CIRCULATING IMMUNE COMPLEXES IN TAKAYASU DISEASE

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Circulating immune complexes in Takayasu disease were investigated to assess the involvement of autoimmune mechanisms. Antibody dependent cell-mediated cytotoxicity (ADCC) and Raji cell assay revealed: The 10 percent inhibition of ADCC in 37 patients was 35.6 ± 5.2, this being a non-statistically significant value as compared with the value of 25.6 ± 3.0 in 33 apparently healthy controls. Raji cell assay values were 30.4 ± 11.5 and 3.5 ± 2.3 for patients and controls, respectively. As 54% of all these patients had a negative reaction and there was no correlation between immune complex levels and BSR, CRP, or ASO, immune complexes, while accelerating or modifying the pathophysiological state, probably are not primary causative factors.

TAKEYASU disease is well known by its characteristic clinical feature of pulselessness and by the epidemiological fact that most patients are young females from Asian or South American areas.1-3 Cause of this disease remains obscure. Different groups discussed the etiology of this morbid condition in relation to immune complex diseases as many patients with Takayasu disease have a past history of rheumatic fever, rheumatoid arthritis or systemic lupus erythematosus.4-7

In fact, many characteristic clinical features such as accentuated BSR, positive reaction of CRP, increase of γ-globulin, fibrilla or arthralgia suggest an immunoreactive mechanism in the etiology of this disease.3,8-10 and Frøvig and Løken and McKusick11,12 reported a case of Takayasu disease complicated with rheumatic disease or SLE.

We investigated circulating immune complexes (ICs) of the sera in Takayasu disease, as such ICs play an important role in inducing damage to tissues in the heart, blood vessels and kidney. Our data are presented herein.

MATERIALS AND METHODS

Blood samples were obtained by venipuncture from 37 (34 females and 3 males) patients with Takayasu disease and 33 young female nurses at the school attached to our university, as a control. The mean ages were 33.5 ± 1.8 and 21.2 ± 0.8 years. These samples were immediately divided into two sterile pyrogen-free test tubes, one for measuring immune complexes and the other for studying clinical inflammatory signs such as C-reactive protein (CRP), blood sedimen-

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Key Words:
- Takayasu disease
- Immune complex
- ADCC
- Raji cell assay

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TABLE I LABORATORY DATA IN TAKAYASU DISEASE AND NORMAL CONTROL GROUP

<table>
<thead>
<tr>
<th>Tests</th>
<th>Normal control (N = 33)</th>
<th>Takayasu disease (N = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSR (mm/1 h)</td>
<td>7.8 ± 0.5</td>
<td>33.0 ± 3.8**</td>
</tr>
<tr>
<td>CRP#</td>
<td>0</td>
<td>1.5 ± 0.3*</td>
</tr>
<tr>
<td>ASLO (Todd)</td>
<td>68.1 ± 11.0</td>
<td>87.0 ± 12.4</td>
</tr>
<tr>
<td>RA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
</tbody>
</table>

** P < 0.01: Takayasu disease vs. normal control
#: (−) 0, (+) +1, (+++) +2, (+++) +3

PERCENT INHIBITION
ADCC ASSAY

CIRCULATING IMMUNE COMPLEX LEVELS IN RAJI CELL ASSAY

Fig.1.

Fig.2.

tation rate (BSR), anti-streptolysin O level (AS0) and rheumatoid factor (RF) test. After the clot had formed in the first tube, the tube was centrifuged and the serum removed aseptically. Sera were stored at −70°C in sterile vials until testing. Antibody-dependent cell-mediated cytotoxicity (ADCC) assay for detecting circulating immune complexes was performed under double blind conditions, using the method described by Larsson et al.13 and modified by Fye et al.14 The tissue culture medium in the inhibition studies was modified by replacing the 5 percent heat-inactivated fetal calf serum with 0.5 percent bovine serum albumin. Normal lymphocytes were adjusted to 6.4 × 10^6 cells/ml in tissue culture medium and incubated for 1 hour at 37°C with an equal volume of heat decomplemented patient’s serum. Fifty microliters of the lympho-

