Cryoglobulinemia in a Horse

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ABSTRACT. Cryoglobulin was isolated from a horse which had glomerulo-nephritis and a history of swelling and skin ulcers of the limbs in the winter. The isolated cryoglobulin showed a single peak on a gel permeation chromatography column with an apparent molecular mass (Mr) of 180,000 which could be divided into two gamma bands by cellulose acetate electrophoresis. Immunoelectrophoretic analysis revealed that the cryoglobulin formed two precipitation lines with anti-horse IgG. Spur formation was observed when the cryoglobulin and the IgG purified from a normal healthy horse were cross-reacted with anti-horse IgG on a double diffusion gel. In addition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis under the reduced conditions showed that the isolated cryoglobulin consisted of two doublets of polypeptides with Mr values of 52,000 and 50,000, and 51,000 and 30,000, corresponding to the heavy chain and the light chain of the horse IgG molecules, respectively. These results suggest that the isolated cryoglobulin might consist of two different IgG molecules, and that the manifestations such as foot swelling with skin ulcers and renal failures were all induced by the cryoglobulin in the serum.—KEY WORDS: cryoglobulin, cryoglobulinemia, horse, mixed cryoglobulin.

Cryoglobulins are serum globulins which precipitate reversibly at temperatures below 37°C. Several types of human cryoglobulins have been demonstrated in association with various diseases [1, 2, 5, 9–11]. In most of these human cases, cryoglobulins appeared to consist of immune complexes and were classified into three types based on their immunoglobulin compositions [2]. In addition, the molecular mechanisms of their temperature-dependent changes in solubility have been partially identified [2, 12]. In contrast, less is known about the characteristics of the cryoglobulin in animals [3, 4, 6]. In the present study, we described the biochemical and immunological characteristics of a cryoglobulin isolated from an Arabian horse which had a history of foot swelling with skin ulcers.

MATERIALS AND METHODS

Case report: A female Arabian horse, 19 years old, exhibited foot swelling with skin ulcers in mid-January. In spite of various treatments by the referring veterinarian, the inflammation did not improve for more than 2 months. In early April, the swelling receded. After that, however, the horse was still anorectic. The horse was admitted to the Veterinary Teaching Hospital of Hokkaido University for further evaluation on June 7.

Physical examinations and laboratory findings showed no abnormalities, except a slight increase of eosinophils in the peripheral blood. However, a white precipitate was formed in the serum from the horse when the serum was allowed to stand at room temperature for about 20–30 minutes (Fig. 1). The precipitate was dissolved on warming to 37°C. Thus, it was confirmed to be cryoglobulin. This cryoglobulin was always detected in the serum until the horse died.

In early August, the horse developed anorexia, diarrhea and weakness. Laboratory examinations showed increases of blood urea nitrogen and serum creatinin, and a decrease of serum total protein. Urine analysis revealed 4+ albumin and 4+ occult blood. In the light of the deteriorating clinical conditions, the horse was killed.

No gross findings were observed, except of anemia and edema of the renal cortex. Histopathological examination revealed cryoglobulinemic glomerulonephritis classified into membranoproliferative glomerulonephritis. Most of glomeruli had intraluminal or subendothelial deposits of hyalin-like substances in the peripheral capillary loops. These deposits were reacted with the antibodies raised against several components in horse serum. More detailed information on these histopathological findings is reported elsewhere [7].
Isolation of cryoglobulin: The patient’s blood was kept at 37°C for 30 min followed by centrifugation at 2,000 × g for 20 min. The serum was kept at 4°C for 2 hours and centrifuged at 4°C. The precipitate was solubilized in phosphate-buffered saline (PBS, pH 7.4) to yield the original volume and was placed at 4°C again. This procedure was repeated 3 more times. After the final washing the resultant precipitate was dissolved in a small volume of 100 mM Tris-HCl (pH 8.0) and dialyzed against the same buffer.

For further purification, the cryoglobulin obtained was loaded onto a gel permeation chromatography column of Sephacryl S-300 (2.6 × 95 cm, Pharmacia-LKB Biotechnology) which had been equilibrated and eluted with 100 mM Tris-HCl (pH 8.0) at 40°C.

Characterization of the cryoglobulin: The protein composition of the isolated cryoglobulin was electrophoretically analyzed on cellulose acetate membranes and sodium dodecyl sulfate (SDS)-polyacrylamide gels. SDS-polyacrylamide gel electrophoresis (PAGE) was carried out according to the method of Laemmli [8] using 10% acrylamide gels.

