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The association between human leukocyte antigens (HLA) and cytoplasmic-antineutrophil cytoplasmic antibody (cANCA)-positive Wegener's granulomatosis in a Japanese population*

Y. Nakamaru, S. Maguchi, M. Takizawa, S. Fukuda, Y. Inuyama

Department of Otolaryngology, Hokkaido University School of Medicine, Sapporo, Japan

SUMMARY

The present study examined the association between various human leukocyte antigens (HLA) and cytoplasmic-antineutrophil cytoplasmic antibody (cANCA)-positive Wegener's granulomatosis (WG) in Japanese subjects to determine whether HLA antigens are involved in the pathogenesis of this disease. The study involved 16 subjects with cANCA-positive WG treated in our department. HLA-typing of the lymphocytes was performed using a lymphocyte microcytotoxicity assay. Of the subjects with cANCA-positive WG, 62.5% (10/16) were positive for HLA-DR9, as compared to 26% of the healthy control subjects. This HLA-DR9 elevation was statistically significant ($p<0.01$, $Pc<0.05$); we also noted a weaker association between HLA-B55 and cANCA-positive WG ($p<0.05$). The results indicate that an association may exist between certain HLA-class allotypes and WG.

Key words: Wegener's granulomatosis, HLA, cANCA, Japanese patients, pathogenesis

INTRODUCTION

Wegener's granulomatosis (WG) was first described by Klinger in 1931, and subsequently characterized by Wegener in 1936 and 1939.

It is characterized by necrotic granuloma with vasculitis of the upper or lower respiratory tract, systemic vasculitis, and focal necrotic glomerulonephritis. The aetiology of WG is still unknown. The presence of anti-neutrophil cytoplasmic antibodies (ANCA; Van der Woude et al., 1989) and the clinically observed responses to various immunosuppressive agents suggest that this disease may be immunologically mediated. Human leukocyte antigens (HLA) are known to be involved in the immune response.

The present study investigated the association between several HLA antigens and cANCA-positive WG, to determine whether any are involved in the pathogenesis of this disease. Previous investigations in this area involved only Caucasian subjects and biopsy-confirmed WG patients (Styrimlan et al., 1978; Katz et al., 1979; Elkon et al., 1983; Murty et al., 1991; Papiha et al., 1992), whereas the presence of cytoplasmic ANCA (cANCA) was not considered.

The present study represents the first investigation into the association between the HLA antigens and cANCA-positive WG in Japanese subjects.

MATERIAL AND METHODS

Patients

Sixteen WG-patients who were treated in our department from January 1990 to July 1995 were studied. All patients (10 males, 6 females) were unrelated Japanese and tested positive for cANCA by an indirect immunofluorescence assay. The mean age was 48.3 years, with a range of 16-65 years. Patients with cANCA-negative WG were not involved in this study. Clinical and pathological data for the 16 subjects are shown in Table 1.

HLA-typing

HLA-typing of lymphocytes was performed using a lymphocyte microcytotoxicity assay. Using a Terasaki antisera tray, the presence of the following specificities was evaluated: HLA-A1-3, 9-11, 19, 23-26, and 28-34; HLA-B5, 7, 8, 12-18, 22, 27, 35, 37-40, 44-46, 48, 51, 52, 54-56, 59-63, 67, and 70; HLA-Cw1-7; and HLA DR1-9.

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Table 1. Clinical and pathological data of the 16 subjects with cANCA-positive WG.

subject No.	sex	age	lesion sites							pathological diagnosis	presence of cANCA
			nose	ear	lung	eye	kidney	larynx	others		
1	m	65	+	+	+	+	+	+	nerve	positive	positive
2	m	57	+	-	-	+	-	-	-	positive	positive
3	m	54	+	-	-	-	-	+	-	suspected	positive
4	m	43	+	+	-	+	-	-	-	suspected	positive
5	m	56	-	-	+	+	-	-	-	suspected	positive
6	m	39	+	-	-	-	-	+	-	negative	positive
7	f	37	+	+	+	-	+	-	-	positive	positive
8	m	48	+	-	-	+	+	-	skin	suspected	positive
9	f	21	+	-	-	-	-	-	joints, skin	suspected	positive
10	m	82	+	-	-	-	-	-	-	negative	positive
11	f	29	+	+	-	+	-	-	-	suspected	positive
12	m	77	+	+	+	-	-	+	-	suspected	positive
13	f	33	+	+	-	+	-	+	-	suspected	positive
14	m	56	+	+	+	-	-	-	joints, skin	suspected	positive
15	f	60	+	+	-	+	-	-	-	suspected	positive
16	f	16	+	-	+	-	-	+	-	suspected	positive

Table 2. Individual HLA types for the 16 subjects with cANCA-positive WG.

subject No.	HLA-A	HLA-B	HLA-Cw	HLA-DR
1	24, 26	44, 55	1, -	8, 9
2	11, 31	51, 61	3, -	2, 4
3	11, 24	39, 62	3, 7	8, 9
4	26, 31	51, 55	1, -	4, 9
5	26, 33	44, 61	3, -	6, -
6	2, 24	54, -	1, 3	4, 8
7	2, 33	44, -	no antigen	6, -
8	24, 33	44, 55	3, -	4, 6
9	2, 26	61, 70	no antigen	9, -
10	24, -	35, 60	3, -	8, 9
11	24, 26	61, 75	3, -	8, 9
12	2, 24	51, 62	no antigen	6, 9
13	2, 26	35, 60	3, 7	2, 4
14	24, -	54, 62	1, 3	1, 9
15	26, 31	51, 55	1, -	8, 9
16	2, 24	46, 61	1, 3	9, -

Statistics

HLA-types were determined in the experimental population and compared to those of a healthy Japanese control population. Data for the control population were taken from 472 healthy Japanese subjects involved in the *3rd Asia-Oceania Histocompatibility Workshop Conference* (Aizawa, 1986). Results were statistically evaluated using Fisher's exact test.

