HLA-DR Expression of Synovium and Correlation with Clinical Features of Patients with Rheumatoid Arthritis

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KUBOTA, H., OKADA, K., SATO, K. and SAGESHIMA, M. *HLA-DR Expression of Synovium and Correlation with Clinical Features of Patients with Rheumatoid Arthritis*. Tohoku J. Exp. Med., 2000, **190** (3), 177–184 — To investigate whether the expression of human leukocyte antigen-DR (HLA-DR) in synovial tissues obtained at synovectomy contributes to the clinical features of patients with rheumatoid arthritis (RA), immunohistochemical analysis was performed to determine the intensity and pattern of HLA-DR expression in synovial tissue from 96 patients with RA. The clinical features before and 1 year after the synovectomy were investigated. At the time of the surgery, duration of morning stiffness was associated with the degree of HLA-DR expression in synovial lining layer, and this synovial lining expression of HLA-DR was more frequently observed in elbow and wrist joints than in knee joint. In patients who underwent knee synovectomy, erythrocyte sedimentation rate and C-reactive protein level one year after the surgery were significantly higher in the patients with intense expression of HLA-DR in the synovial lining. Our findings showed that the expression of HLA-DR in the synovial lining contributes to several clinical features, and the expression in large joint such as knee may related with disease course of patients with RA. ——— rheumatoid arthritis; synovium; HLA-DR © 2000 Tohoku University Medical Press

Rheumatoid arthritis (RA) is an autoimmune disease contributed to some hereditary and environmental factors, and a chronic disease characterized by a variety of disease activities, progresses, susceptibilities, and prognoses. The association between human leukocyte antigen-DR (HLA-DR) and severity of RA has been reported in numerous studies and multiple ethnic groups, and some investigators have reported a high frequency of HLA-DR 4 and a relationship with DR1 and with DRw10 in RA (Young et al. 1984; Bardin et al. 1985; Nepom et al. 1987; Olsen et al. 1988). Histological expression of HLA-DR in rheumatoid

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synovial membrane was described in many studies, and correlation between the expression and rheumatoid inflammation was already reported (Klareskog et al. 1982; Shiozawa et al. 1983; Iguchi et al. 1986). However, the relationship between histological expression of HLA-DR in the tissue obtained at synovectomy and clinical characteristics has not been clarified (Aaron et al. 1986; Olsen et al. 1988; van-Jaarsveld et al. 1998). We investigated the relationship between the intensity and pattern of histological HLA-DR expression and the clinical characteristics including the disease course of RA.

MATERIALS AND METHODS

Synovial tissues were obtained from 96 joints of 43 patients. All patients were diagnosed according to the criteria of The American Rheumatic Association (Arnett et al. 1988). The study group comprised 36 females (82 joints) and 7 males (14 joints), with a median age of 51 years (range 31–69). Synovial tissues were obtained from the 53 knee joints, 26 elbow joints and 17 wrist joints undergoing synovectomy. Synovial specimens were fixed in 10% neutral formalin for 2 days and embedded in paraffin. All samples were then serially sectioned at 3 μ m, and one was stained with hematoxylin and eosin, and another was stained with anti-HLA-DR antibody for immunohistochemical analysis. Monoclonal antibody used was anti-HLA-DR antibody (DAKO, Copenhagen, Denmark). Immunohistochemical staining was performed with a LSAB Kit (DAKO). The slides were reacted in a substrate of 0.01% diaminobenzidine for 2 minutes. The sections were lightly counterstained with hematoxylin and mounted.

Total- and Surface-HLA-DR grades were determined to evaluate the correlation between the expression of HLA-DR and clinical features as follows; Total-HLA-DR grade: The total number of HLA-DR positive cells per square 250 μ m in 20 fields was counted in 96 joints, and were divided into 4 grades as follows: 0, Grade 0; 0–1.0, Grade 1; 1.0–5.0, Grade 2; >5.0, Grade 3. Surface-HLA-DR grade: The ratio of the length of the lining layer in which HLA-DR was positive to the full length of the lining layer was measured in 100 fields of all 96 joints, and divided into three grades as follows: 0, Grade 0; 0–0.1, Grade 1; >0.1, Grade 2.