The antigenic properties of the isolated cryoglobulin were analyzed using the Ouchterlony’s double-diffusion technique and immunoelectrophoresis. The antibodies used were anti-horse whole serum (Cappel), anti-horse IgG (specific to heavy and light chains, Miles), anti-horse IgA (specific to α chain, Bethyl), anti-horse IgM (specific to μ chain, Cappel), and anti-horse C3 (Vector ABC kit, Vector).

RESULTS

The cryoglobulin was isolated from other serum proteins as the precipitate by the simple washing procedure described above. The isolated cryoglobulin showed a single peak with an apparent molecular mass (Mr) of 180,000 on a gel permeation chromatography column (Fig. 2). This value of Mr was in good agreement with that of IgG purified from a normal healthy horse. The immunological and biochemical characterization of the cryoglobulin was performed using the proteins in the peak fraction. No other proteins precipitable at 4°C were observed in the residual serum. Further washing of the precipitate (up to 8 times) caused a slight loss of the cryoglobulin but no alteration in the results of the immunological and biochemical analyses.

The isolated cryoglobulin revealed two gamma bands in a cellulose acetate electrophoresis (data not shown). Figure 3 shows the precipitation patterns of the cryoglobulin reacted with antibodies raised against several components in horse serum. The cryoglobulin formed precipitation lines with anti-horse IgGs as well as with anti-horse whole serum.
but not with the other antisera examined (anti-IgA, anti-IgM, and anti-C3). Figure 3 also shows that spur formation was observed when the isolated cryoglobulin and IgG from a normal healthy horse were cross-reacted with anti-horse IgG. In addition, immunoelectrophoretic analysis showed that two precipitation lines were formed between the cryoglobulin and anti-horse IgG (Fig. 4). These results indicated that there was partial identity between the cryoglobulin and IgG molecules.

As shown in Fig. 5, SDS-PAGE analysis under the non-reduced conditions showed that the cryoglobulin consisted of two major proteins with Mr values of 200,000 and 220,000. When the cryoglobulin was reduced with β-mercaptoethanol, four polypeptides with Mr values of 52,000, 50,000, 31,000, and 30,000 were observed as prominent components. The migrating positions of these proteins well agreed with those of horse IgG under both non-reduced and reduced conditions.

**DISCUSSION**

The immunological and biochemical features of the cryoglobulin found in the present horse indicated that it consisted of two distinct molecules of IgG. We assumed that this cryoglobulin consisted of two monoclonal or polyclonal components of different IgG subclass based on the findings that two distinct bands and precipitation lines were observed on cellulose acetate membranes and immunoelectrophoretic gels (Fig. 4). The formation of a spur between the cryoglobulin-anti-IgG complex and normal IgG-anti-IgG (Fig. 3) supports this assumption. Human cryoglobulins have been classified into three categories [2]. Type I, monoclonal cryoglobulins made of immunoglobulins with only one class or subclass of heavy and/or light chain, type II, mixed
cryoglobulins with a monoclonal component made of immunoglobulins belonging to two different classes, one of which is monoclonal, and type III, polyclonal mixed cryoglobulins which are made of heterogenous immunoglobulin molecules usually belonging to two or more different classes, and sometimes of additional serum proteins [4]. From the above results, the cryoglobulin found in this horse seems to be composed of type II mixed cryoglobulins, IgG-IgG.

We could not determine whether these IgG molecules form the immune complex or not, that is, whether one of them might be an auto-antibody produced against the other one. However, it could be hypothesized that these IgG molecules form the complex within the body, since histopathological examinations in this case revealed the deposition of immunoglobulins in the glomeruli. Glomerular changes are often found in patients with mixed cryoglobulins, especially in those with type II [2]. It has been thought that the deposition of serum cryoglobulins in renal tissue, as immune complexes, may provoke glomerular changes in patients with cryoglobulinemia [1]. Furthermore, it has also been reported that the insolubility of cryoglobulins at low temperatures causes cutaneous injuries, including vascular purpura, skin necrosis, and leg ulcers in such patients [2, 5, 9]. From these reports and the results of the present study, we conclude that the clinical manifestations observed in this horse, such as foot swelling with skin ulcers and renal failures, were all induced by the cryoglobulins in the serum.

REFERENCES


