The Effect of Long-term Administration of Horse Anti-dog Lymphocyte Globulin in Healthy Dogs

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There has recently been a wide spread interest in the use of heterologous anti-lymphocyte serum (ALS) for the prolongation of renal, hepatic and heart allografts in man. This serum is a potent immunosuppressant, particularly when administered over a prolonged period of time. Nevertheless there are a number of theoretical and practical hazards associated with the prolonged administration of any heterologous serum. The present work was undertaken to study the effect of long-term administration of a given pool of anti-lymphocytic globulin (ALG).

MATERIAL AND METHODS

Preparation of anti-lymphocyte Globulin (ALG)

A horse anti-dog lymphocyte serum (HADLS) was prepared according to the method of Iwasaki et al (1967). Suspensions of canine lymph node and spleen cells were prepared from the mesenteric, axillary, and cervical lymph nodes and the spleens of mongrel dogs which had been exsanguinated immediately prior to lymphadenectomy in order to reduce the amount of red cells in the suspension. The lymph nodes and the spleen were minced with scissors and then passed twice through a stainless steel wire mesh into sterile saline. The cells were washed 3 - 6 times in saline and counted. The viability of the lymphocytes was occasionally checked and there were always 90 to 95% viable lymphocytes in the suspension.

A horse was immunized once weekly with subcutaneous injections of lymphocyte suspension. The cell number per injection was increased by 50% on each occasion (from 2 to $60 \times 10^9$). Lymphocyto-toxic and haemagglutinin titres were checked before each injection and after 3 months of immunization the horse was bled out.

The serum was obtained by allowing the blood to clot at room temperature and then to stand in the cold (+4°C) for 3-4 days. The serum was sepa-
rated, decompemented by heating to 56°C for 30 minutes and the haemagglutinin absorption was carried out. This was done by addition of 40-50% of the red cell mass at room temperature to the serum for 30 to 60 minutes. Complete absorption of haemagglutinins (from 1:4096 down to 0) required this procedure to be repeated 5 - 6 times. To prepare the globulin fraction of the ALS, ammonium sulphate precipitation (40%) was carried out. Then the globulin was filtered and stored at +4°C until used.

In vivo immunosuppressive activity of HADLG

The immunosuppressive activity was tested in 6 dogs with kidney allografts. ALG was given in a dose of 1 ml/kg body weight in subcutaneous injections for 5 days before and 6 days after kidney transplantation. Twelve other dogs which received kidney allografts served as controls. Six of them received normal horse globulin (NHG) and the other 6 were untreated. The kidneys, when rejected, were subjected to immunofluorescent studies.

Study of the effect of long-term administration of ALG to healthy dogs

Eight healthy dogs were given ALG in subcutaneous injections daily in a dose of 1 ml/kg body weight. Another group of 8 healthy dogs, receiving normal horse globulin according to the same protocol, served as controls.

The following observations and investigations were carried out in both groups of dogs:

1. Daily general observations (weight, appetite, temperature, reaction to globulin injections)
2. Haematology (RBC, WBC with lymphocyte count, haematocrit, platelet count — every other day; bone marrow biopsy before and after globulin treatment)
3. Total serum proteins and their electrophoretic pattern, aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) activity in the serum, serum alkaline phosphatase — once a week
4. Serum creatinine and blood urea, urinalysis (with special attention to the presence of proteinuria and sediment changes) once a week.

After a period of observation the dogs were sacrificed and routine microscopical examination of the liver, spleen lymph nodes and the kidneys was carried out. In addition immunofluorescent studies of the kidneys were performed.

RESULTS

1. The final product was a globulin with protein content of 3.8 g%, lymphoagglutinin and lymphocytotoxin titres of 1:512 and 1:384 respectively, antibodies against platelets with a titre of 1:16 to 1:512 (depending on the source of antigen) and some antibiotics against dog serum proteins. Finally there was a very delicate precipitation line (agar microdiffusion technique) against kidney antigens which was looked for and found later on during the study.
2. **Immunosuppressive activity of ALG.** The results are presented in Table I. It should be stressed that in all ALG treated dogs the blood urea was normal by the 6th day and rose only to 120-150 mg/100 ml by the 9th day. Presumably the survival time would have been longer if the globulin administration had not been stopped shortly after the operation.

