Cytomegalovirus-Induced Mononucleosis in Guinea Pigs

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The effects of cytomegalovirus (CMV) infection on hematopoietic and lymphoid tissues were studied in guinea pigs. Blood parameters, histopathology, and virus distribution in the bone marrow, spleen, lymph nodes, and thymus were assessed during primary nonlethal acute and chronic guinea pig CMV infection. Transient hematological changes comparable to those seen in human CMV mononucleosis were observed during acute infection. These included anemia and leukocytosis with atypical lymphocytes. Splenomegaly and stimulation of spleen and lymph node T- and B-cell areas were also noted. These changes occurred at the peak of virus recovery from all tissues tested, as well as from macrophages and B- and T-cell–enriched spleen subpopulations. Virus was cleared rapidly from blood and bone marrow; blood counts, spleen size, and histology returned to normal within 1 month after virus inoculation. However, guinea pigs failed to eliminate the virus completely from lymphoid tissues, since virus persisted in splenic macrophage and B-lymphocyte–enriched populations during chronic infection. The data suggest that CMV-infected mononuclear cells play a role in the establishment of generalized acute infection and virus persistence.

Hematological abnormalities occur regularly during acute human cytomegalovirus (CMV) infections. CMV mononucleosis, with atypical circulating lymphocytes, lymphadenopathy, and splenomegaly, constitutes a well-described clinical syndrome and occurs in 5 to 10% of patients who receive massive blood transfusions (20). The isolation of CMV from human lymphoid tissues has been reported (31, 35), and the importance of lymphocytes in the maintenance of CMV latency has been underlined by the occurrence of post-transfusion CMV mononucleosis (8, 20). Furthermore, CMV has also been isolated from lymphoid tissues of mice infected with murine CMV (13, 23, 24, 27–29, 34). Thus, it appears that in both hosts, mononuclear cells can become latently infected with CMV. However, detection of virus in the blood of normal blood donors has rarely been accomplished, and little is known about the involvement of the spleen and bone marrow during human CMV infection.

Similarities have been described between the effects of guinea pig and human CMV on their respective hosts (1, 4, 5, 11, 14–16, 19, 32). Nevertheless, no information is available on the involvement of hematopoietic and lymphoid tissues and cells during CMV infection of guinea pigs, although viremia has been demonstrated during acute infection (14). Through use of the guinea pig model, this study determines the participation of hematopoietic and lymphoid cells in the establishment of acute and chronic CMV infection. Blood parameters, histopathology, and immunological topology were correlated with virus distribution in bone marrow, spleen, thymus, and lymph nodes to define the effects of primary CMV infection on blood, bone marrow, and lymphoid tissues. The information obtained in the guinea pig model may have considerable implications for the elucidation of host defense mechanisms against CMV infection, CMV infections in bone marrow transplant patients, and pathogenesis of CMV mononucleosis.

MATERIALS AND METHODS

Virus strain and animal inoculation. The prototype strain of guinea pig CMV (no. 22122; American Type Culture Collection, Rockville, Md.) was used. The preparation of salivary gland–passaged guinea pig CMV viral stocks has been described previously (1, 11). Animals were inoculated subcutaneously with 1 ml of salivary gland–passaged guinea pig CMV suspension with infectivity titers ranging from 5.5 to 6.5 log 50% tissue culture infective dose (TCID50) per ml. This inoculating dose of virus usually did not cause significant mortality.

One to 2-month-old nonpregnant female Hartley strain guinea pigs were purchased from Camm Research Institute, Wayne, N.J., and from Elm Hill Breeding Laboratories, Chelmsford, Mass. Blood samples were obtained from all animals before inoculation and were tested for the presence of neutralizing antibodies to guinea pig CMV. Animals without preexisting antibodies were inoculated subcutaneously with 1 ml of salivary gland–passaged virus. Guinea pigs inoc-

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ulated with uninfected salivary gland suspensions or uninoculated animals were used as controls.