cyte preparation were then added to 100 μl of labeled chicken erythrocytes (CRBC) in the presence of rabbit anti-CRBC antiserum and ADCC was determined in normal lymphocytes cultured with homogeneic normal sera which served as controls. Percent inhibition was determined by the following formula:

\[
\text{% inhibition} = \left(1 - \frac{\text{tested ADCC}}{\text{control ADCC}} \right) \times 100
\]

Parallel to this study, Raji cell assay was also performed by the method already described by Theofilopoulos et al.15 Here also a double blind condition was adhered to. The concentration of immune complexes was expressed as micrograms of aggregated human IgG equivalent per milliliter. Values lower than 10 μg eq/ml were con-
Correlation between blood sedimentation rate (BSR) and immune complex (ADCC) in Takayasu disease

Fig. 3.

Correlation between ASO and immune complex (ADCC) in Takayasu disease

Fig. 4.

Correlation between blood sedimentation rate (BSR) and immune complex (Raji assay) in Takayasu disease

Fig. 5.

Correlation between ASO and immune complex (Raji assay) in Takayasu disease

Fig. 6.

sidered normal. Parallel to these studies, BSR, CRP and ASO level were also studied, using other samples, in order to check the status of inflammation in these patients. BSR and ASO titers were analyzed statistically.

RESULTS

BSR in young female nurses in normal controls was under 14 mm/h and the mean value was 7.8 ± 0.5 mm/h. ASO titer was also under 166 Todds except two cases with the mean level of 68.1 ± 11.0 Todd Units. Both CRP (except one case) and RF test were negative. On the contrary, in patients of Takayasu disease, the mean value of BSR was 33.0 ± 3.8 mm/h with a statistically significant high level (p < 0.01), as com-
pared with that in normal controls. The value in
twelve patients was over 30 mm/h in BSR, the
highest being 96 mm/h. Seven patients with
Takayasu disease revealed ASO titers of over
166, however, the mean value of 87.0 ± 12.4
Todd Units in Takayasu patients did not show a
significant difference from that of normal con-
trols. Fourteen patients had a positive CRP reac-
tion, the highest being +4. The mean value of
CRP in Takayasu disease was 1.5 ± 0.3, with a
statistically significantly difference as compared
with a negative reaction in normal controls. RF
test was negative in Takayasu as in normal con-
trol groups (Table I). The percent inhibition of
ADCC with the sera of young normal female sub-
jects was 25.6 ± 3.0, such being slightly higher
than normal values determined previously of less
than 20 percent. On the other hand, as shown in
Fig. 1, the percent inhibition in Takayasu disease
ranged from 0% to 97%, the mean percent being
35.6 ± 5.2. There was no statistically significant
difference in the percent inhibition of ADCC
between normal controls and Takayasu disease.

Figs. 3 and 4 show that there was no apparent
relationship among ADCC and BSR or ASO titer.

In the Raji cell assay, 46 percent of patients
with Takayasu disease had positive levels of
circulating immune complexes over 12 µg/ml,
whereas 20 percent of normal controls had values
of over 12 µg/ml. The highest value was 400 µg/
ml in one patient with Takayasu disease who had
so been diagnosed 15 years previously. The
other 54 percent of patients with Takayasu
disease exhibited a negative reaction. However,
the mean level of circulating immune complexes
among patients as a group was 30.4 ± 11.5, this
being a statistically significant high value as com-
pared with 3.5 ± 2.3 in the control group (p <
0.05) (Fig. 2). Accented BSR and high titers of
ASO were not correlated to the concentration of
circulating immune complexes detected by the
Raji cell assay, as shown in Figs. 5 and 6. The
positive CRP reaction did not correlate with the
high concentration of immune complexes in Raji
assay.

