Hypogammaglobulinemia and Thymic Hypoplasia in Horses: a Primary Combined Immunodeficiency Disorder

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A severe combined immunodeficiency disorder was demonstrated in two Arabian foals which were full siblings. The defect in the B-lymphocyte system was shown by hypogammaglobulinemia, lymphopenia, and absence of germinal centers. The almost total absence of thymic tissue in one foal and the lack of thymic dependent lymphocytes in the spleens of both foals demonstrate a T-lymphocyte defect. In a retrospective study of total available Arabian foal cases, 4 of 15 had evidence of immunodeficiency.

The types of immunodeficiencies of man are extremely varied and classification is difficult. When the disorders are viewed from their effect on the lymphoid system, three major groups emerge (10, 12). Some, such as Bruton-type infantile X-linked agammaglobulinemia (3), involve a defect in the B-lymphocyte system which leads to a deficiency of immunoglobulin synthesis, whereas cell-mediated immunity is normal. Other immunodeficiencies, including thymic hypoplasia (DiGeorge's Syndrome) (7), have normal immunoglobulin levels but lack a T-lymphocyte system, resulting in defective cell-mediated immunity. Disorders in the third category involve defects in both antibody production and cell-mediated immunity and are classified as severe combined immunodeficiencies (10). Autosomal recessive type (Swiss type agammaglobulinemia) (15) is an example of this type of disorder.

Few instances of primary defects of the immune system are described in domestic and laboratory animals. The "nude" mouse strain is congenitally athymic with alterations in cell-mediated immunity (8, 22, 24). Nude mice have normal immunoglobulin levels except for immunoglobulin (IgA) but have markedly depressed responses to thymic dependent antigens (5). These mice are an extremely useful model for studying immunodeficiencies as well as a convenient source of athymic experimental animals. A selective immunoglobulin (IgGδ) deficiency in cattle with susceptibility to infection has been reported (21).

This report describes a disorder in Arabian foals with characteristics of a primary combined immunodeficiency disorder. An abstract of this paper has been published (T. C. McGuire and M. J. Poppie, 1973, Fed. Proc. 32:821). Failure to properly identify the disorder in the original case caused the study to be almost entirely retrospective.

MATERIALS AND METHODS

Animals and sera. The initial case (foal A) was a female Arabian foal that died at 7 weeks of age after 3 weeks of progressive pneumonia. The second case (foal B) was a full sibling to foal A which was born 1 year later. This male foal developed a progressive pneumonia at 9 weeks of age. Other foal tissue and histories were acquired from the files of the Department of Veterinary Pathology at Washington State University.

Sera were obtained from foals A and B, their dam and sire, and from seven apparently normal Arabian foals varying from 5 to 8 months of age.

Hematology. Hemoglobin, packed cell volume, total and differential white blood counts, and serum protein were determined by routine methods.

Immunoglobulin quantitation. Serum immunoglobulin levels were determined by single radial immunodiffusion (17). Equine immunoglobulins G, IgG(T), IgM, IgA, and aggregating immunoglobulin (AI) were isolated by techniques that have been previously described in detail (20). The purity of the isolated immunoglobulins was evaluated by immunodiffusion and immunoelectrophoresis. These purified immunoglobulins were used as references after protein concentrations were determined by the Lowry method (18). Specific antisera to IgG, IgG(T), IgM, IgA, and AI were prepared in rabbits and were evaluated and absorbed as previously described (29). The appropriate dilution of each antiserum was...
determined for single radial immunodiffusion and the amount of each immunoglobulin in pooled normal horse serum; it was used as a reference in subsequent determinations of immunoglobulin levels in horse sera.

RESULTS

Clinical and hematologic findings. Foal A (female) developed a clinically evident pneumonia at 4 weeks of age. Bacteriologic examination of nasal exudate revealed the presence of Escherichia coli, Staphylococcus aureus, and Streptococcus pyogenes. Three weeks of extensive antibiotic and supportive therapy were ineffective and the foal died. The most notable hematologic finding (Table 1) was the presence of only 162 to 580 lymphocytes per mm$^3$, whereas the normal range is 2,000 to 6,000 lymphocytes per mm$^3$.

Foal B (male) developed a cough with purulent nasal discharge which required therapy at 9 weeks of age. The total white blood cell count (WBC) on initial examination was 3,000 per mm$^3$ with only 30 lymphocytes per mm$^3$ (Table 1). Three days later, the total WBC count was 12,000 per mm$^3$. A differential count was not done at this time. Despite antibiotic therapy, the foal failed to recover. After 3 weeks of progressive pneumonia, the owners requested euthanasia.

Histopathologic observations. The lungs of foal A were diffusely involved with bronchopneumonia which was superimposed on a well-developed subacute interstitial pneumonia. Bronchial and bronchiolar epithelium had proliferated and epithelial debris was present in the lumina. Near the luminal surface of the epithelium there were large basophilic intranuclear inclusion bodies. These inclusions were typical of those caused by adeno-viruses and have been described in adeno-viral pneumonias of young Arabian foals (16, 19, 28).