**Sampling of blood and tissues for virus recovery in cell culture.** Guinea pig embryo fibroblast monolayer cell cultures were prepared from 20- to 40-day-old Hartley guinea pig embryos as described previously (14, 15) and used for virus assay. Spleen, lymph nodes, and thymus were removed aseptically at sacrifice. Bone marrow flecks were taken from the long bones. Blood samples were collected in Alsever solution (4 parts of blood to 1 part of anticoagulant). Plasma for virus assay was obtained by centrifuging the blood at 600 × g for 20 min. The following procedures were used for virus assay when indicated. (i) Cell suspensions (10%, wt/vol) were prepared from each tissue, and 0.1 ml was inoculated into each of four to five separate guinea pig embryo monolayer cultures. (ii) Cell counts were made from 10% (vol/vol) cell suspensions and adjusted to concentrations of 10⁶ or 10⁵ cells per ml, and infectivity titers were determined by serial 10-fold dilutions cocultivated with guinea pig embryo monolayer cell cultures. (iii) Plasma (0.2 ml) was inoculated into five guinea pig embryo monolayer cell cultures. All samples were observed for 3 to 4 weeks for evidence of virus-induced cytopathic effect. Virus isolates were identified by neutralization tests by using type-specific antisera as described previously (10).

**Hemagglutination titers.** Blood samples were obtained from the ear or the heart. The volume of packed blood cells (hematocrit) was determined in microhematocrit tubes. Leukocyte counts were obtained by microscopic counting with a hemocytometer after lysis of erythrocytes with acetic acid. Differential leukocyte counts were determined after staining the blood smears with Wright stain.

For histological studies, a portion of each tissue was fixed in Bouin fixative or 10% buffered Formalin, dehydrated through graded alcohols to xylene, embedded in paraffin, sectioned, and stained with routine hematoxylin and eosin. Bone marrow sections were decalcified before processing. Bone marrow smears were made directly from bone marrow fragments and stained with Wright stain. Lymph node diameters were determined by measuring the largest diameter of the fixed, stained, and mounted lymph node.

**Spleen and blood cell separations.** Spleens were minced, and the cell suspension obtained was washed, resuspended in RPMI 1640 medium with 20% fetal bovine serum, and separated into cell fractions as described previously (10). The degrees of purity of the B-cell, T-cell, and macrophage fractions were 85, 90, and 90%, respectively (10).

Blood samples were separated as follows. After removal of the plasma, 1 part of blood was diluted with 3 parts of Hanks balanced salt solution. Eight milliliters of this mixture was carefully overlaid over 3 ml of Ficoll-Hypaque. After centrifugation at 400 × g for 40 min at 20°C, the interphase, enriched in mononuclear cells, and the pellet, rich in granulocytes and erythrocytes, were collected separately, washed in Hanks balanced salt solution, and counted.

**RESULTS**

**Blood alterations during primary guinea pig CMV infection.** The effects of CMV infection on several blood parameters were studied. Hematocrits, leukocyte counts, and differential cell counts were obtained in infected and noninfected guinea pigs at various intervals after virus inoculation ranging from 3 days to 4 months. Some guinea pigs were examined once, at the time of sacrifice; others were followed serially over the total study period.

Unlike in the uninfected controls, hematological alterations were repeatedly observed in infected animals during the first 2 weeks after virus inoculation (Table 1). Anemia, as shown by a drop of the hematocrit value, was evident from days 5 to 12, with the values returning slowly to normal after day 14. Leukocyte counts dropped slightly during week 1, increased during week 2, and returned to normal within week 3 after inoculation. Lymphocytosis was apparent as early as 7 days post-inoculation and remained for ca. 2 weeks. During these 2 weeks, atypical lymphocytes were often seen. Some of these lymphocytes were similar to the Downey cells seen in human mononucleosis (Fig. 1). All values returned to normal 1 to 2 months after virus inoculation.

