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— Original Article —

Colonic lymphoid cell subsets and epithelial HLA-DR antigens in familial polyposis coli

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Summary: Although there has been some evidence suggesting that immunological mechanisms are involved in the pathophysiology of familial polyposis coli (FPC), there has not been any report as to whether there is any abnormality of lymphoid cell subsets as background, whether polyps (adenomas) show different lymphoid cell subsets from the normal mucosa, or whether HLA-DR antigens are expressed on the epithelia of adenomas. Lymphoid cell subsets (CD5, CD4, CD8, IgA1, IgA2, IgM, IgD, IgG, and IgE positive cells) in the lamina propria, and HLA-DR antigens on the epithelia were studied in 7 patients with FPC. From each patient, 2 specimens were obtained from both the normal (non-polypoid) area and the polyp. Normal colonic mucosa, taken from 15 patients with conventional polyps or colorectal cancer, served as the normal control mucosa. Lymphoid cell subsets and HLA-DR antigens were identified by indirect immunoperoxidase staining using mouse anti-human monoclonal antibodies. In the normal area of FPC, lymphoid cell subsets were similar to those of normal control mucosa except for an increase in IgD positive (IgD+) cells. However, definite alterations were observed in the polyp. There were significant increases in the number of CD4+ and IgG+ cells, and in the sum of five classes of Ig+ cells compared to the normal area or normal control mucosa. HLA-DR antigens were not expressed in the normal control mucosa or in the normal areas, and only on the epithelia of the polyp in 5 out of 7 specimens (71%). These results clearly demonstrate that immunological reactions are involved in FPC polyps. *Gastroenterol Jpn 1989;24:632–639*

Key words: *familial polyposis coli (adenomatosis coli); HLA-DR antigens; lymphoid cell subsets; colonic epithelium; immunology*

Introduction

Familial polyposis coli (FPC) is characterized by innumerable adenomas throughout the large intestine and malignant change which invariably occurs at an early age. It is inherited as an autosomal, dominant Mendelian character. However, the etiology of the disease is unknown[1]. Natural killer (NK) cells have been suggested to play a major role in the surveillance of tumor development. We have already studied NK cells and their activity (NK activity) of both peripheral blood lymphocytes and colonic mucosal lymphocytes in FPC, and both were found to be normal[2]. It has been reported that there is an increase of the lymph follicles in the terminal ileum in FPC[3,4]. We also recognized a higher frequency of lymph follicles in normal (non-polypoid) areas in FPC than in normal colonic mucosa from healthy individ-

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Table 1 Cases

Patient No.	Sex & age (years)	Approximate number of polyps	Malignant change
1	M 15	30	–
2	F 17	150	–
3	F 31	1600	–
4	F 25	600	Dukes A
5	F 30	1300	Dukes A
6	F 41	700	Dukes B
7	F 28	500	Dukes C

uals[5]. These observations suggested the existence of a local immune response in FPC. However, there has not as yet been any report as to whether there is any abnormality of lymphoid cell subsets as background in FPC.

HLA-DR antigens have been shown to be required in antigen presentation and immunoregulation. Recently, it was reported that HLA-DR antigens are aberrantly expressed on colonic epithelia in cancer of the large bowel[6-12]. However, there has not been any report as to whether HLA-DR antigens are expressed on the colonic epithelia in FPC. To address these questions, therefore, lymphoid cell subsets and HLA-DR antigens on the colonic epithelia were studied in both polypoid (adenomatous) and normal (non-polypoid) areas.

