Monocyte Activation in Horses Persistently Infected with Equine Infectious Anemia Virus

KEITH L. BANKS

Institute of Comparative Medicine and Veterinary Science, Washington State University, Pullman, Washington 99163

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The monocytes of horses infected with equine infectious anemia virus were shown by their failure to migrate from capillary tubes and their increased adherence to erythrocytes to be activated.

Equine infectious anemia (EIA) is a virus-induced disease, which may be fatal during acute infection or animals may become persistently viremic for years with an occasional recurrence of acute disease (reviewed in reference 7). The acute disease is characterized by fever, anemia, hepatitis, and increased titers of circulating virus. Horses with chronic disease become hypergammaglobulinemic with lower serum viral titers and develop immune complex glomerulonephritis. Antibody to virus antigens is produced throughout the infection. Several observations suggest that cells of the monocyte-macrophage type may be important in the pathogenesis of EIA. The virus replicates in monocytes and macrophages both in vivo and in vitro, and the number of monocytes increases during infection. Macrophages of the spleen and liver often contain phagocytosed erythrocytes or erythrocyte breakdown products, including hemosiderin. These observations led to the study of the functional characteristics of monocytes of horses with EIA.

The leukocytes studied were from Shetland ponies infected with the Texas or Wyoming strains of EIA virus and were maintained as previously described (10). Infection was confirmed on the basis of clinical signs and the development of serum antibody to EIA antigens detectable by an immuno-precipitation reaction (7). Horses having a rectal temperature over 101.5°F (ca. 38.6°C) were considered to have the acute disease, whereas afebrile periods were indications of chronic, quiescent disease. Anemia occurred with hematocrit readings of 26 ± 6% (standard deviation) during acute infection and 33 ± 7% (standard deviation) while in the chronic stages. Noninfected horses had hematocrit readings of 40 ± 3% (standard deviation). Five of the horses had been infected for 1 to 3 years and none died during the study. Peripheral blood mononuclear cells and neutrophils were isolated by the Hypaque-Ficoll method as has been described for horses (1, 2). The cells were washed three times with Hanks balanced salt solution and counted. Mononuclear cell preparations isolated from normal horses were 1 to 9% neutrophils and 2 to 17% monocytes, and the remaining cells were lymphocytes. In EIA-infected horses, occasionally more monocytes were observed in the isolated band of cells. These proportions were 1 to 2% neutrophils and 1 to 29% monocytes, and the remaining cells were lymphocytes. Leukocyte preparations rich in neutrophils (98%) were obtained from the pellet of the Hypaque-Ficoll separation.

The mononuclear cells were first examined for their ability to migrate in vitro. The isolated cells were packed into a short segment of a capillary tube, and the migration from the tube into a chamber filled with 80% Leibovitz-15 medium with 20% normal horse serum was measured after 24 h. The details for this test using similar cell populations (3) or peritoneal exudate cells (4) are described elsewhere. Thirteen EIA horses and thirteen normal horses were studied, and each animal was examined one to seven times. A significant decrease in the migration of mononuclear cells (P < 0.001, Student's t test) from horses with EIA was observed when compared with the migration of cells from noninfected horses (Table 1).

Neutrophils and monocytes were then tested for the ability to bind erythrocytes. Small chambers were prepared by attaching Lucite rings to cover glasses, and the cell preparations were added to the chambers. After the adherence of monocytes or neutrophils to the glass, nonadherent cells were removed by washing with Hanks balanced salt solution. Previous studies have shown that only monocytes and neutrophils bind to the glass (1). The leukocytes were incubated at 37°C for 1 h with a 1% suspension...
of autologous, unwashed erythrocytes that were collected the same day as the test was performed. Excess erythrocytes were removed by a gentle washing with Hanks balanced salt solution, and the degree of erythrocyte binding to the leukocytes was scored according to the following scale: ++ + + , rosettes on 70 and 90% of cells; ++ +, erythrocytes on almost every cell and rosettes on about 50% of the cells; + + +, erythrocytes on 50% of the cells; and +, erythrocytes on some cells in every high-power field. In 64% of the observations with EIA monocytes, erythrocytes bound to the monocytes (Table 2). The number of erythrocytes bound was + + + or greater in 34% of the observations. With normal horse monocytes, only 14% of the observations were positive and on only one occasion was this above +. In 7% of the tests, erythrocytes bound to neutrophils of EIA horses whereas neutrophils of noninfected horses were always negative.

