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HLA-DR, DQ and T cell antigen receptor constant beta genes in Japanese patients with ulcerative colitis

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SUMMARY

We studied the T cell antigen receptor (TcR) constant beta chain genes on HLA typed Japanese patients with ulcerative colitis (UC). A TcR constant beta *Eco*RI 6·0-kb fragment was present in all Japanese UC patients ($n=17$) but completely absent in the controls ($n=35$) ($\chi^2=47·6$, $P<0·001$). The frequency of HLA-DR2 antigen was significantly higher in UC patients (85% *versus* 28% in controls, $P<0·001$). Furthermore, HLA-DQw1 antigen was also increased in UC patients (96% *versus* 60% in controls, $P<0·001$). However, HLA-DR4 antigen was significantly decreased in UC patients (12% *versus* 37%, $P=0·02$). HLA-DR1 antigen was not found in UC patients and was present in only 15% of the controls. These results suggest that TcR beta chain and HLA-DQw1 antigen may be important in the pathogenesis of Japanese UC.

Keywords T cell receptor constant beta chain HLA ulcerative colitis restriction fragment length polymorphism

INTRODUCTION

Ulcerative colitis (UC), an inflammatory bowel disease (IBD), is a disorder of unknown aetiology and is characterized by a variety of immunological abnormalities (Kirsner & Shorter 1982). It is associated with HLA-DR2 antigen in Japanese patients (Asakura *et al.*, 1982) and with HLA-B27 antigen in Caucasian patients (Biemond *et al.*, 1986).

T lymphocytes recognize antigens with molecules of the MHC (Zinkernagel & Doherty 1975; Bevan, 1975; Shearer, Rehn & Garbario, 1975). This property, MHC restriction, is the result of a complex structure formed between the T cell antigen receptors (TcR) and the molecules encoded by the MHC (Acuto *et al.*, 1983; Kappler *et al.*, 1983; McIntyre & Allison, 1983). Antigen recognition of T lymphocytes is mediated by TcR, composed of a heterodimer of alpha and beta chains situated on the surface of these immunocompetent cells. The genes that encode these chains are included in variable (V) and constant (C) region segments, which undergo somatic rearrangement to form a complete functional gene. Polymorphism within the constant segments of both the alpha and beta constant chains have been identified (Robinson & Kindt, 1985; Kronenberg *et al.*, 1986), allowing restriction fragment length polymorphism (RFLP) analysis to be carried out. The cDNA clones and genomic clones corresponding to portions of the TcR alpha and beta chains in humans and in mice were isolated from T cell lines (Hedrick *et al.*, 1984; Yanagi *et al.*, 1984; Hood, Kronenberg & Hunkapiller, 1985).

It has recently been reported that the RFLP pattern of the TcR constant beta chain genes on chromosome 7 is associated with some immune disorders (Hoover *et al.*, 1986; Demaine *et al.*, 1987; Millward *et al.*, 1987; Dunckley, Gatenby & Serjeanton, 1988).

Here we report the results of studies on the rearrangement of the TcR constant beta chain genes and on related HLA-DR and DQ antigens.

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MATERIALS AND METHODS

Peripheral blood was collected from 27 unrelated UC patients (14 men and 13 women; mean age $31 \pm 17·6$ s.d., range 10–75; diagnosed in accordance with the standard of the Japanese Ministry of Health and Welfare) and from 54 healthy control subjects (38 men and 16 women, mean age $32·5 \pm 7·6$ s.d., range 24–57). All subjects were Japanese.

Genomic DNA was isolated from the lymphocytes of 17 UC patients and 35 healthy controls by solubilizing the cells overnight in 50 mM Tris (pH 8·0), 100 mM NaCl, 20 mM EDTA, 1% SDS, and 0·15 mg/ml proteinase K at 37°C. Proteins were removed from high molecular weight DNA by 400 mM Tris (pH 8·0) saturated phenol extraction and DNA was recovered by ethanol precipitation. Ten micrograms of DNA from each cell

