Thrombocytopenia
in a Retrovirally-induced
Murine Erythroleukemia*

CHRISTINA GAMBA-VITALO, Ph.D.,†‡
JOSEPH LOBUE, Ph.D.,‡
TORGNY N. FREDRICKSON,‡
SEYMOUR M. E. IEN, Ph.D.,‡
JOSEPH PEDERSEN, Ph.D.,§
ALBERT S. GORDON, Ph.D.,‡
and MATTHEW R. PINCUS, M.D., Ph.D.§

†Department of Pathology,
University of Connecticut
Storrs, CT 06268

and

‡Department of Biology,
1009 Main Building,
New York University,
New York, NY 10003

and

§Department of Pathology,
SUNY Health Center
Syracuse, NY 13210

ABSTRACT

A variant strain of Rauscher leukemia virus (RLV-A) obtained from a transplantable murine monomyelocytic leukemia causes a disease characterized by frank anemia, wasting, hepatosplenomegaly and erythroblastosis. The involvement of platelets in this disease are reported here. The RLV-A induced a severe thrombocytopenia (25 percent of control level) at the terminal stage of disease. This thrombocytopenia was not associated with disseminated intravascular coagulation since the prothrombin times were always within normal limits. The partial thromboplastin time was elevated in the terminal stages of disease and was found to be associated with factor deficiencies, possibly owing to the presence of anti-factor antibodies, in the intrinsic coagulation pathway, especially factor VIII. Further, splenectomy did not abolish the thrombocytopenia, since splenectomized, virally infected animals also developed severe thrombocytopenia (29 percent of control levels). The ensuing splenomegaly during progression of disease was not the cause of the thrombocytopenia. A physiological response to the severe thrombocytopenia was the production of larger size platelets. At terminal stages of the disease, platelet volume increased to 4.2

* Send reprint requests to: Matthew R. Pincus, M.D., Ph.D., Department of Pathology, SUNY Health Center, 750 East Adams Street, Syracuse, NY 13210.
μm³ (normal is 3.0 μm³). An increase in platelet volume was also observed in splenectomized, virally infected animals. Electron microscopy indicated that these circulating platelets contained c-type viral particles. Viral infection was associated with decreased life span of circulating platelets, as measured by 75Se-methionine at mid and terminal stages of the disease. Our results suggest that direct viral infection of platelets and/or megakaryocytes with subsequent cell lysis is a possible cause of the observed thrombocytopenia observed in RLVA-induced disease and may also occur in other retrovirally-induced diseases.

Introduction

Murine leukemias closely resemble many of the neoplastic conditions found in human myeloproliferative diseases. These similarities allow murine models to be used as tools in the understanding of the etiology, progression, and potential treatment of human blood dyscrasias. The variant strain of Rauscher leukemia virus (RLV-A), a retrovirus used in this study, induces in infected BALB/c mice splenomegaly, hepatomegaly, terminal anemia and erythroid infiltration of liver and spleen with concomitant erythroleukemia. At terminal stage of disease, erythrocyte survival is also reduced. In addition, major alterations occur in the pluripotential (CFU-S) and committed stem cell granuloid (CFU-GM) and erythroid (CFU-E) compartment. The observed increase in CFU-S, accompanied by diminished levels of CFU-E suggests a maturation block occurring at the level of committed stem cells. Alterations in the pathophysiology of this disease have been induced by treatment of infected mice with antierythropoietin. These data suggest the existence of a preleukemic population and under certain conditions this preleukemic population may manifest itself as a monomyelocytic leukemia.

Preleukemic conditions have also been associated with alterations in the megakaryocyte-platelet axis. In several leukemic models, the cytoplasm of these megakaryocytes have been observed to contain elevated levels of viral particles. These elevated levels are probably the result of the endomitotic events that occur within the megakaryocyte line. Eventually, viral cDNA is incorporated into platelets and alters their metabolism. This change in platelet metabolism has been documented to cause thrombocytopenia and bleeding episodes.

