REVIEW

Equine Infectious Anemia: A Model of Immunoproliferative Disease

By Robert A. Squire

Equine infectious anemia is a virus disease of horses which was first recognized as a distinct clinical entity more than a century ago. Its natural occurrence is apparently limited to the genus Equus although possible human cases have been reported.\textsuperscript{1,2} The disease may be transmitted by mechanical vectors, e.g., biting insects and hypodermic syringes, but experimental attempts to infect small laboratory animals by several methods have produced only equivocal results.

The most thorough investigation and review of equine infectious anemia (EIA) was reported in the classic monograph by Dreguss and Lombard,\textsuperscript{3} and an excellent review was also presented by Ishii.\textsuperscript{4} Despite the fact that the disease is recognized as a constant threat to the equine industry, there has been little research into the basic nature and pathogenesis of the malady and these remain quite obscure. Efforts, until recently, have been largely limited to the development of a reliable diagnostic test which is not yet available.

EIA is characterized by periodic episodes of fever, anemia, hypergamma-globulinemia, and generalized lymphoid proliferation. The course is usually progressive, but often there are prolonged latent periods. There are many striking clinical and pathologic similarities between the equine disease and the lymphoproliferative or immunoproliferative\textsuperscript{5} diseases of man (Table 1). It is the purpose of this brief review to bring the disease to the attention of medical investigators since, from a comparative standpoint, it may represent an excellent biomedical model for a group of poorly understood human disorders. The descriptions presented are based upon clinical and pathologic observations of twenty affected animals plus the reported findings of other investigators.

CLINICAL ASPECTS

Equine infectious anemia is recognized as occurring in either an acute, subacute, or chronic form, but these categories are arbitrary and all stages are
Table 1.—Comparative Features of Equine Infectious Anemia

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<thead>
<tr>
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<th>Equine Infectious Anemia</th>
<th>Lymphomas</th>
<th>Waldenstrom's Macroglobulinemia</th>
<th>Infectious Mononucleosis</th>
<th>Aleutian Disease</th>
<th>Autoimmune Hemolytic Anemia</th>
<th>Collagen-vascular Diseases</th>
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<td>Evidence for viral etiology</td>
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<td>Response to Corticosteroids</td>
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<td>Serum globulin abnormalities</td>
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Fig. 1.—Lymph node section. Note effacement of architecture and capsular invasion. Hematoxylin and eosin. 40×.
often represented during the course of a disease in a single animal. The disease may be very mild and almost subclinical or, as is more often the case, acute symptoms are noted approximately seven to twenty-one days following exposure or experimental inoculation. Fever, depression, anorexia, anemia, mucosal hemorrhages, and occasionally ventral edema are evident. Clinical recovery may occur after several days or death may result. The subacute and chronic forms are characterized by prolonged clinical signs of lesser severity or by periodic acute attacks. Latent periods lasting weeks, months, or even years intervene between the clinical exacerbations, and death may occur during any attack. In some cases, permanent clinical recovery follows one or several symptomatic periods, although latent infection and viremia apparently persist because the blood of horses once infected is continuously infective to other susceptible animals. As a result, there are probably many unsuspected carrier animals.

Accompanying the above signs and coincident with each febrile period are declines in erythrocyte, hemoglobin, and hematocrit values, and these may
be very precipitous. The anemia is normocytic and normochromic, but reticulocytes or other immature forms are not seen in the peripheral blood regardless of its severity. The unusual absence of this response is apparently characteristic of horses even in the severe hemolytic anemia produced by Babesiosis and, in itself, presents a challenging problem in comparative hematopoietic physiology. It has been demonstrated by the use of $^{51}$chromium labeling that erythrocyte production and half-life in infected animals are both decreased as well as the iron-utilization rate of erythrocytes.\textsuperscript{6,7} There is also a decrease in serum iron and an increase of plasma iron-binding capacity. Lymphopenia may occur initially, but this is often followed by a persistent lymphocytosis in animals which survive. An increase in the number of iron-containing monocytes in the peripheral blood is considered a significant diagnostic feature of the disease. There have been no consistent abnormalities reported in direct or indirect reacting serum bilirubin levels, but these values fluctuate widely in apparently normal horses.

Hypergammaglobulinemia is a relatively constant feature of the disease.