The only lymphoid tissue available for microscope examination from foal A was the spleen. Alterations of the organ were striking: there was an almost complete absence of lymphocytes. Neither periarteriolar lymphocytic sheaths nor germinal centers were present. Plasma cells and lymphocytes were almost totally absent in other areas of the spleen.

The lung alterations in foal B were very similar to foal A including the presence of adeno-virus-like inclusion bodies. In the pancreas, there were ductal epithelial proliferation and necrosis with inclusion bodies similar to those in the lung. The acini surrounding these ducts were disorganized and necrotic with beginning fibrosis. Most islets of Langerhans were intact.

Germinal centers as well as the surrounding mantle zone of lymphocytes were absent in several lymph nodes from foal B (Fig. 1). These lymph nodes were also deficient in lymphocytes and plasma cells in the diffuse cortex and medulla. The cell types populating the nodes were difficult to identify; at least some had the appearance of lymphocytes, whereas many were elongated and similar to fibroblasts. The arterioles of the spleen were surrounded by fibroblastic-type cells instead of a lymphocytic sheath (Fig. 2). Most germinal centers were absent but a few partial ones were detectable. The red pulp contained very few lymphocytes and plasma cells.

Table 1. Results of hematologic examination

<table>
<thead>
<tr>
<th>Findings</th>
<th>Foal A 4 weeks old</th>
<th>Foal A 7 weeks old</th>
<th>Foal B, 10 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g%)</td>
<td>15.5</td>
<td>14.6</td>
<td>ND*</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>41</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Protein (g%)</td>
<td>6.2</td>
<td>6.5</td>
<td>ND</td>
</tr>
<tr>
<td>WBC (count/mm$^3$)</td>
<td>11,600</td>
<td>8,100</td>
<td>3,000*</td>
</tr>
<tr>
<td>Neutrophils (segmented/mm$^3$)</td>
<td>9,048</td>
<td>6,561</td>
<td>2,888</td>
</tr>
<tr>
<td>Neutrophils (band/mm$^3$)</td>
<td>698</td>
<td>405</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophils/mm$^3$</td>
<td>1,276</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Monocytes/mm$^3$</td>
<td>0</td>
<td>972</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes/mm$^3$</td>
<td>580</td>
<td>162</td>
<td>30</td>
</tr>
</tbody>
</table>

* ND, Not done.

a Three days after this white blood cell count, the total was 12,000. A differential count was not made at this time.
The most dramatic morphologic changes in foal B were observed in the thymus. The organ had a yellow color and appeared fatty but retained lobular architecture and was subnormal in size. Seen through a microscope, over 90% of the thymus was adipose tissue. Islands of lymphoid cells were interspersed in the fat and partially formed Hassal's corpuscles could be found in the lymphoid islands (Fig. 3).

**Immunoglobulin levels.** Single radial immunodiffusion did not detect IgM, IgA, or Al in the sera from foals A and B (Table 2). IgG and IgG(T) were present in these sera but at levels below the normal range for Arabian foals (Table 2). Immunoglobulin levels for the dam and sire of the foals were within the normal range.

**Other foals.** After the defect was verified in these two foals, the files of the Washington State University Department of Veterinary Pathology were searched for similar cases. Even though the data was incomplete, at least 4 of the 15 Arabian foal cases available had evidence of immunodeficiency (Table 3). Spleens from three foals lacked germinal centers and periarteriolar lymphocytic sheaths (foals 1, 2, and 3 in Table 3). Hematologic results from two of these demonstrated a lymphocytopenia. The case history from a fourth foal revealed lymphocytopenia and hypogammaglobulinemia (foal 4 in Table 3). No lymphoid tissue was available.

**Table 2. Immunoglobulin levels in immunodeficient and normal Arabian foals**

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Foal A</th>
<th>Foal B</th>
<th>Normal foal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1.50*</td>
<td>2.80</td>
<td>3.46-8.20</td>
</tr>
<tr>
<td>IgG(T)</td>
<td>0.31</td>
<td>0.44</td>
<td>3.00-6.40</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>0.24-0.46</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>0.34-0.88</td>
</tr>
<tr>
<td>Al</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03-0.15</td>
</tr>
</tbody>
</table>

*Values are expressed as milligrams per milliliter and were determined by single radial immunodiffusion.
from this case. This information suggests that immunodeficiency may be common in Arabian foals. These four cases plus three others (no sera, leukocyte counts, or lymphoid tissue were available from the last three cases) had an interstitial pneumonia with basophilic intranuclear epithelial inclusion bodies apparently due to adenovirus infection. This was further complicated by a purulent bronchopneumonia evidently caused by bacteria. The remaining eight Arabian foal cases had no evidence of immunodeficiency or adenovirus infections and had died of a variety of causes. An examination of tissue from 15 non-Arabian foals with pneumonia ranging in age from 3 days to 3 months of age did not reveal evidence of immunodeficiency or adenovirus infection.