The protracted nature of RLV-A disease and its similarities to some erythroid dyscrasias and preleukemic states make it a useful model for studying the mechanisms regulating circulating platelet levels. An understanding of the alterations occurring in the megakaryocyte-platelet compartment may lead to the potential treatment from thrombocytopenia associated with myeloproliferative and preleukemic diseases.

Many of these phenomena observed in the murine models are likewise observed in patients with diagnosed acquired immune deficiency syndrome (AIDS). For example, patients with AIDS are known to be predisposed towards development of lymphomas and leukemic states and to develop thrombocytopenia without evidence of disseminated intravascular coagulopathy (DIC). In this communication, results are presented from murine animal model experiments which suggest that retrovirally-induced thrombocytopenia is at least partially caused by direct viral infection of platelets and/or megakaryocytes and that concomitant coagulation deficiencies
also occur, possibly caused by circulating inhibitors of factors in the intrinsic coagulation pathway.

**Materials and Methods**

**ANIMALS**

BALB/c male weanling mice (12 to 15 g)* were inoculated with RLV-A virus by i.p. administration of 0.1 ml of undiluted viremic plasma as previously described. All mice were housed in a temperature-controlled room (22 ± 2°C) with a 12 hour light/dark cycle and were maintained on Purine lab chow and water *ad libitum.*

As mice became anemic, they were grouped according to their packed red cell volume (PRCV) as: early stage (36 to 42 percent), midstage (26 to 35 percent), and terminal stage (15 to 25 percent) of disease. In all of the following experiments, groups of 15 animals were used at each stage of disease. An equal number of animals were used for appropriate controls.

**Splenectomy Experiments**

Splenectomy experiments were performed to determine the role of the enlarged spleen in the sequestration and regulation of platelet numbers in RLV-A-infected animals. Five-week-old mice were splenectomized by a dorso-lateral method. The splenic vasculature was ligated and the spleen removed. One week post-splenectomy, mice were given 0.1 ml of undiluted viremic plasma and were randomly assorted into a diseased group as previously described. An equal number of mice was sham-splenectomized.

**Hematological Parameters**

Packed red cell volume was obtained by the Strumia micro-micro hematocrit technique. Platelet counts were obtained using the Unopette Test 5855† and platelet size distributions were determined on a ZB Coulter Counter connected in sequence with a Channelizer. A 30 μm aperture was used with amplification 1, aperture current ½, and matching switch 40 k. Each threshold division, following calibration with 2.0 μm³ latex particles, was calculated to be equal to 0.16 μm³.

Various coagulation studies were performed to assess the effect of RLV-A viral infection on blood clotting. The one stage prothrombin time (PT) the activated partial thromboplastin time (aPTT) and plasma fibrinogen levels were assessed to evaluate any association with the observed thrombocytopenia.

**Electron Microscopy**

Platelet rich plasma (PRP) was obtained by centrifuging whole blood at 250 x g for 10 minutes. The PRP was then pelleted and resuspended in cold 2.5 percent glutaraldehyde-Millonig buffer solution for one hour. After rinsing with Millonig buffer, a one percent osmium tetroxide solution was added to the platelets for 90 minutes, and the specimens were washed in distilled water and immersed in 0.5 percent aqueous uranyl acetate overnight. Following ethanol dehydration, the specimens were embedded in Embed 812 (EMS, Pa) and sections were stained with aqueous uranyl acetate and lead citrate and examined using a Phillips 300 electron microscope.

---

* Jackson Labs, Bar Harbor, ME.

† Becton Dickinson, Rutherford, NJ.
**Platelet Survival Studies**

Thrombocytopenia induced by viral infection may be a manifestation of decreased platelet survival.\textsuperscript{30} Normal and RLV-A infected mice were injected i.p. with 2.0 \( \mu \text{Ci/mg of } ^{75}\text{Se-methionine} \) in 0.1 ml isotonic saline. Incorporation of radioactivity into circulating platelets was evaluated every 24 hr.