As the parameters of clinical features, the age, critical age, duration of the disease, part of the operated joint, duration of morning stiffness, Lansbury index, white blood cell count (WBC), hemoglobin (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (IgM-RF) at the time of synovectomy of each patient were reviewed from the patients charts. These laboratory findings were also investigated 1 year after surgery. Plain radiographic findings at the time of surgery were reviewed. In the elbow and knee joints, the joints were divided into 6 grades (Grade 0–5) according to Larsen's system (Larsen et al. 1977), and in the wrist joint, each joint was scored (range; 0–314) by the method described by Sharp et al. (1985).

In the statistical analyses, the mean and standard deviation (s.d.) of the clinical parameters were calculated for each grade of Total- and Surface-HLA-DR. Differences between the grades were considered significant when the p value of comparison by means of one-way factorial analysis of variance (ANOVA) was 0.05 or smaller. In the analysis of the part of the operated joint, chi-square test for independence was used, and considered significant when the p value of comparison was 0.05 or smaller.

RESULTS

HLA-DR was partially positive in hyperplastic synovial lining (Figs. 1 and 2). In 96 joints, Total-HLA-DR grade was as follows: Grade 0, 24 joints; Grade 1, 32; Grade 2, 24; Grade 3, 16. Surface-HLA-DR in 96 joints was as follows: Grade 0, 54 joints; Grade 1, 32; Grade 2, 10.

Analysis of parameters at the time of surgery

The positive (Grade 1 or 2, Figs. 1 and 2) rate of Surface-HLA-DR in each joint was as follows: elbow, 65.4%; wrist, 52.9%; knee, 30.2%. The rate of the wrist or the elbow joint (upper extremity) was significantly higher than that of the knee joint (lower extremity) (chi-square test for independence, $p=0.0029$, Table 1). The mean of the duration of morning stiffness of patients whose Surface-HLA-DR Grade was 0 was 1.02 ± 1.2 (mean $\pm$ s.d.), Grade 1 was 1.7 ± 2.2 , and Grade 2 was 2.5 ± 2.0 . The duration of morning stiffness of patients whose Surface-HLA-DR grade 2 was significantly longer than that of grade 0 (ANOVA,

Fig. 1. Immunohistochemical staining of synovial tissue of rheumatoid arthritis (RA). HLA-DR is excessively expressed on the hyperplastic synovial lining ($\times 170$). A large number of cells, including endothelial cells and lymphocytes, in the sublining layer are also positive for HLA-DR. This patient is estimated Surface-HLA-DR Grade 2, and Total-HLA-DR Grade 3.

Fig. 2. HLA-DR is negative on the synovial lining layer, while many cells in the sublining layer are positive for HLA-DR ($\times 170$). This patients is estimated Surface HLA-DR Grade 0, and Total-HLA-DR Grade 3.

TABLE 1. *Positive rate of surface-HLA-DR*

	Grade 0	Grade 1	Grade 2	Positive rate (%)
Elbow	9	12	5	65.4
Wrist	8	7	2	52.9
Knee	37	13	3	30.2
Total	54	32	10	43.8

Fig. 3. Correlation between the Surface-HLA-DR grade and the duration of morning stiffness. The mean duration of morning stiffness of patients whose Surface-HLA-DR grade 2 was significantly longer than that of Grade 0 (2.5 ± 2.0 vs. 1.02 ± 1.2 hours, mean $\pm$ s.d., ANOVA, $p = 0.0135$)

$p=0.0135$, Fig. 3). Other parameters including age, critical age, duration of disease, Lansbury index, WBC, Hb, ESR, CRP, IgM-RF, and radiographic findings at the time of surgery were not significantly different among the groups of each Surface-HLA-DR grade. All clinical parameters mentioned were not significantly different among the groups of each Total-HLA-DR grade.

Analysis of parameters one year after the surgery

In patients who underwent knee synovectomy, the mean of ESR whose Surface-HLA-DR grade was 0 was 28.5 ± 18.5 , Grade 1 was 39.2 ± 11.2 , and Grade 2 was 50.3 ± 24.0 , and the mean of CRP whose Surface-HLA-DR Grade was 0 was 2.1 ± 1.7 , Grade 1 was 3.3 ± 2.4 , and Grade 2 was 6.3 ± 2.1 . The mean ESR value of the patients whose Surface-HLA-DR Grade was 2 was significantly higher than that of Grade 0 (ANOVA, $p=0.0407$). The mean CRP values of the patients whose Surface-HLA-DR Grade were 2 and 1 were significantly higher than that of Grade 0 (ANOVA, $p=0.0006$, $p=0.0174$, respectively, Fig. 4). In the patients who underwent elbow or wrist synovectomy, ESR and CRP were not significantly different among the groups of each Surface-HLA-DR Grade. Other parameters including age, critical age, duration of morning stiffness, duration of disease, Lansbury index, WBC, Hb, IgM-RF, and radiographic findings one year after the surgery were not significantly different among the groups of each Surface-HLA-DR Grade. All clinical parameters were not significantly different among the