**DISCUSSION**

That a defect is present in the B-lymphocyte system of the foals described is clearly shown by the hypogammaglobulinemia, lymphopenia, and absence of germinal centers in spleens and lymph nodes. The absence of IgM provides considerable support for this conclusion. Previous studies in our laboratory (T. C. McGuire and T. B. Crawford, 1973, Amer. J. Vet. Res., in press) and others (14) demonstrated that pre-suckle foal serum contains IgM, suggesting the foal has the ability to synthesize this immunoglobulin prior to birth. The lack of IgM and IgA with only low levels of IgG and IgG(T) in the serum of 2- and 3-month-old foals is firm evidence of a defect in immunoglobulin synthesis.

The absence of thymus and thymic-dependent lymphoid tissue (periarteriolar lymphocytic sheaths) (4, 23) provides evidence of a T-lymphocyte defect. However, functional studies will be necessary to definitively validate a disorder in cell-mediated immunity.

The primary lesion in the lungs of both foals was an interstitial pneumonia apparently caused by an adenovirus infection. This was complicated by a secondary bacterial-induced bronchopneumonia. Adenovirus inclusion bodies and interstitial pneumonia were present in 7 of 15 other Arabian foal cases in a retrospective study. Four of these seven foals had evidence of an immunodeficiency disorder. Other investigators have described fatal adenoviral infections in Arabian foals with apparently no cases occurring in other breeds (16, 19, 28). Lymphocytopenia and lymphoid atrophy were described as consistent findings (19). We feel that, in the Arabian foal cases we studied and possibly in those reported by others (16, 19, 28), an immunodeficiency disorder was primary and impaired the ability of the foals to combat adenovirus and other infections. This conclusion is based on several considerations. (i) Our studies indicate a high prevalence of immunodeficiency in the Arabian breed. Six of the 17 Arabian foal cases available had evidence of immunodeficiency, whereas none were found in other breeds. (ii) The presence of immunodeficiency in full siblings suggests a genetic origin. (iii) The severity of the involvement of both the B-lymphocyte system (lymphocytopenia, hypogammaglobulinemia and absence of germinal centers) and the T-lymphocyte system (thymic hypoplasia and absence of thymic dependent lymphocytic sheaths in the periarteriolar areas of the spleen) is similar to that described in severe primary combined immunodeficiencies of man (10, 12, 15).

Certain viruses are known to cause immunosuppression and some even cause thymic atrophy. The most notable of these are murine (2, 9) and feline (1, 25) leukemia viruses which cause marked thymic atrophy but the effects on the thymic dependent areas and germinal cen-

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**Table 3. Summary of data from Arabian foals with probable immunodeficiency**

<table>
<thead>
<tr>
<th>Determinations</th>
<th>Foal no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>12 weeks olda</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
</tr>
<tr>
<td>Duration of clinical disease</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Lymphocytopenia (&lt;1,000/mm³)</td>
<td>Yes</td>
</tr>
<tr>
<td>Absence of B- and T-lymphocytes</td>
<td>Yes</td>
</tr>
<tr>
<td>Adenovirus pneumonia</td>
<td>Yes</td>
</tr>
<tr>
<td>Secondary bacterial pneumonia</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypogammaglobulinemia</td>
<td>NA</td>
</tr>
</tbody>
</table>

a Age at time of death.

b NA, Not available.

c Absence of B- and T-lymphocytes was demonstrated by a complete lack of splenic germinal centers and periarteriolar lymphocytic sheaths.
Muters of the spleen and lymph node are not nearly as marked as described in the foals. The severity of lymphoid changes induced by other viruses (13, 27, 28) do not approach those we have described in Arabian foals. Furthermore, we have been unable to find any significant lymphoid lesions described in adenovirus infections of other species. (iv) Precipitating antibody to a group-specific adenovirus antigen has been detected in 43 of 178 horse sera from unknown breeds indicating that adenoviruses are common among horses. It seems likely that these infections are only serious when some type of immunodeficiency occurs. Fatal pulmonary infections due to adenovirus have been reported in human patients with severe combined immunodeficiencies (11).

If a genetic basis for the disorder is assumed, certain important deductions can be made. A sex-linked mode of inheritance seems unlikely since one of the foals was female and the other male. Further, the disorder is apparently sublethal. The dam and sire of the foals had normal immunoglobulin levels and were without histories of recurrent infections. These factors suggest the immunodeficiency was inherited as an autosomal recessive trait.

Defects in the T- and B-lymphocyte systems and a possible autosomal recessive mode of inheritance suggest that this condition in horses is very similar to the Swiss type of severe combined immunodeficiency in man. This condition in horses represents the only currently known example of primary combined immunodeficiency in any animal other than man. A breeding program is being conducted with dams and sires of affected foals. Subsequent studies with this defect in horses should provide important information concerning the basis of the defect and T- and B-lymphocyte function and provide a model for the evaluation of therapeutic procedures which may be applicable to man.

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