The parameters affecting monocyte adherence to large numbers of erythrocytes were examined further. It was observed that rosette formation was dependent upon incubation at 37 C, whereas at 4 C no adherence occurred. Adherence was not prevented by 50% serum, a procedure that blocks the binding of antibody-coated erythrocytes to the Fc receptor of monocytes (1, 9). Rosettes also were formed when washed erythrocytes of noninfected horses, sheep, or rabbits were incubated with the monocytes. Erythrocytes from horses with reactive monocytes did not bind to normal horse monocytes or neutrophils. Therefore, alterations of the monocytes, not changes in the erythrocytes, were responsible for the rosette formation. The greatest number of rosettes was formed when horses were in the acute stages of the disease.

Monocytes from EIA horses had an increased binding to autologous erythrocytes or to erythrocytes from other horses, sheep, and rabbits. In addition, the monocytes migrated more slowly from a cell mass packed by centrifugation in capillary tubes. These findings suggest an increased "stickiness" of the monocytes, a property shown to be characteristic of activated macrophages and monocytes (4, 11, 15). Activation of EIA monocytes may be due to the continuous antigenic stimulation by the persistent virus infection. Macrophages can be activated by products of antigen-stimulated lymphocytes (13, 15) and during the immune response to bacterial (14) or chronic viral infections (5). The significance of activated monocytes in EIA was not determined, although these cells may be partially responsible for the increased extravascular hemolysis that occurs in this disease (10). The involvement of activated macrophages in the pathogenesis of anemia has been suggested by the observation that stimulated macrophages are cytotoxic to syngeneic erythrocytes in vitro (12), and anemia may accompany the stimulation of macrophages in vivo (6). Activated monocytes were present in EIA-infected horses when the anemia was most severe, a stage of the disease during which erythropagocytosis by circulating monocytes has been observed (7). Although these findings suggest a

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**Table 1. Migration of mononuclear cells of EIA-infected and noninfected horses**

<table>
<thead>
<tr>
<th>Disease state</th>
<th>No. of horses</th>
<th>No. of observations</th>
<th>Area of migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute EIA</td>
<td>6</td>
<td>15</td>
<td>0.289 ± 0.13c</td>
</tr>
<tr>
<td>Chronic EIA</td>
<td>7</td>
<td>10</td>
<td>0.301 ± 0.10c</td>
</tr>
<tr>
<td>Noninfected</td>
<td>13</td>
<td>21</td>
<td>0.552 ± 0.19c</td>
</tr>
</tbody>
</table>

* Each horse was examined one to seven times on different days.

**Table 2. Binding of autologous erythrocytes to monocytes and neutrophils of normal and EIA-infected horses**

<table>
<thead>
<tr>
<th>Leukocytes</th>
<th>Disease state</th>
<th>No. of animals</th>
<th>No. of observations</th>
<th>% Observations with erythrocyte binding to leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 + ++ +++ +++++</td>
</tr>
<tr>
<td>Monocytes</td>
<td>EIA</td>
<td>14</td>
<td>92</td>
<td>36 16 14 16 18</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>22</td>
<td>40</td>
<td>86 12 0 2 0</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>EIA</td>
<td>14</td>
<td>74</td>
<td>93 1 0 3 3</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>22</td>
<td>37</td>
<td>100 0 0 0 0</td>
</tr>
</tbody>
</table>

* Each horse was examined one to eight times with each test done on a different day.
relationship of altered macrophages and anemia, direct proof of such a relationship remains to be measured.

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LITERATURE CITED