were digested with the restriction endonuclease *Eco*RI (Boehringer Mannheim, FRG). This treatment was carried out overnight at a concentration of 4 U/μg of DNA at 37°C. The digested samples were then electrophoresed for 18 h at 5 V on 0·7% agarose gel in 1/2 TBE buffer (50 mM Tris, 50 mM boretic acid, 1 mM EDTA; pH 8·3). Fragments of *Eco*RI–*Hin*dIII-digested lambda DNA were used as molecular size markers. It was subsequently denatured in 0·4 M sodium hydroxide and transferred onto nylon membrane (Bio Trace, Gelman Sciences) for 18 h according to the method of Reed & Mann (1985). The filters were prehybridized at 42°C in hybridization solution (50% formamide, 5 SSPE, 5 Denhardt's solution, 10% dextran, 0·1% SDS and 100 μg/ml heat-denatured salmon sperm DNA) for 4 h and hybridized in hybridization solution containing radiolabelled cDNA probe. The TcR constant beta chain probe was derived from the Jurkat T cell line which detects sequences in the TcR beta constant genes on chromosome 7. The probe for hybridization was derived from plasmid which contains sequences complementary to the joining and constant beta regions of T cell antigen receptor messenger RNA (Yanagi *et al.*, 1984). The cDNA clone was digested with PstI and 770 bp fragments were isolated by agarose gel electrophoresis followed by electroelution into dialysis tubing. The purified cDNA probes were labelled with ^{32}P-dCTP (about 111 TBq/mmol; Amersham) to the specific activity of 2×10^9 ct/min per μg of DNA using random primer DNA labelling system (Feinberg & Vogelstein, 1983). Each filter was hybridized with 2×10^7 ct/min for 24 h at 42°C.

The filters were washed twice in SSPE for 5 min at room temperature. Thereafter, they were washed for 15 min at 65°C in SSPE to which 0·5% SDS was added. The final wash was for 15 min at 65°C in 0·5 × SSPE. After the washing, the filters were exposed to Kodak XAR-5 X-ray films with an intensifying screen at −60°C for 4 days.

Tissue typing for HLA antigens was performed according to the standard NIH complement-dependent micro-lymphocytotoxicity test.

The χ^2 test (with Yates' correction) was used for statistical comparison of healthy controls and UC patients.

Table 1. HLA antigen frequencies of Japanese patients with ulcerative colitis (UC)

	UC patients ($n=26$)		Healthy controls ($n=54$)			
HLA	*n*	%	*n*	%	χ^2*	*P*†
DR1	0	0	8	15		
DR2	22	85	15	28	20·6	<0·001
DR4	3	12	20	37	4·0	0·02
DR5	4	15	8	15		
DRw6	3	12	10	19		
DRw8	5	19	8	15		
DR9	10	39	14	26		
DRw52	12	48	31	61		
DRw53	9	36	27	53		
	($n=25$)		($n=54$)			
DQw1	24	96	30	60	11·1	<0·001
DQw3	14	56	26	52		

*With Yates' correction.
†Fisher's exact test.

RESULTS

The phenotype frequencies of HLA antigens are shown in Table 1. The frequency of HLA-DR2 and DQw1 antigens were significantly higher in patients (85% and 96%, respectively) as compared with controls (28% and 60%, respectively; $\chi^2=20·6$ and 11·1, respectively; $P<0·001$ in both cases). HLA-DR4 antigen was significantly decreased in the patients (12%) compared with the control subjects (37%, $\chi^2=4·0$, $P=0·02$). There were no HLA-DR1-positive UC patients but the frequency of this antigen in the control population was also low (15% in our study group).

The TcR constant beta chain probe containing J and C sequences was first used to test whether the hybridization bands of UC patients were different from those of the controls.

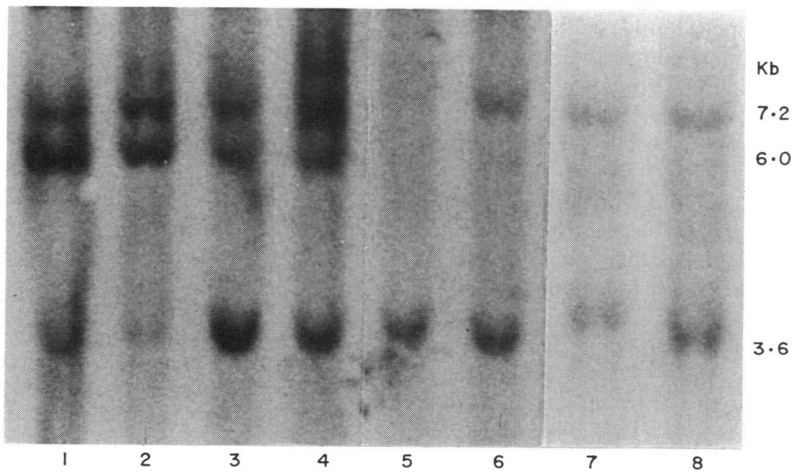

Fig. 1. *Eco*RI genomic DNA restriction fragments obtained by hybridization to T cell antigen receptor constant beta cDNA probe containing J and C regions. Lymphocytes from Japanese ulcerative colitis (UC) patients (lanes 1–4), and from healthy individuals (lanes 5–8); 7·2, 6·0 and 3·6 kb phenotype was observed in all UC patients, but not in any controls. The 6·0-kb fragment was not found in any control subjects.