RESULTS

Of the experimental population, 62.5% (10/16) were positive for HLA-DR9, as compared to 26% of the healthy control subjects. This increase in HLA-DR9 was statistically significant ($p=0.0029$; $Pc=0.026$). There was also a statistically significant increase in HLA-B55 ($p<0.05$). The remainder of the comparisons were not significant, and there were no known patient relatives with WG. HLA-DR9- or B55-positive patients with WG were not distinguishable from HLA-DR9- or B55-negative patients on the basis of organ involvement, response to therapy and clinical course.

Table 3. Frequencies and p-values of the various HLA antigens for the entire population of cANCA-positive WG subjects.

HLA antigens	control subjects positive (%) (n=472)	experimental subjects positive (%) (n=16)	Fisher exact probability
A2	40.7	37.5	p>0.999
A11	17.2	12.5	p>0.999
A24	68.4	56.3	p=0.413
A26	21.2	43.8	p=0.0575
A31	12.5	18.8	p=0.4416
A33	9.5	18.8	p=0.2014
B35	15.5	12.5	p>0.999
B39	7.2	6.3	p>0.999
B44	10.8	25	p=0.0939
B46	10	6.3	p>0.999
B51	14.2	25	p=0.2619
B54	14	12.5	p>0.999
B55	3.8	25	p=0.040*
B60	10.6	12.5	p=0.6839
B61	23.6	31.3	p=0.5494
B62	15.5	18.8	p=0.7247
B70	1.8	6.3	p=0.2858
B75	1.1	6.3	p=0.1821
Cw1	27.6	37.5	p=0.4000
Cw3	49	62.5	p=0.3191
Cw7	23.8	12.5	p=0.3818
DR2	34	12.5	p=0.1036
DR4	41	31.3	p=0.6063
DR6	16	25	p=0.3127
DR8	24.8	37.5	p=0.2500
DR9	26.1	62.5	p=0.0029**

*: $p<0.05$; **: $p<0.01$, $Pc<0.05$.

Individual HLA-types for the 16 subjects with cANCA positive WG are shown in Table 2; frequencies and p-values for the entire population are shown in Table 3.

DISCUSSION

The aetiology of WG remains unknown. Since Van der Woude et al. (1989) have reported the presence of cANCA in WG,

several studies have shown that this auto-antibody has a high specificity and sensitivity for WG (Gross et al., 1986; Savage et al., 1987; Specks et al., 1989). Recently, several relevant findings have been reported. Cytokine expression (i.e., interleukin (IL)-1ß, tumour necrosis factor-α, (TNF-α) and IL-6) was shown to increase in some patients with active WG (Yosida and Nagasawa, 1990; Gross et al., 1991). In addition, proteinase-3, the target antigen of cANCA, was shown to be present in endothelial cells grown in cell culture (Mayet et al., 1992). Anti-proteinase-3 antibodies prevent the inactivation of proteinase-3 by its natural inhibitor, α1-antitrypsin (Van de Wiel et al., 1992). Furthermore, T-cell lymphocytes of WG patients may proliferate in response to proteinase-3 (Van der Woude et al., 1990). These findings indicate that WG is an autoimmune disease caused by cANCA.

In healthy individuals, T cells distinguish HLA antigens from foreign antigens, thereby preventing attack on the body's own cells. The HLA antigens are instrumental in this self-recognition and, therefore, are thought to be important in the pathogenesis of autoimmune diseases, due to the failure of self-recognition. HLA antigens are known to be associated with several autoimmune diseases, including ankylosing spondylitis and rheumatoid arthritis.

Previous investigations of HLA antigens and WG produced contradictory results. In the first investigation, Strimlan et al. (1978) showed no association between WG and the HLA-A and B antigens. In a report by Katz et al. (1979), however, HLA-B8 was significantly elevated in WG patients. In addition, Elkon et al. (1983) showed an increase of HLA-DR2 in patients with WG, while Murty et al. (1991), using restriction-fragment-length polymorphism analysis, reported no association. More recently, Papiha et al. (1992) suggested an association of WG with HLA-DR1. These contradictory results may be due to the less-defined diagnostic criteria used in the earlier studies for confirming WG. In those investigations, WG was confirmed solely on the basis of biopsy results; the diagnostic criteria were not as rigid as the prevailing Japanese criteria. Furthermore, the presence or absence of cANCA was not determined, presenting the possibility that another autoimmune disease may have been present in the subjects used in those studies. The present study evaluated patients with cANCA-positive WG, because cANCA is thought to be involved in the pathogenesis of this disease. As such, the subjects of the present study were defined by a more narrow range of diagnostic criteria than those in the previous reports.

The results of the present study show an increase in the frequency of HLA-B55 and HLA-DR9 elevations in Japanese individuals with cANCA-positive WG. In particular, there is a strong correlation between HLA-DR9 ($p<0.01$, $Pc<0.05$) elevation and this disease. As there are no previous reports suggesting an association between WG and HLA-DR9, it is not clear whether the results presented here are due to our subjects who were all Japanese and cANCA-positive. While the number of subjects used in the present study is small, the results presented here suggest that further investigation into the association between the various HLA antigens and WG is warranted.

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Y. Nakamaru, M.D.
Dept. of Otolaryngology
Hokkaido University School of Medicine
Kita 15, Nishi 7
Kita-ku
Sapporo
Japan