Materials and Methods

Materials

Fourteen specimens from 7 patients (6 females, 1 male) with FPC were obtained either by operation (13) or endoscopic biopsy (1). The ages of cases ranged from 15 to 41 years (mean 27 years). The numbers of polyps in the large intestine ranged from 30 to 1600 (**Table 1**). Four cases had malignant changes, and the Dukes stages of these cases are listed in **Table 1**. All cases had normal levels of serum CEA (less than 4ng/ml) except case 5 in which serum CEA was 7.2ng/ml. In each case, specimens were obtained from both polypoid (adenomatous) areas and neighboring normal (non-polypoid) mucosa. Fifteen specimens were obtained from 15 patients (11, colonic cancer; 2, colonic adenoma; 2, no colonic disease) which served as normal controls. All specimens were obtained from areas which were macroscopically normal in appearance. In cases with colorectal cancer or adenoma, the specimens were obtained from areas far from the lesions.

Methods

Lymphoid cell subsets in the lamina propria, and HLA-DR antigens on the colonic epithelia were identified by the indirect immunoperoxidase staining method. Tissue fixation, tissue mounting and immunoperoxidase staining were performed as previously described[13]. Briefly, tissue specimens were fixed with periodate lysine-4% paraformaldehyde, and then 6 μm serial frozen sections were then made. Endogenous peroxidase activity was blocked with 0.05M periodic acid and 0.03M sodium borohydride. Mouse anti-CD5 (Leu 1)[14], CD8 (Leu 2)[15,16], CD4 (Leu 3)[15,16], IgA1[17], IgA2[17], IgG[18], IgM[18], IgD[19] (Becton-Dickinson, Mountain View, CA, USA), and IgE[20] (Yamasa Shoyu, Choshi, Japan) monoclonal antibodies were applied as the first antibody. Two mouse monoclonal antibodies, anti-HLA-DR[21] (Becton-Dickinson) and Nu-Ia[22] (Nichirei, Tokyo, Japan), were used as the anti-HLA-DR antibody. Peroxidase-labeled anti-mouse Ig (DAKO, Copenhagen, Denmark) was used as the second antibody. For nuclear staining after diaminobenzidine reaction, 0.1% methyl green was used. One serial section, stained in the absence of the first antibody, served as the control specimen. Another section was stained with hematoxylin and eosin.

Positive cells in the lamina propria were counted microscopically under 400× magnification and expressed as cells/mm^2 as previously described[23]. Data were statistically compared using the Wilcoxon test, and $P<0.05$ was considered to be significant.

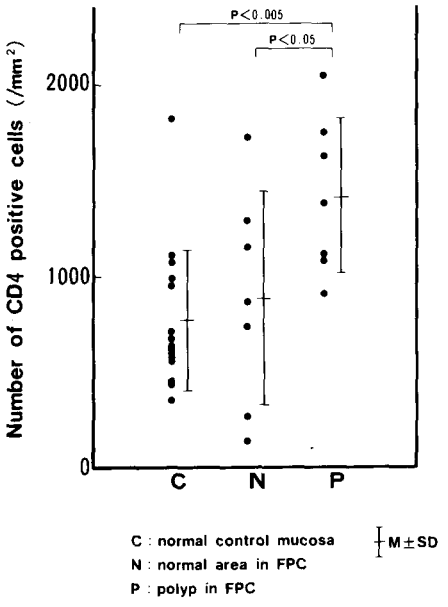

Fig. 1 The number of CD4 positive cells in the lamina propria of the colon.

Results

1. T cells

The number of CD5 positive cells (pan T cells) in the normal control mucosa, normal area of FPC, and polyps of FPC was $1227\pm630/mm^2$, $1485\pm713/mm^2$ and $1873\pm243/mm^2$ respectively, and there were no differences among the three sites. The number of CD8 positive cells (suppressor/cytotoxic T cells) was almost equal in the three sites, with $521\pm343/mm^2$ in the normal control mucosa, $590\pm308/mm^2$ in the normal area, and $515\pm208/mm^2$ in the polyp. The number of CD4 positive cells (helper/inducer T cells) in the polyp was $1418\pm409/mm^2$, which was significantly higher than $769\pm374/mm^2$ in the normal control mucosa ($P<0.005$), and $883\pm565/mm^2$ in the normal area ($P<0.05$) **(Figs. 1 and 2)**. There was no difference in the number of CD4 positive cells between the