Fig. 3.—Spleen section showing absence of lymphoid nodules and diffuse infiltration of pulp. Hematoxylin and eosin. 50 $\times$.\textsuperscript{a}
Electrophoretic peaks in the beta-globulin zone usually accompany each clinical episode, and elevated levels may persist in the chronic or latent cases. This has been shown to represent an abnormal globulin component, but its molecular weight or sedimentation coefficient has not been reported; nor has it been determined whether this is a monoclonal or heterogeneous protein.

Clinical diagnosis is based upon physical and hematologic examination and the inoculation of several horses, some of which are presumed to be susceptible, with suspect blood or serum. The filterable etiologic agent has not been isolated and a specific serologic test is not yet available. There are reports of recently developed diagnostic tests in the literature. However, except possibly for a complement-fixation test developed by Japanese workers, these are apparently nonspecific. Kono and Kobayishia have been successful in growing the virus in equine leucocyte tissue culture with resultant CPE, and their CF test employs infected tissue culture fluid as antigen.

The treatment of EIA has been limited to supportive measures, particularly hematins, and these have no apparent value. The efficacy of corticosteroids or other immunosuppresants has not been reported.

**Pathology**

If death occurs during the initial acute phase, the changes recognized are those associated with septicemia and are not specific. There is increased vascular permeability with associated serosal and mucosal hemorrhages, as well as
microhemorrhages in various tissues. The alterations in lymphoid tissue may be very prominent and consist of atrophy or frank degeneration and necrosis of lymphocytes. This is also a nonspecific change which may be seen in the acute phase of several other animal virus infections.

If an animal survives the acute episode for several days or weeks, the anatomic findings, rather than being degenerative, are characterized by proliferative changes of the lymphoid tissues. Splenomegaly and lymphadenopathy may be noted at gross necropsy examination and, histologically, there is a prominent increase of basophilic cells in all lymphoid tissues. In some cases, this cellular response may efface lymph node and splenic architecture presenting a histologic picture compatible with lymphoma (Figs. 1, 2, and 3). The change is diffuse throughout the cortical pulp and medullary cords of lymph nodes without formation of primary or secondary nodules. There may be infiltration of trabeculae, capsule, and sometimes perinodal fat. Sinusoids, if intact, contain a large and uniform population of the same atypical cells. The cells are mainly medium to large lymphocytes with prominent basophilic cytoplasm, and many show distinct plasma cell characteristics including nuclear eccentricity and clear perinuclear golgi areas (Fig. 4). They are similar in appearance to the hemocytoblasts and plasma cell precursors of immunologic response, and also to certain of the atypical lymphocytes which are characteristic of infectious mononucleosis. Ishitani has demonstrated by electron microscopy a well developed rough endoplasmic reticulum in the cells. Some of the

Fig. 5.—Bone marrow section showing hypercellularity. Hematoxylin and eosin. 130 ×.
more differentiated plasma cells exhibit strongly positive periodic acid-Schiff cytoplasm similar to that observed in Waldenström's macroglobulinemia where it apparently represents the high hexose content of the abnormal globulin.\textsuperscript{14}

The PAS-positive material usually occurs as globular inclusions which fill the cytoplasm like the Russell bodies in \textit{Mott cells}. We have not observed PAS-positive inclusions in the nuclei of the cells in the equine disease.

Ironically, the tissue which has been studied least is the bone marrow. There is hypercellularity which is generally attributed to erythroid hyperplasia in response to the anemia, but in most cases these observations have not been supported by precise histologic studies. In this author's experience, the marrow does not usually exhibit a normal response. There is, in fact, often a paucity of erythrocyte precursors. The most striking finding is a relative increase in lymphoid cells, and this appears to reflect the same proliferative response noted in peripheral lymphoid and other tissues (Figs. 5 and 6).

Liver involvement is very characteristic and consists of portal and sinusoidal lymphoid infiltration, bile duct proliferation, and occasionally centrlobular
Parenchymal degeneration (Fig. 7). Another histologic feature which is quite constant is the accumulation of hemosiderin in cells of the reticuloendothelial system. Kupffer cells and other fixed and free macrophages throughout the body are often enlarged, apparently increased in number, and contain Prussian-blue-positive pigment.

The heart generally shows a slight to moderate lymphoid infiltration. There is also an increase in Anitschkow cells in and around the adventitia of blood vessels and fibrinoid degeneration of perivascular connective tissue (Fig. 8). These interstitial changes are sometimes most severe in the auricular appendages. The kidneys also characteristically exhibit a prominent interstitial infiltration of lymphocytes and plasma cells.

