Gastroenterologia Japonica
Copyright © 1992 by The Japanese Society of Gastroenterology

Vol. 27, No. 4
Printed in Japan

An HLA study on 149 Japanese patients with Crohn's disease

Hiroaki MATAKE[1], Nobuo OKABE[1], Setsuya NAITO[2], and Tsuneyoshi YAO[3]
[1]First Department of Internal Medicine and [2]Second Department of Internal Medicine, School of Medicine, Fukuoka University, Fukuoka, Japan; and [3]Department of Gastroenterology, Chikushi Hospital, Fukuoka University, Chikushino, Japan

Summary: To search for possible immunogenetic roles in the pathogenesis of Crohn's disease, we examined the HLA-A, -B, -C, -DR and -DQ locus antigens in 149 Japanese patients with Crohn's disease. All patients were living on Kyushu island. We also examined the HLA of 136 healthy controls who resided in Kyushu. The results were compared with both controls throughout Japan and Kyushu controls. In Japanese patients with Crohn's disease, HLA-DR4, especially -DR4.1, and -DQ4 were more frequent than in the controls throughout Japan and in Kyushu. In light of these observations, an immunogenetic factor may have some role in the development of Crohn's disease. The susceptibility to Crohn's disease may relate to HLA-DR4, especially -DR4.1, and -DQ4, in Japanese patients. *Gastroenterol Jpn 1992;27:496-501.*

Key words: *Crohn's disease; HLA; inflammatory bowel disease.*

Introduction

Sixty years ago, Crohn et al.[1] reported the first evidence of a new clinical entity. Numerous studies have been performed to elucidate the pathogenesis of Crohn's disease (CD)[2], however little progress has been made. Immunological mechanisms appear to be important in the pathogenesis of CD[2,3].

To examine the immunogenetic background of CD, we studied the relationship between HLA systems and CD, and noted a strong positive association with HLA-DR4 in Japanese patients with CD[4]. In the international literature, a number of investigators failed to obtain evidence for a significantly increased risk of CD with any particular HLA phenotype[3].

In our ongoing studies of CD[5-11], we examined the immunological aspects of CD. In 108 CD patients, we found an increased incidence of HLA-DR4, especially DR4.1[12]. We have now acquired data on HLA antigens from a large number of Japanese patients with CD and compared them with controls throughout Japan and with control subjects living on Kyushu island.

Patients and Methods

Patients

Between October 1987 and March 1990, 149 Japanese patients with CD (106 men and 43 women) were treated in our hospitals. The diagnosis was made on the basis of clinical manifestations and radiological and histological findings. They were classified to three types (45 ileitis, 25 colitis and 79 ileocolitis type).

Controls

Four hundred and seventy-two unrelated, healthy Japanese who were examined at the Third Asia and Oceania Histocompatibility Workshop served as the controls for a comparison of HLA antigen frequencies (Japanese controls)[13,14]. In addition, we also examined the HLA of 136 unre-

Received December 16, 1991. Accepted March 27, 1992.
Address for correspondence: Hiroaki Matake, M.D., The First Department of Internal Medicine, School of Medicine, Fukuoka University, Fukuoka 814-01, Japan.

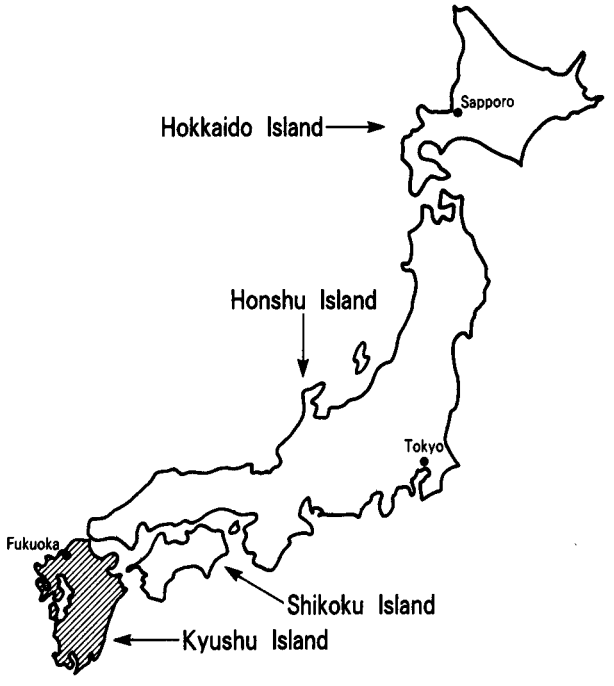

Figure 1. Map of Japan.

lated, healthy controls who were living on Kyushu island and compared them with HLA of patients with CD (Kyushu controls).