Blood samples were obtained from lightly ether-anaesthetized mice by cardiocentesis. The whole blood was immediately placed in polycarbonate test tubes and was centrifuged for 15 minutes at 250 \( \times g \). Platelet rich plasma was aspirated with plastic pipettes and placed in polycarbonate tubes. The red blood cells and buffy coat were resuspended in saline and recentrifuged at 250 \( \times g \) for 10 minutes. The supernatant plasma and saline mixture were combined and centrifuged at 1300 \( \times g \) for 13 minutes. The platelet button obtained from this supernatant was then washed three times at room temperature; first in one percent ammonium oxalate to lyse containing red blood cells and twice more in platelet washing fluid (CA\textsuperscript{+}+ -Mg\textsuperscript{2+} free Tyrode’s Solution). The pellet was resuspended to a volume of one ml in platelet washing fluid, and a platelet count was made. Samples were counted in a Baird-Atomic 709 scintillation counter and radioactivity was expressed as cpm per 10\textsuperscript{9} platelets.

Platelet survival data were analyzed by an arithmetic plot of cpm per 10\textsuperscript{9} platelets against time, in days. Survival time was estimated as the interval, in days, between the 50 percent point on the ascending and descending limb of the curve.\textsuperscript{18} To eliminate or reduce the reutilization of label in the control platelet studies, cold methionine (2.0 mg per 0.2 ml) was injected one and eight hours following injection of labeled methionine. This time schedule eliminated reutilization of label from day four to day seven.\textsuperscript{18}

All statistical analyses were performed as reported previously.\textsuperscript{14} Tests for statistical significance were performed for platelet volumes as described in a previous paper.\textsuperscript{14}

**Results**

**Effects of Viral Infection on Platelet Counts**

The circulating platelet counts in RLV-A, intact and splenectomized mice are given in table I. In non-splenecto-

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Circulating Platelet Count in Rauscher Leukemia Virus Infected and Control BALB/c Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet Count (10\textsuperscript{9}/mm\textsuperscript{3})</td>
</tr>
<tr>
<td><strong>RLV-A INFECTED</strong></td>
<td></td>
</tr>
<tr>
<td>Early stage</td>
<td>1.18 ± 0.06*</td>
</tr>
<tr>
<td>Mid stage</td>
<td>0.85 ± 0.08</td>
</tr>
<tr>
<td>Terminal stage</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td><strong>RLV-A INFECTED SPLENECTOMIZED</strong></td>
<td></td>
</tr>
<tr>
<td>Early stage</td>
<td>1.51 ± 0.01</td>
</tr>
<tr>
<td>Mid stage</td>
<td>1.17 ± 0.01</td>
</tr>
<tr>
<td>Terminal stage</td>
<td>0.64 ± 0.09</td>
</tr>
<tr>
<td><strong>RLV-A INFECTED SHAM-SPLENECTOMIZED</strong></td>
<td></td>
</tr>
<tr>
<td>Mid stage</td>
<td>0.97 ± 0.12</td>
</tr>
<tr>
<td>Terminal stage</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td><strong>CONTROLS</strong></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>1.80 ± 0.04</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>2.19 ± 0.05</td>
</tr>
<tr>
<td>Sham-splenectomized</td>
<td>1.95 ± 0.06</td>
</tr>
</tbody>
</table>

RLV = Rauscher leukemia virus

*Values represent mean ±1 standard error of the mean of 15 experimental animals per group.

\[\text{\textsuperscript{4}Amersham, England, Sp. Act. 1-10 \( \mu \text{Ci per mg seleno-methionine.}\)}\]
mized RLV-A-infected animals, platelet counts decreased to 65 percent and 46 percent of the control level during the early and mid-stages of disease, respectively whereas splenectomized RLV-A infected animals demonstrated platelet counts that decreased to 69 percent and 53 percent of the appropriate control, respectively. At the terminal stage of disease, severe thrombocytopenia was noted for intact and splenectomized viral-infected animals (platelet counts decreasing to 25 and 29 percent of the appropriate control, respectively). Although the platelet level was consistently higher in the splenectomized, infected groups, the percentage of the appropriate control for each group was similar at each stage of the disease.