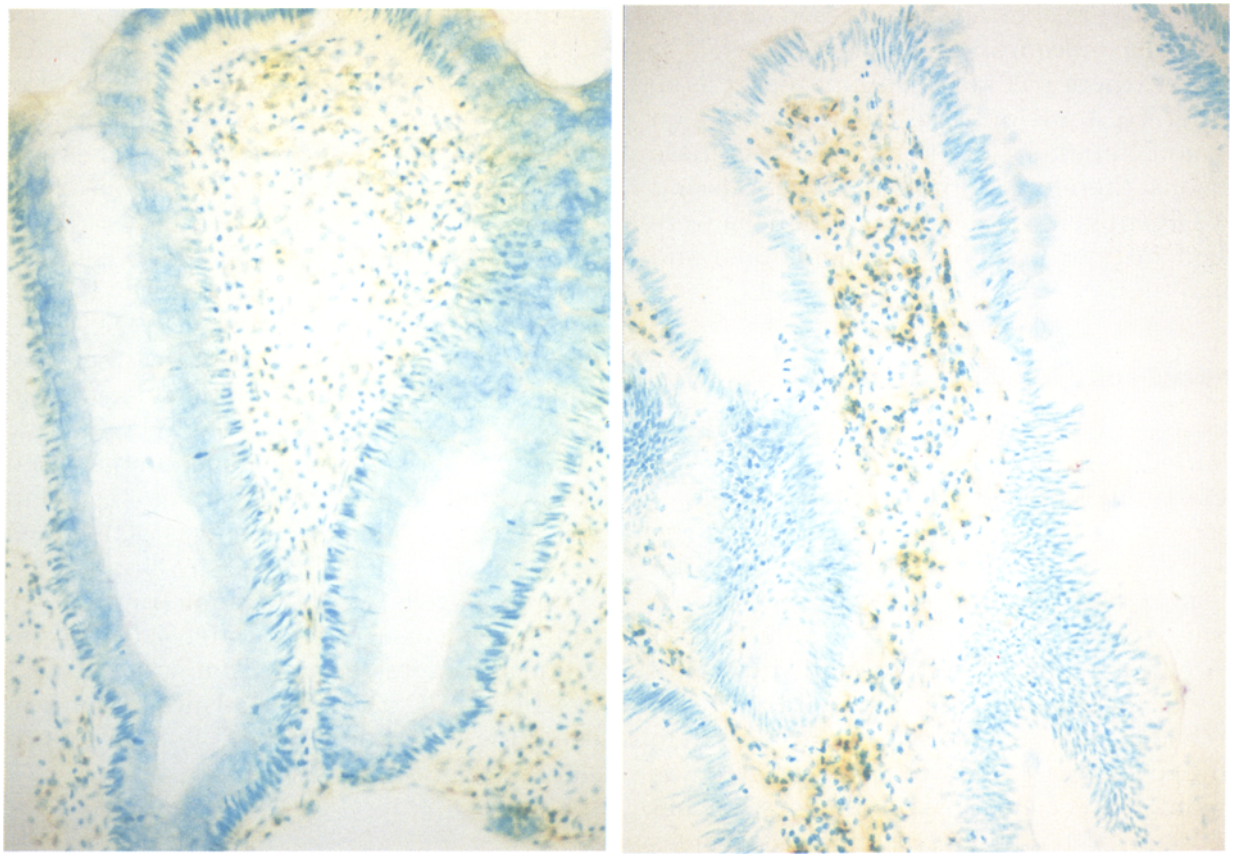

Fig. 2 CD4 positive cells in the lamina propria of the colon in familial polyposis coli. left: normal area (×58). right: polyp (×58).