HLA typing

HLA-A, -B, -C, -DR and -DQ typings were performed by the method of Terasaki's microdroplet lymphocyte cytotoxicity test[15], using antisera distributed at the Third Asia Oceania Histocompatibility Workshop. B lymphocytes isolated by the nylon wool method were used for detection of HLA-D locus antigens.

Statistical analysis

For evaluation of possible HLA associations the chi-squared test was employed. Fisher's exact test was used when the number of the case studied was less than five. P-values were corrected for the number of antigens studied (class I antigens: n=50, class II antigens: n=13) and then termed Pc[16]. A Pc of less than 0.05 was considered to be significant. Odds ratio (OR) was calculated by the formula $(P^+ \times C^-)/(P^- \times C^+)$, where P^+ or P^- denote the number of patients positive or negative for a specific antigen, and C^+ or C^- denote the number of controls positive or negative for this antigen.

Table 1. Frequency of HLA class I antigens in CD compared with controls throughout Japan

HLA	AF(%) CD n=149	AF(%) J-C[13] n=472	χ^2	Pc	OR (Cl_{95})
A2	44.3	40.7	0.6	NS	
A11	24.2	17.2	3.6	NS	
A24	55.0	68.4	9.0	NS	
A26	18.8	21.2	0.4	NS	
A31	16.1	12.5	1.3	NS	
A33	14.8	9.5	3.2	NS	
B7	3.4	12.9	9.9	NS	
B13	2.7	3.8	0.2	NS	
B27	1.3	0.4	0.4	NS	
B35	10.7	15.5	2.1	NS	
B39	8.7	7.2	0.4	NS	
B44	12.8	10.8	0.4	NS	
B46	13.4	10.2	1.2	NS	
B48	4.0	4.7	0.1	NS	
B51	23.5	14.2	7.1	NS	
B52	14.1	23.5	6.0	NS	
B54	22.8	14.0	6.5	NS	
B55	5.4	3.8	0.7	NS	
B59	2.0	7.0	4.3	NS	
B60	14.8	10.6	1.9	NS	
B61	21.5	23.5	0.3	NS	
B62	15.4	15.5	0.0	NS	
B67	2.0	1.1	0.2	NS	
Cw1	32.9	27.5	1.6	NS	
Cw3	42.3	49.4	2.3	NS	
Cw4	5.4	7.2	0.6	NS	
Cw7	22.8	23.7	0.1	NS	

AF: antigen frequency. J-C: Japanese controls. NS: not significant. Pc: corrected P (P multiplied by the number of antigens tested). OR: odds ratio. Cl_{95}: 95% confidence interval.

Results

1. Comparison between patients with CD and controls throughout Japan

In **Table 1**, the frequency of HLA-A, -B and -C antigens is shown for patients with CD and Japanese controls. There were no significant differences between patients and Japanese controls. **Table 2** presents the distribution of the frequency of HLA-DR and -DQ loci. Particular attention was accorded to the distribution of antigen DR4

Table 2. Frequency of HLA class II antigens in CD compared with controls throughout Japan

HLA	AF(%) CD n=149	AF(%) J-C[13] n=472	χ^2	Pc	OR (Cl_{95})
DR1	2.7	12.3	10.6	Pc<0.025	0.197(0.115-0.338)
DR2	18.8	34.3	12.9	Pc<0.005	0.443(0.399-0.491)
DR4	61.7	41.7	18.2	Pc<0.00025	2.253(2.171-2.338)
DR4.1	45.0	24.4*	23.2	Pc<0.000025	2.536(2.352-2.735)
DR5	22.8	18.6	1.3	NS	
DR11	5.4	6.1	0.1	NS	
DR12	14.1	7.6	5.7	NS	
DR6	20.8	16.1	1.8	NS	
DR13	15.4	6.1	12.7	Pc<0.005	2.788(2.346-3.313)
DR14	4.0	5.5	0.5	NS	
DR8	31.5	24.8	2.7	NS	
DR9	18.8	26.1	3.3	NS	
DR52	64.4	52.3	6.7	NS	
DR53	69.8	64.8	1.2	NS	
DQ1	46.3	64.4	15.5	Pc<0.00025	0.477(0.445-0.512)
DQ3	38.9	54.7	11.2	Pc<0.025	0.529(0.477-0.587)
DQ7	20.8	18.6	0.3	NS	
DQ4	43.0	10.2	82.3	$Pc<1.5\times10^{-18}$	6.651(6.024-7.344)