**Virus recovery from plasma and blood cell fractions.** Virus distribution was determined in plasma and blood cell fractions ob-

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**Table 1. Peripheral blood values during guinea pig CMV infection**

<table>
<thead>
<tr>
<th>No. of days after guinea pig CMV inoculation</th>
<th>No. of guinea pigs tested</th>
<th>Hematocrit packed-cell volume (mean ± SE)</th>
<th>Leukocyte counts/mm³ (mean ± SE)</th>
<th>% Lymphocytes (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>44.7 ± 0.9</td>
<td>6,010 ± 548</td>
<td>63.7 ± 4.5</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>40.6 ± 0.9</td>
<td>4,654 ± 436</td>
<td>54.4 ± 11.1</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>39.3 ± 0.9</td>
<td>3,157 ± 408</td>
<td>68.5 ± 5.7</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>37.8 ± 1.6</td>
<td>3,364 ± 304</td>
<td>74.8 ± 1.5</td>
</tr>
<tr>
<td>9–10</td>
<td>4</td>
<td>38.7 ± 1.6</td>
<td>5,100 ± 1,198</td>
<td>85.5 ± 5.8</td>
</tr>
<tr>
<td>11–12</td>
<td>6</td>
<td>36.8 ± 1.0</td>
<td>11,957 ± 2,186</td>
<td>81.0 ± 5.2</td>
</tr>
<tr>
<td>13–14</td>
<td>6</td>
<td>40.8 ± 0.8</td>
<td>11,388 ± 2,368</td>
<td>87.3 ± 2.6</td>
</tr>
<tr>
<td>21</td>
<td>7</td>
<td>47.0 ± 0.9</td>
<td>8,692 ± 1,665</td>
<td>81.7 ± 3.7</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>46.3 ± 1.2</td>
<td>5,760 ± 189</td>
<td>76.3 ± 8.0</td>
</tr>
<tr>
<td>≥60</td>
<td>5</td>
<td>47.2 ± 1.7</td>
<td>8,440 ± 1,203</td>
<td>79.8 ± 3.8</td>
</tr>
</tbody>
</table>
tained at various intervals after inoculation to assess the possible association of guinea pig CMV with a specific peripheral blood cell fraction (Table 2). Guinea pig CMV was recovered from the plasma only during week 1 after virus inoculation. The granulocyte-erythrocyte fractions harbored the virus from days 1 to 11. The mononuclear fraction contained virus from days 1 to 16 and harbored virus more frequently than did any other blood fraction.

Spleen weight, virus content, and virus infectivity titers in spleen cell subpopulations. We observed that splenomegaly occurred regularly in guinea pigs during acute CMV infection. Hence, spleen weights of infected animals expressed as a percentage of the spleen weight of control animals were correlated with virus infectivity titers of unseparated spleen cell suspension (Fig. 2). The mean weight of control animals was 1.18 ± 0.07 g (mean of 20 animals ± the standard error [SE]). This is shown in Fig. 2 as 100 ± 6%. Spleens of infected animals were significantly enlarged from day 7 to 15 and harbored guinea pig CMV from days 3 to 30. The highest virus titers (1.50 log10 TCID50/106 cells) were measured on day 7. In addition, virus was recovered from the spleen most frequently on days 5 to 13 after inoculation with 18 out of 21 guinea pigs showing virus. Virus infectivity titers declined thereafter; by day 16, only very low titers could be measured in some animals.

Virus infectivity titers were determined in splenic macrophage and B- and T-lymphocyte-enriched fractions obtained from the spleens of infected guinea pigs to determine which cell type harbored guinea pig CMV during acute and

TABLE 2. Recovery of guinea pig CMV from plasma and blood cell fractions

<table>
<thead>
<tr>
<th>Days after guinea pig CMV inoculation*</th>
<th>No. of guinea pigs with virus in:</th>
<th>Erythrocyte-granulocyte-enriched pellet</th>
<th>Mononuclear cell-enriched interphase</th>
<th>Total no. of guinea pigs showing viremia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3 (21)</td>
</tr>
<tr>
<td>5-7</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>10 (71)</td>
</tr>
<tr>
<td>9-11</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>6 (43)</td>
</tr>
<tr>
<td>13-16</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3 (21)</td>
</tr>
</tbody>
</table>