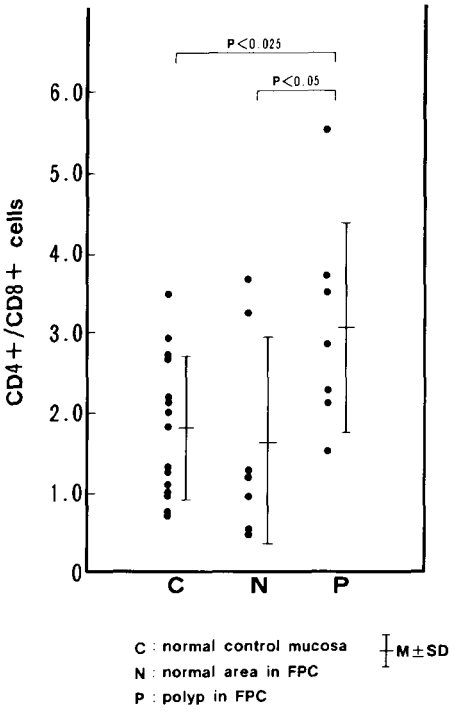

Fig. 3 The ratio of CD4+/CD8+ cells in the lamina propria of the colon.

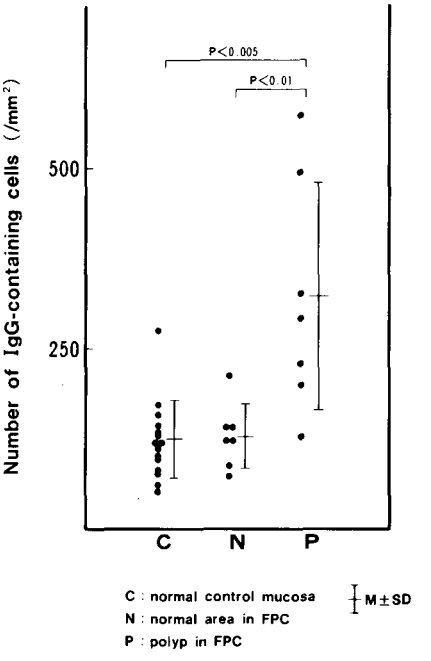

Fig. 4 The number of IgG-containing cells in the lamina propria of the colon.

normal control mucosa and the normal area (**Fig. 1**). The CD4+/CD8+ ratio in the polyp was 3.06±1.34, which was significantly higher than 1.81±0.87 in the normal control mucosa ($P<0.025$) and 1.64±1.29 in the normal area ($P<0.05$) (**Fig. 3**).

2. Ig-containing cells

The number of IgG-containing (IgG+) cells in the polyp was 323±158/mm^2, which was significantly higher than the 125±54/mm^2 in the normal control mucosa ($P<0.005$) (**Fig. 4**), and 130±45/mm^2 in the normal area ($P<0.01$) (**Figs. 4 and 5**). There was no difference in the number of IgG+ cells between the normal control mucosa and the normal area (**Fig. 4**).

The number of IgA1+ cells was greatest in the polyp at 992±324/mm^2 followed by 732±280/mm^2 in the normal area and least in the normal control mucosa at 630±286/mm^2. However, these differences were not statistically significant.

The number of IgA2+ cells was greatest in the normal area at 1480±369/mm^2 followed by 1363±411/mm^2 in the polyp and was the least in the normal control mucosa at 999±456/mm^2. The difference in the number of IgA2+ cells between the normal control mucosa and the normal area was significant ($P<0.025$).

The numbers of IgM+, IgD+, and IgE+ cells in the normal control mucosa were 182±109/mm^2, 157±66/mm^2, and 53±45/mm^2, respectively. The corresponding values were 216±137/mm^2, 342±135/mm^2 and 75±57/mm^2 in the normal area, and 289±95/mm^2, 587±491/mm^2, and 147±75/mm^2 in the polyp. There was no difference in the number of positive cells of these Ig classes among the three sites, except for IgD+ cells in the normal control mucosa and the normal area ($P<0.05$).