AF: antigen frequency. J-C: Japanese controls. *: quote from the data of Aparicio et al.[14]. NS: not significant. Pc: corrected P (P multiplied by the number of antigens tested). OR: odds ratio. Cl_{95}: 95% confidence interval.

(χ^2=18.2, Pc<0.00025, OR=2.253). HLA-DR4.1 was the more frequent subtype of HLA-DR4 (χ^2=23.2, Pc<0.000025, OR=2.536). On the other hand, compared to the Japanese controls, the incidence of HLA-DR1 and -DR2 decreased, possibly due to the increased frequency of -DR4. As shown in **Table 2**, HLA-DQ4 was significantly (χ^2=82.3, $Pc<1.5\times10^{-18}$, OR=6.651) higher than that in the healthy controls, DQ1 and DQ3 were less frequent in CD (χ^2=15.5, Pc<0.00025, OR=0.477 and χ^2=11.2, Pc<0.025, OR=0.529).

2. *Comparison between patients with CD and Kyushu controls*

Table 3 shows the frequencies of HLA-A, -B and -C locus antigens in 149 patients with CD. The results were compared with those of 136 Kyushu controls. There were no significant differences between patients and Kyushu controls. **Table 4** summarizes the results of the HLA class II antigens compared with data on the Kyushu controls. HLA-DR4 incidence in CD was greater than in Kyushu controls (χ^2=13.8, Pc<0.005, OR=2.451). HLA-DR4.1 was the more frequent subtype of HLA-DR4 (χ^2=23.0, Pc<0.000025, OR=3.628). DQ4 was more frequent in CD compared with Kyushu controls (χ^2=22.7, Pc< 0.000025, OR=3.699), while the incidence of DQ1 was decreased.

3. *Comparison between Japanese controls and Kyushu controls*

We found no statistically significant difference between Japanese controls and Kyushu controls (data not shown).

Discussion

HLA, the major histocompatibility system in humans, is one of the most polymorphic genetic systems and locates on chromosome 6[17]. In the past two decades, strong associations between HLA and susceptibility to certain diseases have been demonstrated and the biological role of HLA

Table 3. Frequency of HLA class I antigens in CD compared with Kyushu controls

HLA	AF(%) CD n=149	AF(%) K-C n=136	χ^2	Pc	OR (CI_{95})
A2	44.3	35.3	2.4	NS	
A11	24.2	16.9	2.3	NS	
A24	55.0	68.4	5.3	NS	
A26	18.8	29.4	4.4	NS	
A31	16.1	14.7	0.1	NS	
A33	14.8	10.3	1.3	NS	
B7	3.4	10.3	4.4	NS	
B13	2.7	4.4	0.2	NS	
B27	1.3	0.7	0.0	NS	
B35	10.7	14.0	0.7	NS	
B39	8.7	5.9	0.8	NS	
B44	12.8	11.0	0.2	NS	
B46	13.4	5.9	4.6	NS	
B48	4.0	5.9	0.5	NS	
B51	23.5	20.6	0.3	NS	
B52	14.1	23.5	4.2	NS	
B54	22.8	15.4	2.5	NS	
B55	5.4	5.1	0.0	NS	
B59	2.0	5.9	1.9	NS	
B60	14.8	14.0	0.0	NS	
B61	21.5	22.1	0.0	NS	
B62	15.4	19.1	0.7	NS	
B67	2.0	4.4	0.7	NS	
Cw1	32.9	27.2	1.1	NS	
Cw3	42.3	51.5	2.4	NS	
Cw4	5.4	5.9	0.0	NS	
Cw7	22.8	19.9	0.4	NS	
Cw9	18.1	19.9	0.1	NS	
Cw10	20.8	27.2	1.6	NS	
Cw11	13.4	4.4	7.0	NS	

AF: antigen frequency. K-C: Kyushu controls. NS: not significant. Pc: corrected P (P multiplied by the number of antigens tested). OR: odds ratio. CI_{95}: 95% confidence interval.

may prove to be of great significance. These considerations led to the search for HLA-disease associations, particularly among patients with disease of unknown etiology. In studies on the HLA of these patients, many associations were found[18,19]. In 1979, Thorsby and Lie[20] reported findings of a relationship between HLA systems and CD. In Dutch patients with CD, the antigen B18 showed an increased frequency[21] and an incidence of HLA-B12 was reported to be increased in Viennese patients[22]. However, a few studies revealed some statistically significant association between HLA and CD in Caucasians[3].

