(6) Autoimmune Hemolytic Anemia

A Review of Clinical Features of Autoimmune Hemolytic Anemia in Japan and Immunoserological Studies on Anti-erythrocyte Autoantibodies

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Autoimmune hemolytic anemia (AIHA) is characterized by the presence of the autoantibodies directed to red cells and the shortening of red cell survival in vivo.

The autoantibodies are varied from one patient to the next with temperature requirement, specificity, chemical nature and complement dependency. The mechanisms by which, in the presence of the autoantibodies, red cells are lysed intravascularly or sequestered and destroyed in the reticuloendothelial tissues are not completely understood.

This paper deals, firstly with the clinical features of AIHA in Japan and, secondly, with immunoserological studies of autoantibodies with special reference to their roles played in the hemolysis in vivo.

Material and Methods

I. Patients: In 1966, questionnaires were sent to university hospitals in Japan to inform the author of the clinical data on patients with AIHA¹⁾. From these data, 100 cases were collected. In 1972, clinical records of 100 cases were obtained from case reports in Japanese medical literatures published from 1967 to 1971²⁾. A total of 244 cases, including 44 observed at Kyoto University Hospital, were investigated in this study. Analyses were done on these cases according to sex, age, type of autoantibodies and, in secondary AIHA, underlying diseases. Factors which initiated to develop the disease or which aggravated the clinical course were also analyzed.

II. Immunoserological study: The following tests were performed on patients admitted to Kyoto University Hospital.

1) Direct Coombs' test was carried out with antiglobulin (broad-spectrum), anti-immunoglobulin (IgG, IgA and IgM) and anti-complement antisera.

2) Indirect Coombs' test: Patients' sera and red cell eluates were tested using the panel red cells (Identigen, Ortho).

3) Quantitative assay of autoantibodies: Eluates were prepared from patients' red cells according to Kochwa's method³⁾. Amounts of IgG in them were measured by radio-immunoassay which the author developed and reported previously⁴⁾.

4) Leukocyte migration inhibition test (LMT)⁵⁾: Sjöborg's method⁵⁾ was employed.

Red cell membranes were obtained from the healthy donors of O, CcDEe. After washing and sonication, these were used as the antigen⁶⁾.

Result

I. 1) Classification: A total of 244 patients with AIHA were classified clinically depending upon the presence or absence of the underlying diseases. There were 162 cases of idiopathic AIHA, 74 of secondary AIHA and 8 of paroxysmal cold hemoglobinuria. From the laboratory view point, 222 cases of them were comprised of 194 warm-reactive, 22 cold-reactive and 6 mixed autoantibody types.

Among the underlying diseases in secondary AIHA, SLE, malignant lymphoma and liver diseases were in that order in frequency. Age of the patients was distributed from the first to seventh decades, but there was a sharp peak in the third decade in females and a small peak in the sixth decade in males. The ratio of female to male was about 2 to 1.

2) Clinical features: Anemia of moderate to marked severity was the most common manifestation. Red cell counts were 1 or less, 1 to 2, 2 to 3 and 3 or more millions per cu. mm in 17.4, 51.0, 23.5 and 8.2 percent of the total patients with idiopathic AIHA, respectively. Reticulocyte counts were over 10 percent in more than a half of them. Anemia plus leukopenia were found in 9.4, anemia plus thrombocytopenia in 13.1, and pancytopenia in 1.3 percent of the patients. Splenomegaly was found in about a half of them.

II. Autoantibodies: Warm-reactive autoantibodies are mainly IgG in immunoglobulin class, but these are varied in biological activities from one case to another. This fact is shown in the following cases.

Case 1: 19-year-old, male. The patient had been well until one year previously when he noticed anemia and jaundice. Laboratory findings showed RBC 3.2 millions, reticulocytes 20%, total bilirubin 2.0 mg/dl, and indirect bilirubin 1.0 mg/dl. Direct Coombs' test was strongly positive and indirect test was negative. Red cells were agglutinated by anti-IgG but not by anticomplement antisera. The autoantibodies eluted from the red cells had anti-e specificity. The patient's blood type was A, CcDEe. The survival times (TI/2) of transfused patient's red cells and of normal red cells with A, CCDee were 4.8 and 1.2 days, respectively. Steroids were given and the patient improved, even though direct Coombs' test remained positive. At that time, survival studies were reexamined. Life span of the patients' red cells and that of normal red cells with A, ccDEE were 16.5 and 31 days, respectively. From these observations, the autoantibodies found in this patient seemed to exert destructive effect upon the red cells with e-antigen(s) in vivo.