The sum of Ig+ cells of all five classes, namely IgG+, IgA1+ and IgA2+, IgM+, IgD+, and IgE+ cells was greatest in the polyp at 3701±944/mm^2 followed by 2994±362/mm^2 in the normal area, and least in the normal control mucosa at 2145±792/mm^2 (**Fig. 6**). These differences in the total number of Ig+ cells between

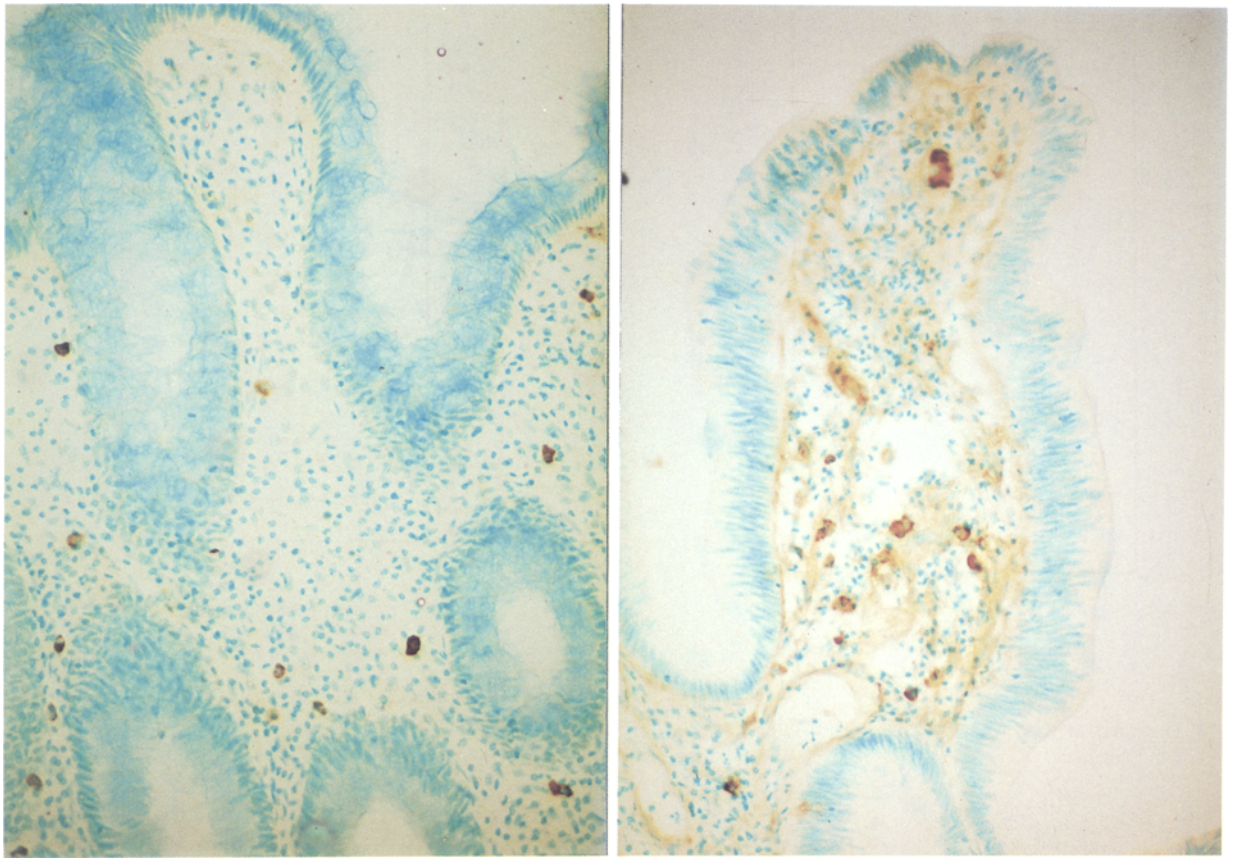

Fig. 5 IgG-containing cells in the lamina propria of the colon in familial polyposis coli. left: normal area (×58). right: polyp (×58).

the three sites were statistically significant **Fig. 6**).

