Autoantibodies and Red Blood Cell Membrane Proteins in a Case of Canine Autoimmune Hemolytic Anemia

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(Received 12 December 1989/Accepted 9 September 1990)

ABSTRACT. Autoantibodies and erythrocyte membrane proteins were analyzed in a case of a dog with autoimmune hemolytic anemia (AIHA). In crisis phase, antiglobulin test was positive. The eluate from the erythrocytes of the dog with AIHA gave agglutination against autologous erythrocytes. Immunoglobulin subclasses in the eluate were revealed to be IgG and IgA by the double diffusion test. Comparing the SDS polyacrylamide gel electrophoretic patterns of erythrocyte membrane proteins between the crisis and remission phases, there was a change in the protein on protein 4.1 region. However, there were no changes in blood group typings in two phases.—KEY WORDS: autoimmune hemolytic anemia, dog, erythrocyte membrane protein.

Autoimmune hemolytic anemia (AIHA) caused by autoantibodies against the patient’s erythrocytes has been well reported in dogs [1, 5, 13]. Autoantibodies against erythrocytes in AIHA have the different physical properties, reflecting the different clinical syndromes of AIHA. Recently, the antigens on the membrane of erythrocytes were studied in the human cases of AIHA and acquired alterations of blood group antigens were confirmed [6–9, 12, 14]. In some cases of AIHA in human, the acquired alteration on the surface membrane of erythrocytes was detected in accordance with the appearance of the autoantibody. In this paper, we studied the autoantibodies and the membrane proteins of erythrocytes in a dog with AIHA.

MATERIALS AND METHODS

Patient: A 2-year-old male setter was admitted to the hospital with a history of pale mucous membranes and weakness. Initial blood examination showed hematocrit 27% and WBC 8900/μl. Direct antiglobulin (Coombs’) test was positive. The patient was treated with dexamethasone and antibiotics. After the treatment for about 3 months, the dog was recovered from anemia.

Venous blood samples were collected from the patient and several normal dogs. Erythrocytes were washed three times in physiological saline and prepared to 2% cell suspensions. Direct and indirect antiglobulin test were performed by the standard techniques. Determination of blood group typing was performed according to the procedure described by Ejima et al. [3]. Nine blood group systems examined in the present case were DEA 1, DEA 5, DEA 6, D, J1, J2, J3, J4 and J5.

Elution of the antibody against autologous erythrocytes was performed by the method of Landsteiner and Miller [11]. Immunoglobulin subclass in the eluate from erythrocytes was determined by the double diffusion method using anti-dog IgM, -IgG and -IgA (Miles Scientific, U.S.A.).

The ghosts of erythrocyte membrane were prepared as described by Dodge et al. [2]. The electrophoretic separation of erythrocyte membrane proteins was performed in a 5–15 gradient polyacrylamide gel according to the method of Laemmli [10]. The major bands were named according to the nomenclature in human described by Fairbanks et al. [4].

RESULTS

Erythrocytes from the dog with AIHA were agglutinated with the eluate from autologous erythrocytes. In addition, the eluate gave weak agglutination against the erythrocytes of normal dogs. However, the eluate from the erythrocytes of a normal dog gave no agglutination against the erythrocytes of the AIHA and normal dogs. Immunoglobulin subclasses in the eluate from the dog with AIHA were revealed to be IgG and IgA by the
Fig. 1. The patterns of erythrocyte membrane proteins separated by SDS polyacrylamide gel electrophoresis. A: The dog with AIHA, B, C: Normal dogs. An arrow shows an increased band in the red cell membrane proteins of the dog with AIHA.

Fig. 2. The patterns of red blood cell membrane proteins of the dog with AIHA separated by SDS polyacrylamide gel electrophoresis. A: Crisis phase, B: Remission phase. An arrow shows the increased band in the dog with AIHA.

double diffusion test using anti-dog immunoglobulin. After remission of AIHA, direct antiglobulin test became to be negative.

Erythrocyte membrane proteins were separated by SDS polyacrylamide gel electrophoresis (SDS PAGE) as shown in Fig. 1. A protein which located on the region of protein 4.1 of erythrocyte membrane increased in the sample from the dog with AIHA. The molecular weight was estimated around 81,000 by comparing with marker proteins on SDS PAGE. While, the increased band on protein 4.1 region was not found in remission phase of AIHA (Fig. 2). Therefore, the patterns of erythrocyte membrane proteins of the dog with AIHA was different between in the crisis and remission phases. Besides, the other variation such as band 3 and minor bands were observed in the patterns of erythrocyte membrane proteins on SDS PAGE. The band on protein 4.1 region of erythrocyte membrane was observed to be of variety among ten healthy dogs on the patterns of SDS PAGE. As confirming test, blood samples were obtained from one healthy mongrel dog three times at intervals of about a week and prepared erythrocyte ghosts. There were no remarkable changes of the bands among those samples. Blood group antigens of the dog with AIHA have no difference in the crisis and remission phases.

DISCUSSION

A peptide associated with AIHA in human was detected from the supernatants of dispase-treated erythrocytes of the patients with AIHA [8]. These peptide and autoantibody disappeared simultaneously after chemotherapy. In the present case of AIHA in a dog, there were changes in the membrane proteins of erythrocytes. The protein on protein 4.1 region increased in crisis phase and decreased in remission phase in accordance with the disappearance of autoantibodies. This finding would suggest that the increased band might be associated
with the hemagglutination detected in the present case of AIHA.

Acquired alterations of blood group antigens have been reported in patients with AIHA in human [6–9, 12, 14]. Some patients with AIHA caused by the antibodies having recognizable blood group specificity showed transient depression of the activity of the corresponding antigen [12, 14]. In addition, the acquisitions of several glycoporphin-like antigens were found in AIHA [6, 7]. In the present case of AIHA in a dog, the increased band associated with the crisis of AIHA were detectable in erythrocyte membrane. However, the band seemed not to be carried blood group antigens.

This study suggested the alterations of erythrocyte membrane in the dog with AIHA. The analyses of erythrocyte membrane proteins may lead us to elucidate the pathogenesis of AIHA in dogs.

REFERENCES