**Effects of Viral Infection on Platelet Size**

Accompanying the progressive thrombocytopenia in intact RLV-A-infected mice was an increase in modal platelet volume (figure 1). At early and mid-stage of disease the modal platelet volume was 3.8 μm³; at terminal state, platelet volume was increased to 4.2 μm³ (normal is 3.0 μm³). Although these differences in modal volume were not statistically significant, comparisons of total distributions indicate a shift to a larger platelet size. An increase in modal platelet volume was also evident in RLV-A infected splenectomized mice (figure 2). The modal value for early and mid-stage was 3.7 μm³. At terminal state, the platelet volume was lower (3.4 μm³). At the terminal stage of the disease, the platelet volume distribution became bimodal (figure 2). This finding is discussed further in the Discussion Section. Nonetheless, when compared with the splenectomized control modal platelet volumes (3.0 μm³), all stages of disease in splenectomized animals exhibited increased platelet volume distributions. Sham-splenectomized animals were found to have platelet volume distributions that did not differ from normal or splenectomized platelet volume distributions (data not shown).
EFFECTS OF VIRAL INFECTION ON COAGULATION FACTORS

One possible cause of the observed thrombocytopenia was disseminated intravascular coagulopathy (DIC). The effects of viral infection were explored on both the aPTT and PT to determine whether these values were increased in infected animals. An analysis of any alterations in coagulation parameters (table II) was complicated by the increase in blood volume known to occur during mid- and terminal stage of disease. With progression of disease, the PT values were not significantly different from control levels (table II). If increased blood volume is taken into consideration, the actual level of factors involved in the PT assay may have been elevated. The A-PTT values increased with the progression of disease (table II), indicating that factor levels may have been decreased. Plasma fibrinogen levels remained constant with progression of disease. If the increase in blood volume is again considered, fibrinogen levels were found actually to increase slightly.

These findings indicate that DIC was not a contributory cause of the observed thrombocytopenia in infected animals.

Since the aPTT was found to increase significantly during the course of disease, the effects of viral infection were investigated further on the coagulation profiles of animals infected with RLV-A. A summary of the effects of adding different amounts of normal control plasma to the

<table>
<thead>
<tr>
<th>PT (sec)</th>
<th>a-PTT (sec)</th>
<th>Fibrinogen (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.2 ± 0.2</td>
<td>32.1 ± 1.3</td>
</tr>
<tr>
<td>Early stage</td>
<td>9.9 ± 0.2</td>
<td>34.7 ± 1.7</td>
</tr>
<tr>
<td>Mid stage</td>
<td>9.5 ± 0.4</td>
<td>37.4 ± 2.0</td>
</tr>
<tr>
<td>Terminal stage</td>
<td>9.6 ± 0.6</td>
<td>39.0 ± 2.0</td>
</tr>
</tbody>
</table>

Determination of values were obtained for 15 experimental animals per group for prothrombin time (PT) and accelerated partial thromboplastin time (a-PTT). Fibrinogen levels were determined on separate groups of 15 animals.
PLATELETS IN MURINE ERYTHROLEUKEMIA

TABLE III

Effects on Accelerated Partial Thromboplastin Time of Mixing Normal Plasma with Rauscher Leukemia Virus-A Infected Plasma