In 1984, we examined the HLA of 62 Japanese patients with CD and noted a strong positive association of HLA-Bw46, B51, DR4 and DR5[4]. Further, in 1989, we examined the relationship between HLA systems in 108 CD patients and noted an increase in the frequency of HLA-DR4, especially DR4.1[12]. In the present study, we again noted the strong positive association of DR4 and DQ4 with CD compared with both the all-Japan controls and the Kyushu controls. In a study on CD patients living in the Tokyo area, Yagita et al.[23] found the association of HLA-DR4 and DQw3. In the further study from their laboratories, HLA-DR4, DR53 and DQw3 were reported to be significantly associated antigens[24]. In addition, a large study conducted in Japan showed that HLA-DR4 is more frequent in Japanese patients with CD. Thus, it may be concluded that HLA-DR4 is more frequent in the Japanese patients with CD. As for the DQ locus antigen, there have been three reports[23-25] which conflicted with our results. HLA-DQ4 is a newly recognized antigen and is thought to be linked to HLA-DR4.1[14]. Indeed, we found that the frequency of both HLA-DR4.1 and DQ4 is statistically higher than that of both the controls.

All patients we tested were living on Kyushu island. Therefore, we established a control group (Kyushu controls) as living on Kyushu island rather than the control group living in various parts of Japan. The HLA distribution in the Kyushu controls was not significantly different from the Japanese controls. Within the Japanese population, there is no regional difference in the HLA antigens. The Japanese are a relatively homogenous race, and this factor may explain the positive association observed in Japanese patients.

CD has generally been considered to have a higher incidence among people of Jewish origin[26,27]. In addition, the involvement of genetic factors has also been suggested by familial aggregation. In Caucasians, familial occurrences have been reported[28-31]. In Japan, an increasing attention has been directed to the familial occurrences and many cases were reported[32-34]. Hiwatashi et al.[34] reported that the familial incidence of CD in Japan is more frequent than the expected preva-

Table 4. Frequency of HLA class II antigen in CD compared with Kyushu controls

HLA	AF(%) CD n=149	AF(%) K-C n=136	χ^2	Pc	OR (CI_{95})
DR1	2.7	10.3	5.7	NS	
DR2	18.8	36.8	11.6	Pc<0.01	0.398(0.344-0.461)
DR4	61.7	39.7	13.8	Pc<0.005	2.451(2.182-2.752)
DR4.1	45.0	18.4	23.0	Pc<0.000025	3.628(3.126-4.211)
DR5	22.8	16.2	2.0	NS	
DR11	5.4	2.9	0.5	NS	
DR12	14.1	8.8	1.9	NS	
DR6	20.8	19.9	0.0	NS	
DR13	15.4	12.5	0.5	NS	
DR14	4.0	7.4	1.5	NS	
DR8	31.5	25.0	1.5	NS	
DR9	18.8	30.9	5.6	NS	
DR52	64.4	52.9	3.9	NS	
DR53	69.8	62.5	1.7	NS	
DQ1	46.3	62.5	7.5	NS	
DQ3	38.9	47.1	1.9	NS	
DQ7	20.8	18.4	2.3	NS	
DQ4	43.0	16.9	22.7	Pc<0.000025	3.699(3.164-4.323)

AF: antigen frequency. K-C: Kyushu controls. NS: not significant. Pc: corrected P (P multiplied by the number of antigens tested). OR: odds ratio. CI_{95}: 95% confidence interval.

Table 5. HLA study in Japanese patient with CD

Author	n	HLA	Year	References
Fujita et al.	62	Bw46, B51, DR4, DR5	1984	4
Yagita et al.	47	A31, B51, Bw61, DR4, DRw53, DQw3	1986	23
Asakura et al.	36	DQw3	1988	25
Matake et al.	108	DR4 (DR4.1)	1989	12
Kobayashi et al.	30	DR4, DRw53, DQw3	1990	24
Present study	149	DR4 (DR4.1), DQ4	1992	

lence rate. Thus, an increase of familial incidence suggests the involvement of an immunogenetic influence in the pathogenesis of CD.