Fig. 4. Correlation between Surface-HLA-DR grade and ESR and CRP one year after the knee synovectomy. The mean ESR value of the patients whose Surface-HLA-DR grade 2 was significantly higher than that of Grade 0 (50.3 ± 24.0 vs. 28.5 ± 18.5 mm, mean $\pm$ S.D., ANOVA, $p=0.0407$). The mean CRP values of patients whose Surface-HLA-DR grades were 2 and 1 were significantly higher than that of Grade 0 (6.3 ± 2.1 vs. 2.1 ± 1.7 , ANOVA, $p=0.0006$, and 3.3 ± 2.4 vs. 2.1 ± 1.7 , ANOVA, $p=0.0174$).

groups of each Total-HLA-DR Grade.

DISCUSSION

While the contribution of HLA typing to establishing the diagnosis of RA is limited, HLA-DR genotyping, DR4 subtype determination, and the two regions in MHC provide valuable markers for the prognosis of joint destruction in RA (Hameed et al. 1997; Wagner et al. 1997; Singal et al. 1998; Seidl et al. 1999). However, these evaluation need large efforts, while histological expression of HLA-DR is easily evaluated in the routine processed tissue. Several studies on histological expression of HLA-DR revealed the correlation of the expression in the synovial membrane and rheumatoid inflammation (Klareskog et al. 1982; Shiozawa et al. 1983; Iguchi et al. 1986). However, the relationship between intensity and pattern of the histological expression of HLA-DR in the tissue obtained at synovectomy and clinical characteristics has not been clarified (Aaron et al. 1986; Olsen et al. 1988; van-Jaarsveld et al. 1998).

In synovial tissue of RA, HLA-DR is expressed in hyperplastic synovial lining, endothelial cells, infiltrated lymphocytes, and dendritic cells. In this study, ESR and CRP level one year after the knee synovectomy were significantly higher in the patients with intense expression of HLA-DR in the synovial lining, although these data were not significantly different in the patients who underwent elbow or wrist synovectomy. HLA-DR expression in synovial lining of large joint such as knee may related with the disease course of patients with RA. In RA, correlation between expression of HLA-DR and production of proinflammatory cytokines such as interleukin-1 and tumor necrosis factor- α by synovium has been well known (Miyasaka et al. 1988; Isomaki et al. 1996), and HLA-DR positive T cells were identified in hyperplastic synovial lining and lymphoid follicles (Itoh et al. 1992). On the other hand, the Total-HLA-DR grade, which is an index of HLA-DR positivity of all synovial components, was not related to clinical characteristics. Therefore, we believe that the HLA-DR expression on the synovial lining layer has a more significant relationship with the pathogenesis of RA. In the study of *in situ* hybridization (Firestein et al. 1991), HLA-DR mRNA was markedly observed in the synovial lining layer, suggesting that the expression in the synovial lining layer is important in the immunohistochemical study of HLA-DR.

Among the clinical features, the duration of morning stiffness, which is a clinical feature of the upper extremity, exhibited a relationship with the Surface-HLA-DR grade. In addition, the positive rate of Surface-HLA-DR was significantly higher in the joints of the upper extremities than that of the lower extremities. These findings indicated that the Surface-HLA-DR grade may be associated with the inflammation degree especially in the upper extremities. While, most parameters employed as the indices of RA inflammation were not correlated with intensity and pattern of HLA-DR expression. These parameters

are those of systemic condition, and are not considered to be directly related to the degree of inflammation of the local joints which show varying disease conditions. Another reason for these poor relationships was considered to be a large number of modifying factors such as drugs used in the therapeutic course of RA. Wagner et al. (1997) examined HLA genotyping using polymerase chain reaction-based methods of RA patients, and showed that DR4-positive patients had a significantly increased risk of developing bony erosions and that HVR3 epitope-positive DR4-positive individuals had significantly higher Larsen indices than did epitope-negative patients. A more detailed study including an evaluation of these different shared epitope allotypes should be further necessary.

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