Table 2. *Eco*RI restriction endonuclease fragments detected by the T cell receptor constant beta probe

Fragments	UC patients ($n=17$)		Healthy controls ($n=35$)		χ^2*	P†
	n	%	n	%		
7·2 kb	17	100	33	94		
6·0 kb	17	100	0	0	47·6	<0·001
3·6 kb	17	100	35	100		

*With Yates' correction.
†Fisher's exact test.

The four filters were used to study both the patients and the controls. Using this probe with the restriction endonuclease *Eco*RI, 7·2, 6·0, and 3·6 kb fragments were present in all UC patients (Fig. 1, lanes 1–4). All UC patients showed the same RFLP pattern. Control subjects, however, displayed two different RFLP patterns: 7·2/3·6 kb or —/3·6 kb (Fig. 1, lanes 5–8). The frequencies of these fragments differed between UC patients and the control subjects (Table 2). Perhaps the fragment difference was with respect to the *Eco*RI 6·0-kb fragment: it was present in all UC patients and missing in all controls ($\chi^2=47·6$, $P<0·001$). The 3·6-kb fragment was derived from the CB2 region and was found in all individuals.

DISCUSSION

In previous studies, it has been reported that the HLA-Bw52-DR2 haplotype was significantly increased in Japanese UC patients (Asakura *et al.*, 1982) and the HLA-B27 antigen in Caucasians (Biemond *et al.*, 1986). In the present study, the HLA-Bw52, DR2 and DQw1 antigens showed a strong association with Japanese UC compared with healthy Japanese subjects. Among these antigens, DQw1 showed the strongest association with UC. However, HLA-DR4 was significantly decreased in the patients compared with the control subjects. In our study groups, there were no HLA-DR1-positive Japanese UC patients, but the frequency in the heathly controls was 15%. These data suggest that a common epitope sharing with both HLA-DR1 and DR4 might induce a suppressive factor. The HLA-DQw1 antigen associated with the HLA-DR2 may induce a helper-like factor, or it may not be allowed to induce a suppressive factor for Japanese UC.

The importance of the TcR genes as a potential diagnostic tool has been emphasized by several investigators (Berliner *et al.*, 1985; Robinson & Kindt, 1985). Demaine *et al.* (1987), using the restriction endonuclease *Bgl*II, detected a significant association between a TcR constant beta chain RFLP phenotype and insulin-dependent diabetes mellitus, membranous nephrophaty and Graves' disease. All of these diseases are associated with the HLA-DR3 in Caucasians, but this antigen is not found in Japanese subjects. In addition, Hoover *et al.* (1986) found that TcR beta chain polymorphism is linked to diabetes mellitus. In systemic lupus erythematosus, Dunckley *et al.* (1988) could not identify TcR beta chain polymorphism with the disease.

When digested with restriction endonuclease *Eco*RI, we observed the same rearrangement pattern (7·2, 6·0 and 3·6 kb) in Japanese UC patients. The 3·6-kb (CB2) fragment was found in all individuals (Table 2) and the absence of the 11·0-kb (CB1) fragment indicates the polyclonal nature of the population (Savill *et al.*, 1987). We suggest that the 6·0-kb fragment is derived by rearrangement of the 11·0-kb (CB1) fragment. The 6·0-kb fragment was found in all patients but not in healthy control subjects. It is likely that the 7·2-kb fragment is derived from a joining region.

The second non-HLA-linked locus, the T cell antigen receptor constant beta chain gene located on chromosome 7, is associated with UC (Fig. 1). Therefore, the TcR constant beta chain RFLP phenotype is also associated with susceptibility to UC. Since we did not test a large number of individuals, additional population and family analysis may be required to determine the exact mode of inheritance of both HLA-DQ and the T cell antigen receptor genes in Japanese UC. The association of HLA-DQw1 or DR2 antigen and specific TcR constant beta gene may reflect the associative recognition of MHC class II molecules by the rearrangement of favoured TcR beta chain genes. Although the role of the genes associated with the rearrangement of the TcR beta chain genes described here is still unclear, the use of this RFLP marker for a gene located on chromosome 7 as well as similar markers for the HLA-DQw1 antigen should be helpful for predicting high-risk individuals in UC. Furthermore, the ability to type individuals for two unlinked loci involved in the susceptibility to UC should significantly enhance our understanding of the genetic and environmental factors involved in this disease. The interaction between the TcR constant beta chain with such rearrangement (7·2, 6·0 and 3·6 kb phenotypes) and the HLA-DQw1 antigen may be important in this disease of the autoimmune system, though the mechanism of the interaction is still unclear.

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REFERENCES

Acuto, O., Husey, R.E., Fitzgerald, K.A., Protrntis, J.P., Meuer, S.C., Schlossman, S.F. & Reinherz, E. (1983) The human T cell receptor: appearance on ontogeny and biochemical relationship of a and b subunits on IL-2 dependent clones and T cell tumors. *Cell*, **34,** 717.

