117

Buchanan WW, Cox AG, Harden RMC G, Glen AIM, Anderson JR, Gray KG (1966) Gastric studies in Sjögren's syndrome. Gut 7:351–355

Doniach D, Roitt IM, Taylor KB (1963) Autoimmune phenomena in pernicious anaemia: serological overlap with thyroiditis, thyrotoxicosis and systemic lupus erythematosus. Brit Med J I:1374–1377

Duchateau A, Zeitoun P (1983) Parietal cell autoantibodies: localization of their binding sites on isolated gastric glands. Clin Exp Immunol 53:406–412

Edwards FC, Coghi'l NF (1966) Aetiological factors in chronic atrophic gastritis. Brit Med J II:1409–1413

Finlayson NDC, Fauconnet M, Krohn K (1972) In vitro demonstration of delayed hypersensitivity to gastric antigens in pernicious anemia. Am J Dig Dis 17:631–636

Hämmerling GL (1976) Tissue distribution of Ia antigens and their expression on lymphocyte subpopulations. Transplant Rev 30:64–68

Hanafusa T, Chiovato L, Doniach D, Pujol-Borrell R, Russel RCG, Bottazzo GF (1983) Aberrant expression of HLA-

DR antigen on thyrocytes in Graves' disease: Relevance for autoimmunity Lancet II:1111–1118
Hoedemaker PJ, Ito S (1970) Ultrastructural localization of gastric parietal cell antigen with peroxidase-coupled antibody. Lab Invest 22:184–192
Ito H, Pitchumoni CS, Glass GBJ (1978) Detection of cellular immunity derangements in chronic gastritis by a skin window test. Am J Dig Dis 23:919–925
Jansson R, Karlsson A, Forsum U (1984) Intrathyroidal HLA-DR expression and lymphocyte phenotypes in Graves' thyrotoxicosis. Hashimoto's thyroiditis and nodular colloid goitre. Clin Exp Immunol 58:264–278
Jeffries GH, Sleisenger MH (1965) Studies of parietal cell antibody in pernicious anemia. J Clin Invest 44:2021–2025
Klareskog L, Forsum U, Peterson PA (1980) Hormonal regulation of the expression of Ia antigens on mammary gland epithelium. Eur J Immunol 10:958–966
Lindahl G, Hedfords E, Klareskog L, Forsum U (1985) Epithelial HLA-DR expression and T-lymphocyte subsets in salivary glands in SJögren's syndrome. Clin Exp Immunol 61:475–482
Moutsopoulos HM, Hooks JJ, Chou CC, Dalavanga YA, Skoupouli FN, Detric B (1986) HLA-DR expression by labial minor salivary gland tissues in SJögren's syndrome. Am Rheum Dis 45:677–683
Morson BC, Dawson IMP (1979) Gastrointestinal pathology. Blaeckell Scientific Publications, Oxford, pp 95–108
Norton AJ, Ramsay AD, Smith SH, Beverley PCL, Isaacson PG (1986) Monoclonal antibody ($UCHL_1$) that recognises normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol 39:399–405
Pujol-Borrell R, Hanafusa T, Chiovato L, Bottazzo GF (1983) Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. Nature 304:71–75
Pathbone BJ, Wyatt JI, Worsley BW (1986) Systemic and local antibody responses to gastric Campylobacter pyloritis in non-ulcer dyspepsia. Gut 27:642–648
Salupere V, Nutt H, Järve R (1972) Lymphocyte blast transformation test in vitro in case of chronic gastritis. Scand J Gastroenterol 7:215–221
Scott H, Solheim BG, Brandtzaep P, Thursby E (1980) HLA-DR like antigens on the epithelium of the small intestine. Scand J Immunol 12:77–81
Selby WS, Janossy G, Goldstein G, Jewll DP (1981) T-lymphocyte subsets in human intestinal mucosa: The distribution and relationship to MHC-derived antigens. Clin Exp Immunol 44:453–458
Selby WS, Janossy G, Mason DY, Jewell DP (1983) Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. Clin Exp Immunol 53:614–619
Todd I, Pujol-Borrell R, Hammond LJ, Bottazzo GF, Feldmann M (1985) Interferon-y induces HLA-DR expression by thyroid epithelium. Clin Exp Immunol 61:265–273
Tsutsumi Y, Nagura H, Watanabe K (1984) Immune aspects of intestinal metaplasia of the stomach: an immunohistochemical study. Virch Arch A (Pathol Anat) 403:345–359
Uibo RM, Krohn KJE (1984) Demonstration of gastric cell autoantibodies in antral gastritis with avidin-biotin Complex antibody technique. Clin Exp Immunol 58:341–347
Uibo R, Krohn K, Villako K, Tammur R, Tamm A (1984) Parietal cell, gastric cell and thyroid autoantibodies in a population sample, with relationship to the state of gastric mucosa. Scand J Gastroenterol 19:1075–1081
Uibo R, Salupere U (1976) Leukocyte migration inhibition and parietal cell antibody test in chronic gastritis without pernicious anemia. Acta Med Acad Sci Hung 33:321–326
Vandelli C, Bottazzo GF, Doniach D, Francheschi F (1979) Autoantibodies to gastric producing cells in antral (Type B) chronic gastritis. N E J Med 300:1406–1411
Vecchi M, Berti E, Primignani M, Monti M, Agupe D, Torgano G, Arosio de Francis R (1985) In situ identification of immune competent cells in gastrointestinal mucosa: an evaluation by immunoelectronmicroscopy. Virch Arch A (Pathol Anat) 406:407–415
Wyatt JI, Rathbone BJ, Heatley RV (1986) Local immune response to gastric Cambylobacter in non-ulcer dyspepsia. J Clin Pathol 39:863–868

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