Several immunological studies were carried out in patients with inflammatory bowel disease (IBD). In CD, investigation of lymphocyte functions showed a normal response to mitogenic stimuli[5,35] and no evidence of deficient helper T cell function for pokeweed mitogen stimulated immunogloblin synthesis[36]. Studies on lymphocyte phenotype revealed a normal proportion of helper/suppressor T cells[8,37]. Thus, there is little evidence that CD is a classical immune disease of "autoimmune" type.

The notion that ulcerative colitis (UC) may be some form of an autoimmune reaction also has been given attention. Antibodies able to bind to colonic epithelial cells were first described in 1959[38]. This finding has been confirmed and expanded upon by many investigators[2,3]. Consideration must be given to the results of HLA distribution in Japanese patients with UC. The frequency of Bw52 and DR2 in Japanese patients with UC is high[39]. Thus, among IBD, there must be a clear and definite difference between CD and UC regarding immunological aspects and an immunogenetic background.

This study was supported by a grant from the Research group of Intractable IBD by Japanese Ministry of Public Welfare. This work was presented in part at the 9th World Congress of Gastroenterology, Sydney, Australia, August, 1990, and at an International Symposium on Genetic intervention in Diseases with Unknown Etiology, Tokyo, Japan, November, 1990. The authors thank Dr. Ogahara for comments on the manuscript, K. Okamoto for excellent secretarial assistance and Y. Tsukasaki for skilled technical assistance.