<table>
<thead>
<tr>
<th></th>
<th>Number of dogs</th>
<th>Mean survival time (days)</th>
<th>6th day blood urea</th>
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<tbody>
<tr>
<td>ALG treated dogs</td>
<td>6</td>
<td>13.6</td>
<td>below 50 mg/100 ml</td>
</tr>
<tr>
<td>NHG treated dogs</td>
<td>6</td>
<td>11</td>
<td>30 - 120 mg/100 ml</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>6</td>
<td>8.7</td>
<td>40 - 360 mg/100 ml</td>
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3. **The effect of long-term administration of ALG.** All dogs survived during the time of observation, in good condition without symptoms of wasting. In 5 of 8 dogs given ALG local abscesses developed. No similar abscesses were observed in NHG treated dogs.

There was slight anaemia in all dogs of both groups, but no significant decrease of RBC or haemoglobin level was observed. Bone marrow biopsy taken before the treatment was normal. After 4 weeks of treatment with ALG the bone marrow in all but one of the dogs showed hypercellularity with normoblastic regeneration. The white cell population showed evident shift to the left. An increased percentage of myelocytes and promyelocytes was found. In one dog an acute hyperplastic reaction in the white cell population was observed. The bone marrow in NHG treated dogs showed no changes except some normoblastic regeneration.

In ALG treated dogs there was a marked increase of WBC, presumably due to the abscesses. The increase of WBC in NHG treated animals was only moderate (Figure 1). A sharp decrease in absolute lymphocyte count in the peripheral blood appeared on the next day after ALG injection and profound leucopenia developed which lasted for at least 2 weeks longer than ALG injections. There was no lymphopenia in NHG treated dogs (Figure 2). Despite the presence of antiplatelet antibodies there was no evident thrombocytopenia in ALG treated dogs (lowest count 120,000/mm³). The platelet count in NHG treated animals was normal.

Electrophoretic pattern of serum proteins in both groups of animals was normal. There was some increase in aspartate aminotransferase activity in ALG treated dogs which occurred 4 weeks after the commencement of the treatment (Figure 3). The alanine aminotransferase activity was normal. No changes in these enzyme activities were observed in NHG treated dogs.

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Figure 1. White blood cell count in dogs receiving ALG (continuous line) and NHG (interrupted line).

Figure 2. Absolute lymphocyte count in ALG treated dogs (continuous line) and NHG treated animals (interrupted line).
In 3 of 8 dogs proteinuria appeared in the third week of treatment. This was accompanied by the presence of erythrocytes in the sediment and must have been due to some damage to the basement membrane. Two to three weeks after the ALG treatment was stopped the proteinuria disappeared. No similar changes were observed in NHG treated animals. In all dogs the blood urea and the serum creatinine were normal.

Routine haematoxylin-eosin staining of the kidney slices showed thickening of glomerular walls in ALG treated dogs, and similar changes in NHG treated animals. Immunofluorescent studies of the kidneys in the ALG treated group showed trace amounts of fluorescent subendothelial deposits of dog gamma globulin in capillary loops. Staining for the presence of horse globulin revealed also trace amounts of fluorescent material diffusely distributed throughout the capillary tufts (Figure 4, A, B).

Figure 4.
A. Granular and lobular deposits of dog gamma globulins in glomerular tufts. Staining with fluorescein isothiocyanate-labelled rabbit globulin anti-dog gamma globulin. x 300
In NHG treated dogs distinct amounts of dog globulin localized under the epithelial cells and trace amounts of horse gamma-globulin were detected in subendothelial glomerular deposits (Figure 4, C, D).

CONCLUSIONS
The long-term administration of ALG caused some impairment of kidney function in 3 of 8 treated dogs, and in all animals, dog and horse gamma
globulin deposits in the glomeruli were found. Similar deposits were found in NHG treated animals. It may well be that, because of the presence of some anti-kidney and anti-dog protein antibodies this particular serum was specially toxic. Similar observations were made by Alexandre et al (1968), Shanfield et al (1968) and Guttman et al (1967). This finding emphasizes the necessity for some standardization of ALS production, in the source of the antigen for immunization. Some workers claim ALS prepared from thoracic duct lymphocytes has no detectable antibodies against serum proteins, the kidney or the liver antigens. If such antibodies do occur they should be absorbed.

Despite the presence of some antiplatelet antibodies there is no necessity for their absorption since thrombocytopenia has never occurred.

The whole problem has been further complicated by the lack of an in vitro test for determining the immunosuppressive as well as the toxic potential of a given pool of ALS so the optimal antigen immunization schedule and bleeding time cannot be predicted.

REFERENCES


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