* Fourteen guinea pigs were tested in each group.
chronic infection (Fig. 3). Virus was detected in the macrophage and B- and T-cell fractions as early as 3 days post-inoculation. Infectivity titers peaked at 7 days post-inoculation in all cell fractions. Guinea pig CMV infectivity titers decreased first in the macrophage fractions; by day 13, titers were low to undetectable. Both B- and T-lymphocyte fractions showed a slower decline in virus titer after day 7. However, guinea pig CMV was completely eliminated from the T-lymphocyte fraction by day 16; thereafter, virus could no longer be recovered from that fraction. Guinea pig CMV was found to persist longer in the B-cell fraction, although on day 60, virus was recovered only occasionally, and in low titers, from the B-lymphocyte and the macrophage fractions of two of six animals tested.

**Spleen histopathology.** In comparison to that of normal guinea pigs, the splenic red pulp of guinea pig CMV-infected animals showed a two- to threefold increase of erythrocyte precursors from 3 to 13 days after inoculation. Scattered, small areas of eosinophilic necrosis and histiocytes with abnormal nuclei and ingesting erythrocytes were present. No differences were seen between control animals and animals infected for 1 month or longer.

No stimulation was seen in the B- and T-cell areas before day 5 after virus inoculation. The B-cell–dependent lymphoid nodules developed some germinal centers on days 5 and 7; the germinal centers became numerous and large, with many mitoses and tingible bodies on days 11 and 13, but were seldom seen at 2 months and thereafter. The T-cell–dependent periarteriolar lymphoid sheath cells, on the other hand, were very active from days 5 to 11, with mitoses, tingible bodies, and eosinophils, but returned to normal on day 13 and thereafter. Distinct virus-induced inclusions were not seen in the spleen, although the bizarre nuclei of histiocytes in the red pulp area on days 3 to 11 were compatible with virus infection.

**Virus recovery from bone marrow, thymus, and lymph nodes.** The frequency of guinea pig CMV recovery from bone marrow, thymus, and cervical lymph nodes is shown in Table 3. Virus was recovered from bone marrow on days 1 to 13 only. In contrast, the thymus and superficial ventral cervical lymph node harbored virus for 60 days and longer. Maximum virus infectivity titers were obtained on day 7 in the thymus, lymph nodes and bone marrow. Thereafter, virus titers decreased rapidly. Virus remained present in very low titers in the thymus and lymph nodes.

In separate experiments, virus distribution in the axillary, superficial ventral cervical left and right lymph nodes, as well as in the jejunal and cranial mesenteric nodes, was determined on days 5, 15, and 30 after inoculation. Guinea pig CMV was recovered from cervical and axillary lymph nodes tested from day 5 to day 30, whereas virus was present in the visceral lymph nodes on days 5 and 13 only.

**Bone marrow, thymus, and lymph nodes histopathology.** Examination of decalcified bone marrow sections showed that there were no large-scale abnormalities. All cell lines were

### Table 3. Recovery of guinea pig CMV from bone marrow, thymus, and lymph nodes

<table>
<thead>
<tr>
<th>No. of days after guinea pig CMV inoculation</th>
<th>No. of guinea pigs (%) with virus in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone marrow</td>
</tr>
<tr>
<td>1-3</td>
<td>10</td>
</tr>
<tr>
<td>5-7</td>
<td>9</td>
</tr>
<tr>
<td>9-13</td>
<td>12</td>
</tr>
<tr>
<td>22-30</td>
<td>15</td>
</tr>
<tr>
<td>≥60</td>
<td>10</td>
</tr>
</tbody>
</table>

![Fig. 3. Virus infectivity titers in spleen cell subpopulations enriched in macrophages, B lymphocytes, or T lymphocytes. Virus titers are expressed as log10 TCID50 per 10^6 cells; average of three experiments ± SE.](http://iai.asm.org/)
present at all times tested, and no inclusions were seen. However, examination of Wright-stained bone marrow smears showed that the myeloid-erythroid ratios (M/E ratio) were decreased in CMV-infected guinea pigs during the first 2 weeks after inoculation. The mean M/E ratio ± SE of control guinea pigs was 1.69 ± 0.11, whereas values in guinea pig CMV-infected animals were 0.59 ± 0.04, 0.40 ± 0.10, and 2.25 ± 0.51 on days 7, 14, and 28 post-inoculation, respectively. Abnormalities and inclusions were not noted at any time in the thymus of the infected animals.