DISCUSSION

In 1908, M. Takayasu reported peculiar
changes in the retinal vessels, and this form of
vasculitis, so reported for the first time, has come
to be known as Takayasu disease.16 The etiology
of this disease has never been entirely clarified
and factors such as tuberculosis,1718 rheumatic
fever,19 syphilis20 atherosclerosis11,21 and auto-
immunopath911 have all been linked to the
proposed mechanisms involved in the disease.

In 1962, Judge et al. suggested the involve-
ment of autoimmunopathy22 as their patients
with Takayasu disease had a high gammaglobulin,
elevated BSR, leucocytosis, positive CRP, fibrilla
and arthralgia and a high titer for circulating anti-
body against the aortic wall23. Utilizing
immunological processes, Saito produced in the
arterial wall of rabbits changes similar to those
seen in Takayasu patients.24

Ikeda found a positive antiglobulin reaction in
tanned red cells from a patient with Takayasu
disease.25 Other workers have found disorders
such as rheumatic fever, rheumatoid arthritis,
SLE, polyarthritis, scleroderma, giant cell
arteritis, ankylosing spondilitis, etc.247 to be
associated with Takayasu.

On the other hand, Wakisaka et al.26 and
Hirsch et al.27 reported negative circulating anti-
bodies against human aorta. Strachen28 also
reported negative results in tests for thyroid
antibody, antinuclear antibody, and in precipi-
tation test for nonorgan-specific tissues in
Takayasu disease, suggesting that Takayasu
arteritis does not occur in a group of subjects
specially prone to the development of the organ-
specific autoimmune tissue disease. Attempts to
produce experimentally relative changes in rabbit
aorta have also failed.29

Newly designed assay systems for immune
complexes provide precise information and auto-
immune diseases such as periarteritis nodosa, SLE
and nercotizing rheumatoid vasculitis have been
clarified as to the role of immune complexes in
these pathophysiological conditions.3032 Theofilopoulos et al. reported that over 60% of
their patients with SLE or nercotizing vasculitis
showed a positive reaction in the Raji assay and
a good correlation between ADCC inhibition and
Raji cell assay has been confirmed.15

In our patients with Takayasu disease there
was not a high percent inhibition of ADCC in the
sera and the Raji cell assay proved to be negative
in 54% of our patients.

In light of our data obtained using Raji cells
and the ADCC tests it would appear that immune
complexes are not a "primary" causative factor
in this condition, although such may to some ex-
tent accelerate or modify the pathophysiological
state. It is generally considered that the high
levels of immune complexes are associated with
the active stage and that there is a complete
decline in 6 weeks after initiation of chemother-

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apy. In patients with Takayasu disease, there was no correlation between the level of immune complexes and the stage of the disease, nor was there a correlation between BSR, CRP or ASO and the level of immune complexes. Some patients who had had Takayasu disease for over 10 years and in whom the obvious signs of inflammation would suggest the inactive stage, revealed a high level of immune complexes and some patients who had an elevated BSR and a positive CRP showed a negative reaction in the Raji assay. Such inconsistencies of clinical signs and immune complexes also suggest that these complexes seem not to be a primary factor.

Our recent findings on HLA factors in Takayasu disease led to the postulation of a possible genetic factor. Both population and family studies revealed a statistically significant association of one haplotype of A9–B5, as compared with that in healthy Japanese. We found an association of the HLA antigen in D-locus antigen (DHO) however, there was an even closer relationship of BW-52, a subslocus of B5. All these observations suggest that there is another gene located close to B-locus which regulates factors in Takayasu disease, as it is generally considered that the immune response gene is located closer to the D-locus than the B-locus gene. The degree of association between this gene which is located closer to B-locus with Ir gene may determine the characteristic features of Takayasu disease. Studies on these associations are underway in our laboratories.

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