3. The expression of HLA-DR antigens on the colonic epithelia

HLA-DR antigens were not expressed on the colonic epithelia in any of the 15 specimens from the normal control mucosa, or in the 7 specimens from the normal area of FPC. However, the antigens were expressed in 5 out of 7 specimens (71%) from the polyp (**Fig. 7**). The antigens were expressed mainly on the epithelia of the top or upper part of the polyp. The antigens were observed similarly with both Nu-Ia and anti-HLA-DR antibodies, although former generally gave clearer expression.

Discussion

To our knowledge, there has not been any study on lymphoid cell subsets of colonic mucosa in FPC. In this study lymphoid cell subsets in the normal colonic area of FPC were compared to those in the normal control mucosa. For the T cell population, CD4+ cells predominated over CD8+ cells in both mucosae. Among the Ig-containing cells, IgA+ cells comprised the largest population and IgE+ cells the smallest population in both mucosae. IgA2+ cells predominated over IgA1+ cells in both mucosae. In the normal area of FPC, IgD+ cells comprised the second largest population, followed by IgM+ and then IgG+ cells, whereas in the normal control mucosa, the IgD+ and IgM+ cells rankings were reversed. However, since the number of IgD+ and IgM+ cells were almost

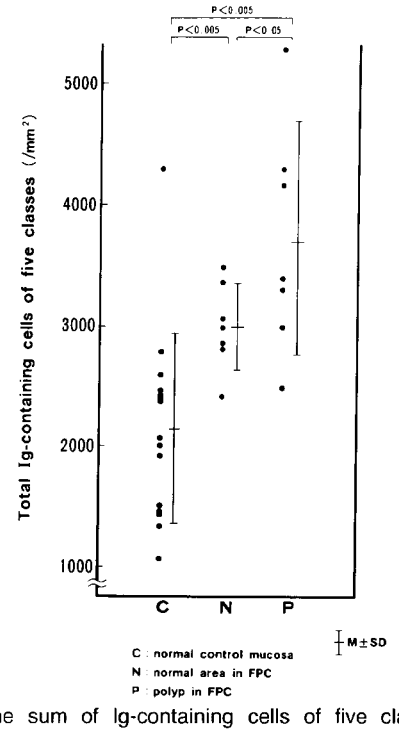

Fig. 6 The sum of Ig-containing cells of five classes in the lamina propria of the colon.