<table>
<thead>
<tr>
<th>Stage of Disease</th>
<th>Control</th>
<th>RLV-A Only</th>
<th>0.9 ml Nml + 0.1 ml RLVA-A</th>
<th>0.5 ml Nml + 0.5 ml RLVA-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage</td>
<td>33.2 ± 1.0</td>
<td>35.1 ± 0.9</td>
<td>33.9 ± 1.2</td>
<td>34.4 ± 1.6</td>
</tr>
<tr>
<td>Mid-stage</td>
<td>32.6 ± 1.4</td>
<td>37.6 ± 1.8</td>
<td>35.9 ± 1.4</td>
<td>36.9 ± 0.3</td>
</tr>
<tr>
<td>Terminal stage</td>
<td>34.6 ± 1.8</td>
<td>43.6 ± 1.3</td>
<td>37.9 ± 1.0</td>
<td>39.4 ± 2.3</td>
</tr>
</tbody>
</table>

*Each value is the mean ± the standard error for 30 experimental animals. All incubations were performed for five minutes.

plasma of infected animals at progressive stages of disease is shown in table III. As can be seen from this table, addition of greater amounts of normal plasma tends to correct the aPTT of plasma from infected animals but not completely. These corrections were found to be stable, i.e., the aPTT measured after five minutes and after one hour (not shown) were close to one another in value.

At 1:1 ratios of normal plasma to RLV-A plasma from infected animals, there was a slowly increasing elevation of the aPTT which was lower than that for the unmixed (RLV-A-infected) plasma. It is significant that even at high ratios (9:1) of normal to RLV-A-infected plasma, there was also a slow increase in the aPTT with progressive disease. This incomplete correction of the aPTT by normal plasma suggests that, in addition to possible coagulation factor deficiencies, a circulating inhibitor to elements of the intrinsic system may also be present in the plasma of infected animals.

To document further specific coagulation factor deficiencies, mixing experiments were performed in which the plasma of infected animals was mixed with factor VIII-deficient plasma at 1:1 ratios. The results of this study are shown in Table IV, where it can be seen that, even with 1:1 mixtures of factor VIII-deficient plasma, there was a steady rise in the aPTT. At terminal stages of the disease, the aPTT increased 10.8 seconds over the control. This value can be compared with the aPTT for the mixing experiments shown in table III. The increase in the aPTT for terminal stage of the disease over the control for infected plasma mixed with normal plasma was 3.6 seconds for the 9:1 ratio, while that for the 1:1 mixing experiments was 4.8 sec-

TABLE IV

Effects of Mixing Rauscher Leukemia Virus-A Plasma with Factor VIII-deficient Plasma on the Accelerated Partial Thromboplastin Time*

<table>
<thead>
<tr>
<th>Condition</th>
<th>aPTT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.4 ± 1.4</td>
</tr>
<tr>
<td>0.5 ml early RLV-A + 0.5 ml factor VIII-deficient plasma</td>
<td>34.5 ± 0.6</td>
</tr>
<tr>
<td>0.5 ml mid-stage RLV-A plasma + 0.5 ml factor VIII-deficient plasma</td>
<td>37.3 ± 0.8</td>
</tr>
<tr>
<td>0.5 ml terminal stage plasma + 0.5 ml factor VIII-deficient plasma</td>
<td>44.2 ± 1.3</td>
</tr>
</tbody>
</table>

*All values are expressed as the mean ± standard deviation from the mean.
For unmixed plasma, the increase was nine seconds, almost twice that observed for the 1:1 mixing experiments. Thus, factor VIII-deficient plasma was found not to correct the increase in aPTT observed for plasma from infected animals.

**Effects of Viral Infection on Platelet Survival**

In order to evaluate the effects of retroviral infection on platelet lifetimes, $^{75}$Se-methionine incorporation experiments were performed in which the incorporation of this label into platelets was measured as a function of time. It has been demonstrated previously$^{18}$ that, if reutilization of this label by megakaryocytes and platelets is not appreciable, the curves for label incorporation are bell-shaped, and the mean platelet lifetime can be computed by measuring the width of the uptake curve at half of the peak height. It was further shown previously$^{18}$ that reincorporation of the label by platelets causes skewing of the descending limb of the uptake curve. This reutilization effect, however, can be largely eliminated by chasing the label with two doses of cold Se-methionine at one and eight hours after administration of the label in the uptake experiments. This schedule eliminates the reutilization of label from day four through day seven.$^{18}$