A significant pathologic feature of the disease in addition to the cellular proliferation is damage to blood vessels. There is fibrinoid necrosis and periarteritis of small muscular arteries and arterioles, particularly those in the kidneys and heart (Fig. 9). The glomeruli also show distinct involvement,
Fig. 8.—Coronary arteriole showing early fibrinoid degeneration and several Anitschkow Cells. Hematoxylin and eosin. 650 x.

and this is characterized by increased cellularity in the mesangium (Fig. 10). There is apparently no basement membrane thickening.

The chronic disease is characterized by progressive anemia and lymphoid hyperplasia. There is a greater infiltration of non-lymphoid tissues in the later stages, and the cells are predominantly small lymphocytes and plasma cells. Liver portal triads and sinusoids may show very marked infiltrates and, indeed, the histologic picture may be similar to that of lymphocytic leukemia or lymphosarcoma in this organ. The kidney involvement is also more severe, and there may be progressive hyalinization of glomeruli. Less frequently there is a meningeal and perivascular cellular infiltrate in the central nervous system similar to that in the Bing-Neel syndrome of macroglobulinemia (Fig. 11). The hemosiderin deposits which may be very prominent in earlier stages are usually less evident in the chronic stage.

Discussion

A striking and significant aspect of EIA is the widespread proliferation of
lymphoid tissue which, although self limiting, may anatomically border upon neoplasia. This bears similarity to human disorders which appear to be abnormal reactions of the immune system. Such lymphoproliferative diseases include infectious mononucleosis, Waldenström's macroglobulinemia, certain acquired hemolytic anemias, lymphomas, and myeloma. EIA may be included with infectious mononucleosis, several animal lymphomas, and Aleutian disease of mink and ferrets as examples with apparent viral etiology. Further, the vascular lesions, the renal and myocardial involvement, and the hypergammaglobulinemia give definite indication that the equine disease also belongs in the group of naturally occurring hypersensitivity disorders. The relationships between autoimmune disease and lymphoma have been reported. Leader et al. emphasized the allergic features of EIA and indicated possible mechanisms in their discussion of Aleutian disease of mink, which is strikingly similar to the equine disease. The proliferation of immunologically competent cells in EIA may be the result of direct viral mutation or transduction, or continued antigenic stimulation derived from either virus-altered tissue components or the persistent virus protein itself. None of these, however, explain the chronic course and the intermittent and prolonged latent periods. These are significant features of the disease and require investigation of factors in the host-virus relationship which allow for this type of biologic behavior.

The nature of the anemia in EIA has long been the subject of controversy.
In consideration of the clinical and pathologic manifestations, it appears that hemolysis is a major factor. The rapid fall in hemoglobin, the shortened life-span of erythrocytes, and the hemosiderosis certainly support this view. However, the same evidence also allows for the possibility of an aplastic anemia with the resultant decrease in iron utilization which has been demonstrated, and considering the cellular histology of the marrow, a myelophthisic anemia may also be considered. If hemolysis is involved, which appears to be a reasonable assumption, the evidence points to this being the result of an auto-antibody with affinity for antigenic determinants on erythrocytes as well, perhaps, as other tissue components. It is unlikely, based upon our current understanding of viral infectivity and replication, that there is a direct hemolytic action by the virus, for mature erythrocytes themselves could hardly serve as target cells. (However, erythrocyte precursors containing nuclear material could serve this function, and infection at this cellular stage might possibly explain an aplastic anemia.) In support of an autoimmune hemolytic anemia, Gainer et al. reported positive direct Coombs’ tests, and Moore, using
fluorescent methods, observed that 20 percent of the erythrocytes of affected horses were coated by an abnormal globulin. In any case, if the globulin fraction is functional antibody at all, the persistence of viremia, apparently throughout the life of the animal, would indicate that it does not have specific affinity for viral antigen.

Finally, the evidence for vector transmission of EIA is quite conclusive and presents another tempting comparison to human disease. The Burkitt tumor of African children, which is certainly a lymphoproliferative disorder, has been suspected of being a vector-borne infectious disease. The fact that EIA is apparently a virus disease introduces many possibilities for experimental manipulations to further study basic factors in pathogenesis.

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REFERENCES


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