Axillary, cervical, and mesenteric lymph nodes were examined histologically. The mean size ± SE of 16 lymph nodes obtained from four control guinea pigs was 2.9 ± 1.4 mm. In contrast, lymph nodes from infected guinea pigs measured 3.4 ± 0.6 mm, 5.9 ± 0.6 mm, and 6.2 ± 0.9 mm at 5, 13, and 30 days post-inoculation, respectively. (Detailed information on the lymphadenopathy will be reported separately.) Histiocytes with abnormal nuclei were present in lymph nodes of infected guinea pigs on days 5 through 13, including a few with Cowdry type A inclusions. These histiocytes were seen throughout the node, especially in the medulla, and were actively phagocytizing erythrocytes. The draining subaxillary nodes near the site of virus inoculation were very large on day 7 and contained many of these histiocytes. Small areas of coagulative necrosis were also noted. The predominately B-cell-dependent outer cortex was not stimulated until day 7 in some of the animals; by day 11 and for the rest of the first month, many germinal centers were noted in all lymph nodes examined. Animals examined at intervals from 2 to 12 months after inoculation had outer cortices resembling those seen in uninfected guinea pigs. The predominately T-cell-dependent paracortical areas of most nodes were stimulated as early as 5 days and up to 1 month after inoculation. By 2 months or more after infection, the T-cell areas had returned to normal.

**DISCUSSION**

Several animal models are available for the study of CMV infection. Previous studies have shown that the guinea pig model of CMV infection is unique because its pathogenesis resembles that of human CMV infection, the occurrence of intrauterine infections during primary maternal guinea pig CMV infection being of special importance (4, 11, 16, 19). The present study demonstrates that guinea pig CMV infection in the adult randomly bred Hartley guinea pig involves the hematopoietic and lymphoid tissues, resulting in a condition similar to human CMV mononucleosis.

CMV mononucleosis in humans has been observed in otherwise healthy individuals (18) or in subjects who have received blood transfusions. It may present with leukopenia, but the peak period of leukocytosis is usually found relatively late in the disease (18). Atypical lymphocytes are commonly observed, and the acute disease is generally mild and limited. Occasionally, a more severe clinical picture is seen in children when autoimmune hemolytic anemia is associated with acute CMV infection (6, 31, 35).

In such patients, severe anemia, thrombopenia, and neutropenia are sometimes noted, along with splenomegaly, lymphadenopathy, and circulating atypical lymphocytes. Osborn and Shahidi (26) showed that in mice infected with murine CMV, anemia, leukocytosis, and thrombocytosis occurred during week 1 after inoculation of sublethal doses of murine CMV. The hematological alterations observed in guinea pigs during acute guinea pig CMV infection resemble those described for human and sublethal murine CMV infections. The appearance and regression of these hematological changes correlated with the highest virus infectivity titers and/or frequency of virus in the blood, bone marrow, spleen, thymus, and lymph nodes. In addition, enlargement of lymph nodes and spleen was noted. Increased spleen mass included both an increase in erythropoietic tissues and in immune-activated lymphoid tissue. The alterations of splenic erythrocyte precursors and bone marrow myeloid-erythroid ratios appear to reflect an increase in erythropoiesis. Although it is possible that many changes in the peripheral blood parameters are due to the direct effect of virus infection, the increased phagocytic activity in many histiocytes of the lymph node and spleen might also account for the anemia by removal of circulating erythrocytes. It is not known whether the mononucleosis-like syndrome observed in guinea pigs is due to an immunological response to CMV infection involving B- and T-cell interactions as has been suggested in Epstein-Barr virus mononucleosis (30, 33).