References

1. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis, a pathologic and clinical entity. JAMA 1932;99:1323-1329.
2. Kirsner JB, Shorter RG. Recent developments in nonspecific inflammatory bowel disease (Second of two parts). N Engl J Med 1982;306:837-848.
3. Elson CO. The immunology of inflammatory bowel disease. In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. Philadelphia: Lea & Febiger, 1988;97-164.
4. Fujita K, Naito S, Okabe N, et al. Immunological studies on Crohn's disease 1. Association with HLA systems in the Japanese. J Clin Lab Immunol 1985;16:155-161.
5. Fujita K, Okabe N, Yao T. Immunological studies on Crohn's disease 2. Lack of evidence for humoral and cellular dysfunctions. J Clin Immunol 1985;16:155-161.
6. Okabe N, Fujita K, Yao T. Immunological studies on Crohn's disease 3. Defective natural killer activity. J Clin Lab Immunol 1985;17:143-146.
7. Okabe N, Fujita K, Takenaka K, et al. Immunological studies on Crohn's disease 4. Decreased ADCC activity. J Clin Lab Immunol 1986;19:77-81.
8. Okabe N, Fujita K, Yamasaki M, et al. Immunological studies on Crohn's disease 5. Enumeration of circulating lymphocytes subsets using monoclonal antibodies. Gastroenterol Jpn 1985;20:431-435.
9. Okabe N, Kuroiwa A, Fujita K, et al. Immunological studies on Crohn's disease 6. Increased chemiluminescent response of peripheral blood monocyte. J Clin Lab Immunol 1986;21:11-15.
10. Okabe N, Matsuoka Y, Ueda I, et al. Immunological studies on Crohn's disease 7. Reduced immunologic activity in monocytes. J Clin Lab Immunol 1988;25:69-72.
11. Okabe N, Ikura S, Uchida Y, et al. Immunological studies on Crohn's disease 8. Diminished phagocytic activity in peripheral blood monocytes. J Clin Lab Immunol 1990;32:29-31.
12. Matake H, Naito S, Ogahara S, et al. HLA study in 108 Japanese patients with Crohn's disease. Jpn J Gastroenterol 1989;86:1448-1454. (in Japanese)
13. HLA in Asia-Oceania. The proceedings of the Third Asia-Oceania Histocompatibility Workshop conference. Aizawa M, ed. Sapporo: Hokkaido University Press, 1986;1080-1081.
14. Aparicio J, Wakisaka A, Takada A, et al. HLA-DQ system and insulin-dependent diabetes mellitus in Japanese. Immunogenet 1988;28:240-246.
15. Terasaki PI, Bernoco DB, Park MS, et al. Microdroplet testing for HLA-A, -B, -C and -D antigens. Am J Clin Path 1978;69:103-120.
16. Svejgaard SS, Ryder LP. Association between HLA and disease, In: Dausset J, Svejgaard A, eds. HLA and Disease. Copenhagen: Munksgaard, 1977;46-71.
17. Morris PJ. HLA and disease. In: Irvine WJ, ed. Medical immunology. Edinburgh: Teviot Scientific Publications, 1979;69-76.
18. Svejgaard A, Platz P, Ryder LP. HLA and disease 1982-A survey. Immunological Rev 1983;70:193-218.
19. Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: The Failure of self tolerance. Science 1990;248:1380-1388.
20. Thorsby E, Lie SO. Relationship between the HL-A system and susceptibility to disease. Transpl Proc 1971;3:1305-1307.
21. Van den Berg-Loonen EM, Dekker-Saeys BJ, Meuwissen SGM, et al. Histocompatibility antigens and other genetic markers in ankylosing spondylitis and inflammatory bowel diseases. J Immunogenet 1977;4:167-175.
22. Smolen JS, Gangl A, Polterauer P, et al. HLA antigens in inflammatory bowel disease. Gastroenterology 1982;82:34-38.
23. Yagita A, Kitajima M, Takekawa I, et al. HLA and Crohn's disease in Japan. In: Aizawa M, ed. HLA in Asia-Oceania Histocompatibility Workshop Conference. Sapporo: Hokkaido University Press, 1986;797-801.
24. Kobayashi K, Atoh M, Yagita A, et al. Crohn's disease in the Japanese is associated with the HLA-DRw53. Expl Clin Immunogenet 1990;7:101-108.
25. Asakura H, Hibi T. Immunological abnormalities in ulcerative colitis and Crohn's disease. Igaku-no-ayumi 1988;147:371-374. (in Japanese)
26. Asheson ED. The distribution of ulcerative colitis and regional enteritis in United States veterans with particular reference to the Jewish region. Gut 1960;1:291-299.
27. Rogers BHG, Clark LK, Kirsner JB. The epidemiology and demographic characteristics of inflammatory bowel disease: an analysis of a computerized file of 1400 patients. J Chron Dis 1971;24:743-750.
28. Mayberry JF, Rhodes J. Familial prevalence of inflammatory bowel disease in relatives of patients with Crohn's disease. Br Med J 1980;280(6207):84.
29. Achord JL, Gunn CH, Jackson JF. Regional enteritis and HLA concordance in multiple siblings. Dig Dis Sci 1982;27:330-332.
30. Lifton LJ, Stafford JM. Crohn's disease affecting three generations—case reports and genetic typing. Am J Gastroenterol 1883;78:159-161.
31. McConnel RB, Shaw JM, Whibley EJ, et al. Inflammatory bowel disease: A review of previous genetic studies and the Liverpool family data. Front Gastrointest Res 1986;11:1-11.
32. Tamura T, Kawahito M, Wada D, et al. Crohn's disease in siblings. Jpn J Gastroenterol 1983;80:2584-2587. (in Japanese)
33. Kubo T, Nishida K, Fujita K, et al. Crohn's disease in siblings. Jpn J Gastroenterol 1987;84:1825-1829. (in Japanese)
34. Hiwatashi N, Nakajima K, Yamazaki H, et al. Familial prevalence of inflammatory bowel disease. J Jap Soc Colo-proctol 1987; 40:889-893. (in Japanese)
35. Auer 10, Roder A, Frohlich J. Immune status in Crohn's disease. 6. Immunoregulation evaluated by multiple, distinct T-suppressor cell assays of lymphocyte proliferation, and by enumeration of immunoregulatory T-lymphocyte subsets. Gastroenterology 1984; 86:1531-1543.
36. James SP, Fiocchi C, Waldermann TA. Immunoregulatory function of lamina propria T cells in Crohn's disease. Gastroenterology 1985;88:1143-1150.
37. Selby WS, Jewell DP. T lymphocyte subsets in human ulcerative colitis. J Exp Med 1959;110:657-674.
38. Broberger O, Perlmann P. Autoantibodies in human ulcerative colitis. J. Exp Med 1959;110:657-674.
39. Inoue M, Morita N, Shiwaku Y. Immunogenetics in inflammatory bowel disease. Saishin-Igaku 1982;37:1202-1210. (in Japanese)