As shown in figures 3 and 4, reutilization of label was a complicating factor in platelets taken from control animals and those at the early stage of retroviral infection, respectively. To obtain estimates of the lifetimes of platelets from these animals, cold methionine was administered one and eight hours after injection of labeled methionine. For control (normal) platelets, as shown in figure 3, linear regression analysis was applied to days three, four and five data points and the slope computed. The average survival of normal circulating platelets was estimated to be about 4.5 days (figure 3). At early stages of the disease, reutilization of label was found that was substantially higher than that found in the control platelets (figure 4). This increased reutilization may have been due to increased protein production in the liver and spleen. Thus, despite use of cold methionine chase doses, accurate survival values could not be determined for these platelets.

However, as can be seen in figure 4, at mid- and terminal stages of disease, reutilization was not found to be significant until after the sixth day obviating the cold chase experiments. Consistent with this finding, there was a 60 percent increase in label per platelet at mid- and terminal stage of disease (compare peak heights of the three curves in figure 4). Survival of mid- and terminal stage platelets was estimated from the curves in figure 4 to have been decreased to approximately 2.5 to 3.0 days from the control value of 4.5 days (figure 3).
PLATELETS IN MURINE ERYTHROLEUKEMIA

Because of the noted effects on platelet count, size, and average lifetime that were not related to DIC, the possibility that platelets may have been directly infected by the virus was further investigated. Such a phenomenon may be responsible for the observed effects on platelets from infected animals. Figure 5A shows the results from EM studies of platelets from infected animals in the early stages of the disease. In this figure, the arrow points to type C-viral inclusion bodies found in platelets. In figure 5B, budding viral particles from the platelet membrane are shown that were discovered in the platelets of infected animals in late stages of the disease. It is clear that direct retroviral infection of platelets and/or megakaryocytes occurs in these animals and that these viral particles are shed, leading to possible further viral infection of platelets.

Discussion

It is clear from the previous results that platelet levels of RLV-A-infected animals decrease with progression of the disease. Platelets from these animals are larger and have shorter survival times than those found for platelets from control animals.

In response to the severe thrombocytopenia observed in intact and splenectomized viral-infected mice, there was an increase in both the modal platelet volume and the size distribution. This increase possibly could be due to elevated levels of the glycoprotein, thrombopoietin, a moiety known to regulate platelet production.28,30 The bimodal pattern of platelet volume distributions observed in splenectomized animals (figure 2) at the terminal stage was probably a result of hemolytic debris and fragmented platelets. Karpatkin14 has observed this phenomenon in hemolytic and thrombocytopenic states.

The observed thrombocytopenia in infected animals could have been caused by an accompanying splenomegaly, by disseminated intravascular coagulopathy, by circulating antiplatelet antibodies, by direct viral infection, or by any combination of these phenomena. Although there are data to support a direct correlation between splenomegaly and the observed thrombocytopenia in viral-infected animals,5,6 the observed splenomegaly in RLV-A disease was not the critical cause of the decrease in circulating platelets. The very similar decreases in platelet counts in both non-splenectomized and splenectomized animals at each stage of disease suggests that the enlarged spleen
FIGURE 5. Electron micrographs of circulating platelets from Rauscher leukemia virus-A (RLV-A)-infected animals. A. Platelet from an infected animal showing intra-cellular viral inclusion body (arrow). B. Platelet from an infected animal showing budding viral particle from the platelet membrane (arrow).
was not sequestering appreciable numbers of circulating platelets. In support of this premise, Bergson et al.\textsuperscript{1} demonstrated a 70 percent reduction in the splenic circulation at the terminal stage of the disease. It seems clear, therefore, that the thrombocytopenia in RLV-A-infected animals cannot be attributed to splenic sequestration.