Human CMV has been isolated from lymph nodes and spleen during acute infection, but has not been recovered frequently from the blood of healthy donors (8, 26). In the mouse model, CMV has been isolated from lymphoid tissues during acute infection (13, 23, 24, 29). It has been demonstrated that the virus is able to inject and persist in mouse macrophages (2, 3, 25) as well as in mouse B- and T-lymphocytes (27, 34). Our observations of guinea pigs appear comparable
to these results. The rapid spread of infectious virus to blood, bone marrow, spleen, thymus, and lymph nodes was demonstrated by the peak of virus infectivity titers and the highest frequency of virus isolation which were observed on day 7. This suggests that the infected mononuclear cells may be responsible for the distribution of infectious virus to the hematopoietic and lymphoid tissues. Although virus was cleared from the blood and bone marrow after 2 weeks, guinea pig CMV assays in spleen subpopulations indicated that after an initial general virus distribution in the various mononuclear cells, infectious virus persisted in a limited number of cells during chronic infection. Our data indicate that macrophages and B lymphocytes may be involved in guinea pig CMV persistence. The reappearance of virus in guinea pig lymphoid cells long after the primary infection has resolved may represent a mechanism whereby healthy human blood donors harbor infectious virus in their blood. Further, the data obtained from guinea pigs and mice on the persistence of CMV in cells involved in host immune responses suggest that a similar situation may occur in humans during CMV infection.

Histological signs of active viral infection were limited to days 3 to 11 post-inoculation, when altered histiocytes were seen in lymphoid tissues with a few cells containing intranuclear inclusions or foci of eosinophilic necrosis. The timing and sequence of T-cell area stimulation, followed by B-cell area activation, observed during guinea pig CMV infection seemed to duplicate the pattern seen in guinea pigs and other species when animals were immunized with any antigen, classically with sheep erythrocytes (12, 17, 21, 22). Lymphoid tissues appeared to differ from the salivary gland in that fully developed intranuclear inclusions were rarely seen in lymphoid tissue, although small inclusions have been observed in the lymph nodes of 3 out of 50 guinea pigs with natural CMV infection (7). In general, salivary gland tissue contains well-developed intranuclear and intracytoplasmic inclusions from day 9 up to 2 months after infection (9). The response to CMV may be modified in lymphoid cells as compared to salivary gland cells. The infecting dose, the route of inoculation, and the strain of animal may also play important roles in the involvement of lymphoid tissues during CMV infection. Indeed, Mims and Gould (24) have shown that necrosis of the spleen with intranuclear inclusions were seen only with certain strains of mice and after inoculation of large doses of murine CMV. Significant replication of murine CMV was necessary for splenic necrosis to develop. Further, lymph node histopathology was observed only after footpad inoculation, but not after intraperitoneal inoculation (24). The present study is concerned specifically with the nonlethal, self-limited CMV infection of guinea pigs, which generally does not produce significant tissue necrosis, with intranuclear inclusions. However, we have reported previously that death occurs in 17 to 28% of guinea pigs inoculated during pregnancy (11). In addition, other studies in progress in our laboratory have indicated that intranuclear inclusions could be found in the spleen, lymph nodes, and bone marrow of severely ill animals inoculated with large doses of infectious virus. The latter will be reported separately.

The present report has demonstrated that CMV infection in guinea pigs involves the blood, bone marrow, and lymphoid tissues, producing a syndrome analogous to human CMV mononucleosis. The consequences of CMV infection in cells of the immune system on host defense mechanisms remain to be determined. The guinea pig model of CMV infection may be a useful tool for studying host immune responses during CMV infection. Furthermore, questions regarding the pathogenesis of CMV mononucleosis and CMV infection in marrow transplant recipients may also be ultimately answered.

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LITERATURE CITED