Asakura, H., Tsuchiya, M., Aiso, S., Watanbe, M., Kobayashi, K., Kibi, T., Ando, K., Takata, H. & Sekiguchi, S. (1982) Association of the human lymphocyte-DR2 antigen with Japanese ulcerative colitis. *Gastroenterology*, **82,** 413.

Bevan, M.J. (1975) The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. exp. Med.* **142,** 1349.

Berliner, N., Duby, A.D., Morton, C.C., Leder, P. & Seidmean, J.G. (1985) Detection of a frequent restriction fragment length polymorphism of the human T cell antigen receptor beta chain locus. *J. clin. Invest.* **76,** 1283.

Biemond, I., Burnham, W.R., D'Amaro, J. & Langman, M.J.S. (1986) HLA-A and -B antigens in inflammatory bowel disease. *Gut*, **27,** 934.

Demaine, A., Welth, K.I., Hawe, B.S. & Farid, N.R. (1987) Polymor-

phism of the T cell receptor β chain in Graves' disease. *J. clin. Endocrinol. Metab.* **65,** 643.

DUNCKLEY, H., GATENBY, P.A. & SERJEANTON, S.W. (1988) T-cell receptor and HLA class II RFLPs in systemic lupus erythematosus. *Immunogenetics*, **27,** 392.

FEINBERG, A.P. & VOGELSTEIN, B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high activity. *Anal. Biochem.* **132,** 6.

HEDRICK, S.M., COHEN, D.I., NIELSEN, E.A. & DAVIS, M.M. (1984) Isolation of cDNA clones encoding T-cell specific membrane-associated proteins. *Nature*, **308,** 149.

HOOD, L., KRONENBERG, M. & KUNKAPILLER, T. (1985) T cell antigen receptor and the immunoglobulin supergene family. *Cell*, **40,** 225.

HOOVER, M.L., ANGELINI, G., BALL, E., FERRARA, G.B. MARKS, J., RASKIN, P., ROSENSTOCK, J., STASTNY, P., TOSI, R. & CAPRA, J.D. (1986) HLA-DQ and T cell receptor genes in insulin-dependent diabetes mellitus. In *Cold Spring Harbor Symposium on Quantitative Biology*, p. 803. Cold Spring Harbor Laboratories, Cold Spring Harbor. NY.

KAPPLER, J., KUBO, R., HASKINS, K., WHITE, J. & MARRACK, P. (1983) The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. *Cell*, **34,** 727.

KIRSNER, J.B. & SHORTER, R.G. (1982) Recent development in nonspecific inflammatory bowel disease. *N. Engl. J. Med.* **306,** 837.

KRONENBERG, M., SIU, G., HOOD, L. & MALISSEN, B. (1986) The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Annu. Rev. Immunol.* **4,** 529.

MCINTYRE, B.W. & ALLISON, J.P. (1983) The mouse T cell receptor: structural heterogeneity of molecules of normal T cells defined by xenoantiserum. *Cell*, **34,** 7393.

MILWARD, B.A., WELSH, K.I., LELSIE, R.D.G., PYKE, D.A. & DEMAINE, A.G. (1987) T cell receptor beta chain polymorphisms are associated with insulin-dependent diabetes. *Clin. exp. Immunol.* **70,** 152.

REED, K.C. & MANN, D.A. (1985) Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res.* **13,** 7207.

ROBINSON, M.A. & KINDT, T.J. (1985) Segregation of polymorphic T-cell receptor genes in families. *Proc. natl. Acad. Sci. USA*, **82,** 3804.

SAVILL, C.M., DELVES, P.J., KIOUSSIS, D., WALKER, P., LYNYARD, P.M., COLACO, B., SHIPLEY, M. & ROITT, I.M. (1987) A minority of patients with rheumatoid arthritis show a dominant rearrangement of T-cell receptor β chain genes in synovial lymphocytes. *Scand. J. Immunol.* **25,** 629.

SHEARER, G.M., REHN, T.G. & GARBARIO, C.A. (1975) Cell-mediated lympholysis of trinitrophenylated-modified autologous lymphocytes. Effector cell specificity to modified cell surface components controlled by H-2K and H-2D serological regions of the major histocompatibility complex. *J. exp. Med.* **141,** 1348.

YANAGI, Y., YOSHIKAI, Y., LEGGETT, K., CLARK, S., ALEKSANDER, I. & MAK, T.W. (1984) A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature*, **308,** 145.

ZINKERNAGEL, R.M. & DOHERTY, P.C. (1975) H-s compatibility requirement for T cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus: different cytotoxic T-cell specificities are associated with structures coded from H-2K or H-2D. *J. exp. Med.* **141,** 1427.