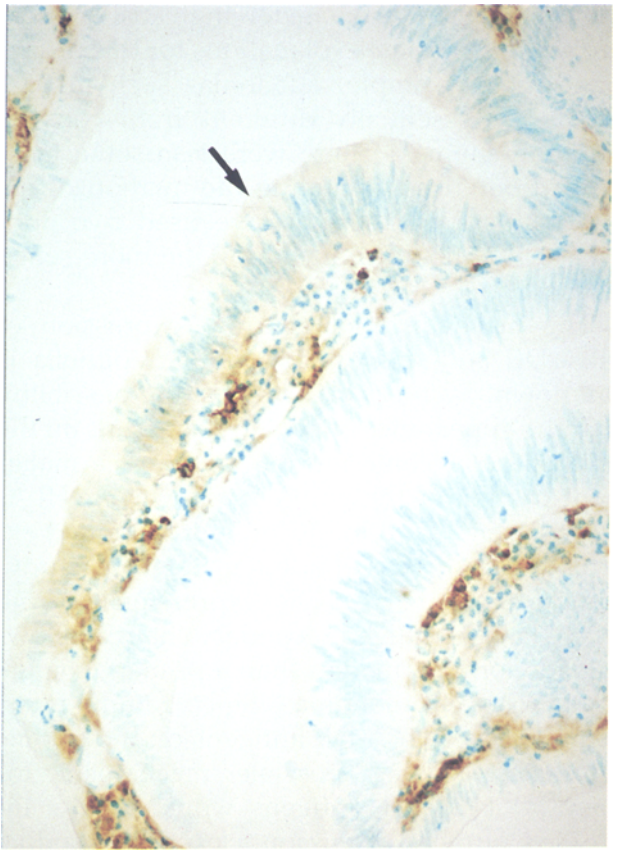

Fig. 7 The epithelia of a polyp (adenoma) stained with anti-HLA-DR antibody (×58). Epithelia expressing HLA-DR antigens are indicated by arrows.

equal in the normal control mucosa, it remains to be resolved whether the reversed ranking in the number of IgM+ and IgD+ cells in the normal area has any significance.

Lymphoid cell subsets in the polyp of FPC were compared to those in the normal area of FPC. There were several common features in the both mucosa; 1) CD4+ cells predominated over CD8+ cells, 2) IgA+ cells comprised the largest population among Ig+ cells, 3) IgA2+ cells predominated over IgA1+ cells, 4) IgD+ cells made up the second largest population, and 5) IgE+ cells comprised the smallest population. In the polyp, IgG+ cells were the third largest population followed by IgM+ cells, while in the normal area, these rankings were reversed. There were significant increases in the number of CD4+ cells, IgG+ cells and sum of five classes of Ig+ cells in the polyp compared to the normal area. These findings suggest that, in the polyp of FPC, CD4+ cells stimulated the activation of B cells which then resulted in antibody-production by Ig-containing cells. This may indicate that an immune-response was occurring in the polyp of FPC.

There has not, as yet, been any report about HLA-DR expression on the epithelia of adenomas in FPC. In this study, HLA-DR antigens were expressed in 5 of 7 adenomas (71%). There are 7 reports[6-12] about HLA-DR expression on the epithelia of both colorectal cancer and adenomas. The frequency of HLA-DR expression in colorectal cancer has been reported to range from 13.3% to 93.5%. For colorectal adenoma, Thompson et al[6] were unable to detect HLA-DR antigens on the epithelia of 5 specimens, while van den Ingh et al[12] detected the antigens in only 1 out of 12 specimens (8%). In our study, it was clear that the epithelia of adenomas in FPC expressed HLA-DR antigens in 71% of the cases/specimens; differing from the results of

Thompson et al[6] and van den Ingh et al[12]. There are several possible explanations for this discrepancy. For example, adenomas seen in FPC would be essentially different from conventional adenomas. There were also some technical differences, including the method of tissue fixation. Furthermore we used two anti-HLA-DR antibodies, including the more sensitive antibody, Nu-Ia.

The mechanism of aberrant expression of HLA-DR antigens on the colonic epithelia is unknown. Daar et al[7] suggested the possibility that the appearance of HLA-DR antigens on the epithelia of colonic mucosa precedes a malignant change of the epithelia. However, HLA-DR antigens were found only on a limited extent of the surface of adenomas in our study, indicating that HLA-DR expression on the epithelia was rather a secondary change following adenoma formation than a primary change that produced adenomas. Since gamma-interferon induces HLA-DR antigens on the colonic epithelia, another possible mechanism for aberrant HLA-DR antigens would be the increased number of mononuclear cells producing gamma-interferon. Epithelia of adenomas may be defective in their defence system against the entry of antigens in the lumen, thereby resulting in inflammation of the surface of the adenoma. There may also be mechanical injuries to the protruding adenoma, followed by inflammation of its surface. These inflammations may provide the mononuclear cells that produce gamma-interferon.

In conclusion, our study clearly demonstrates immunological changes, including T (CD4+) cells, B (Ig-containing) cells, and HLA-DR antigens, on the epithelia of polyps in FPC. The significance of these immunological changes in FPC remains to be resolved.

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