Case 2: 62-year-old, female. The patient had suffered from rheumatoid arthritis and chronic thyroiditis for some ten years. She was hospitalized because of dyspnea due to hypothyroidism. Anemia was not found. Survival time of patient's red cells was entirely normal. But direct Coombs' test was strongly positive and indirect test

was negative. The autoantibodies found on red cells were IgG type. Eluate from red cells was strongly reactive to all of the panel red cells (panagglutinin). In this case, no destructive effect upon the red cells was exerted by the autoantibodies.

1) Quantitative assay of autoantibodies: Amounts of IgG eluted from 1 ml of packed red cells was less than 100 ng in normal controls and 200 to 5,000 ng in patients with Coombs' positive AIHA. The quantities of IgG were not inversely correlated with the severity of anemia in the patients. This implied that the qualitative difference of the autoantibodies and other factors such as complement and functions of lymphocytes and macrophages determine the degree of hemolysis *in vivo*.

2) Complement dependency: With the use of anti-IgG and anti-complement antisera, red cells from the patients were tested and IgG+C type was found in 15, IgG type in 19 and C alone in 1 out of 35 patients. Red cell counts of patients with IgG+C type were 1.5 millions and of those with IgG type 1.9 millions on the average. No significant difference was found between both patients' groups. The capability of the autoantibodies to fix complement component did not necessarily reflect the severity of hemolysis *in vivo*.

3) Serum complement level: CH50 was determined in 16 patients with AIHA. It was found to be depressed in 8 and normal in the remaining 8 cases. It was observed that CH50 was more markedly depressed in IgG+C type than in IgG type. There were some patients with IgG type who had normal CH50 levels in spite of severe hemolysis. In these cases, there was no apparent evidence that complement took an active part in the hemolysis.

4) Lymphocyte function: With the purpose of finding possible mechanisms by which lymphocytes would be involved in the hemolysis *in vivo*, the leukocyte migration inhibition test was performed using the red cell membranes as antigen. It showed a positive inhibition in 1 and weakly positive in 3 out of 7 patients. And it was also found weakly positive in 1 out of 2 patients with Coombs-negative AIHA⁶. From this observation, it was proved that the lymphocytes in some patients were sensitized with red cells and were capable of liberating the chemical mediators (lymphokines) on contact with the antigens. Therefore, it is suggested that the lymphocytes play some part in the hemolysis *in vivo*, probably through migration inhibition and phagocytosis enhancement of the macrophages.

III. Analysis of initiating or aggravating factors in AIHA: As mentioned before, SLE and malignant lymphoma were frequently the underlying diseases of secondary AIHA.

On the other hand, the etiology of idiopathic AIHA was entirely unknown. The author made an attempt to find any factors by which the disease had been initiated or aggravated in its course.

From this study, several factors were found in 34 out of 94 cases reported in Japan. Among them, pregnancy and delivery, drug intake, and genetic background were considered important.

As to pregnancy and delivery, the disease was initiated and aggravated during

the 1st and 2nd trimesters in 7 out of 9 cases. Remission was usually brought after delivery. Both warm- and cold-reactive type were found to be about equal in frequency. Evans syndrome was relatively common.

α -Methyldopa and L-Dopa have been well known to induce positive Coombs' test and, in some cases, overt hemolysis. The author and his collaborators⁷⁾ reported a case which developed Coombs' positive hemolytic anemia after long-term administration of a laxative named oxyphenisatin.

As to genetic factors, immunological studies were done on families of the patients with AIHA. Abnormal immunoglobulin levels and positive test for autoantibodies such as antithyroid, RA-factor, ANF were more frequently found in the first degree relatives of the patients than in general population.

Oyagi and the author⁸⁾ reported a family in which a mother and her daughter suffered from AIHA.

IV. Therapy and prognosis:

The mainstay for treatment of the warm antibody type of AIHA was the administration of corticosteroid hormones in the author's and other hospitals. About two thirds of the patients responded well to the corticosteroids with improvement of anemia. But the remaining number responded incompletely or did not respond at all. Recently, immunosuppressive drugs such as 6MP, azathioprine and cyclophosphamide have increasingly been used. In order to evaluate the effectiveness of the immunosuppressive drugs, a comparison was made between patients treated with corticosteroids alone and those with corticosteroids plus 6MP. No significant difference was found between them in terms of amelioration of clinical and serological findings.

Prognosis: The mortality rate of patients with IAIHA within one year from the onset was 28.6 percent and that of patients who survived over two years was 8.6%.

Summary

A clinical review of patients with autoimmune hemolytic anemia in Japan and results of immunoserological studies on antierythrocyte autoantibodies (warm-type) were presented in this paper. Quantitative amount of the autoantibodies did not necessarily reflect the degrees of hemolysis *in vivo*. The author postulated that the lymphocytes played an important role in the destruction of red cells probably through liberating the humoral factors (lymphokines) for migration inhibition and phagocytosis enhancement of the macrophages.

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