Disseminated intravascular coagulopathy (DIC), which can occur in retrovirally-infected hosts, and might account for the observed thrombocytopenia, can be ruled out because no decrease in plasma fibrinogen level and no elevation in the PT were observed at any stage of the disease (table II). A progressive, selective rise in the aPTT was observed, however. This rise cannot be explained by DIC and is not likely due to plasma dilution of coagulation factors. While it is known that at mid- and terminal stages of RLV-A disease the blood volume can be elevated up to 11 percent over normal blood volume,\textsuperscript{20} this dilution would be expected to affect PT values as well.

The increased aPTT in RLV-A-infected animals could be stably partially corrected when the plasma of RLV-A-infected animals was combined with normal plasma in ratios of 1:1 or at greater dilutions with normal plasma\textsuperscript{12} (table III). Thus, an acquired factor deficiency may occur with this disease. However, even with addition of high (9:1) ratios of normal to infected plasma, the aPTT was not fully corrected. These findings can be interpreted as suggesting the presence of a coagulation factor inhibitor such as an antibody to one or more coagulation factors in the intrinsic pathway. Factor VIII might be a target of such a circulating antibody since factor VIII-deficient plasma failed to correct the aPTT in the plasma of infected animals. This conclusion is based on the data in tables III and IV that show that an increase of the aPTT for the undiluted plasma of infected animals in the terminal stage of the disease was nine seconds over the control value, while that for infected plasma from animals in terminal stages of the disease mixed 1:1 with factor VIII-deficient plasma was over 10 seconds. Antibodies selective for factor VIII have been described in a number of disease states, predominantly in autoimmune diseases.\textsuperscript{13} It is further possible that either an anti-factor VIII antibody or another circulating antibody may be responsible for destruction of platelets in RLV-A-induced disease. This possibility is being investigated currently.

**DIRECT VIRAL INFECTION AS A CAUSE OF THROMBOCYTOPENIA**

In addition to the possibility of circulating anti-platelet antibodies as a cause of thrombocytopenia, direct viral infection of platelets may also be responsible for this phenomenon. Indeed, both types A and C viral particles were observed by electron microscopy in platelets from animals with RLV-A disease (figure 5). Throughout the disease, budding viral particles were found in numerous platelets from infected animals (figure 5). These observations support the conclusion that direct viral infection, probably at the megakaryocytic level, with proliferation of viral particles, ultimately causing platelet rupture, is an important cause of the observed progressive thrombocytopenia.

**EFFECTS OF VIRAL INFECTION ON PLATELET SURVIVAL**

C-type viruses in platelets such as those found in our study have been shown to alter the metabolism and fragility of platelets.\textsuperscript{4} For this reason, the average platelet life span was evaluated using \textsuperscript{75}Se-methionine. This compound labels platelet precursors in the bone marrow; however, all actively protein
synthesize organs (e.g., liver and spleen) also utilize and release this label. For these reasons, it was difficult to evaluate the platelet survival of normal and early stage viral-infected animals based on the usual approach, which is the span between the 50 percent point on the ascending and descending limb of the survival curve.\textsuperscript{18} At mid- and terminal stages of disease, the average survival had decreased to approximately 2.5 to 3.0 days. Control circulating platelet survival was estimated to be 4.5 days. The biphasic survival curve with increased utilization observed at the early stage of the disease can be explained in at least two ways: (1) It is possible that there are two populations of megakaryocytes in the bone marrow, each with a different transit time. Each population would release labeled platelets (one population normal, the other viral infected) producing the observed survival pattern. (2) Since a population of platelets contains viral particles, the fragility of these platelets could be possibly altered, and the biphasic curve would be an indication of increased destruction of this virally infected population. Subsequent release of the label would be used by the next cohort of megakaryocytes in the bone marrow. The increased radioactivity per platelet can be attributed to a physiological response to the thrombocytopenia. The increased platelet size makes more protein available to incorporate the label.

Acknowledgment

This work was supported by grants CA-12815 (J.L.) and CA-42500 (M.R.P.) from the National Cancer Institute, N.I.H., V.S.P.H.S. and NCI.

References


