Infection of Megakaryocytes by Human Immunodeficiency Virus in Seropositive Patients With Immune Thrombocytopenic Purpura

By Fawzia Louache, Ali Bettaieb, Annie Henri, Eric Oksenhendler, Jean-Pierre Farce, Philippe Bierling, Maxime Seligmann, and William Vainchenker

Twenty-one human immunodeficiency virus (HIV)-positive patients, including 11 acquired immunodeficiency syndrome (AIDS)-free patients with immune thrombocytopenic purpura (ITP), were studied. Because patients, including 11 acquired immunodeficiency syndrome (AIDS)-free patients with immune thrombocytopenic purpura (ITP), were studied to determine whether the megakaryocyte/platelet lineage was infected by HIV. Because purification of platelets did not reach a level sufficient for unequivocal results by the polymerase chain reaction, in situ hybridization was thus performed. Purified marrow megakaryocytes (MK) from 10 HIV-infected ITP patients were studied using a 35S HIV riboprobe, antisense of an HIV ENV sequence. HIV transcripts were clearly detected in MK from five of these 10 patients, although heterogeneity among MK was observed. In three of these five cases, small amounts of HIV glycoproteins were detected in MK by means of immunofluorescence. In addition anti-HIV antibodies could be eluted from platelets of all patients. In contrast, HIV transcripts were not detected in MK derived from colony-forming units-MK (CFU-MK) cultured in suspension, suggesting either that MK are infected by HIV during terminal differentiation or that HIV-infected CFU-MK are unable to differentiate in vitro. In conclusion, this study suggests that HIV infection of MK may be implicated in the pathogenesis of thrombocytopenia of HIV-positive patients.

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Materials and Methods

Patients. Twenty-one HIV-infected patients were included in this study after giving their informed consent. Their clinical and immunologic characteristics are given in Table 1. Fifteen patients had a clinical course of ITP with platelet counts below therapy of less than 50 x 10^9/L. Four of these 15 patients had AIDS (cases 15, 16, 20, and 21), while the others were AIDS-free patients (patients 1 through 10 and 13) and fell into stage II, III, or IVA of the Centers for Disease Control (CDC) classification. The remaining six patients had a mild thrombocytopenia (between 77 x 10^9/L and 120 x 10^9/L) (cases 11, 12, 14, 17, 18, and 19) and four of them showed a high p24 serum level (patients 11, 14, 17, and 19). None of the patients were receiving zidovudine at the time of the study.

Bone marrow (BM) aspirates were obtained from the patients and from normal donors for BM transplantation (BMT) and from two HIV-uninfected patients with idiopathic ITP.

Cell separation. BM was drawn into calcium and magnesium-free phosphate-buffered saline (PBS) containing 13.6 mmol/L Na citrate, 11.1 mmol/L glucose, 1 mmol/L adenosine, 2 mmol/L theophylline, 2.3 x 10^-10 mol/L prostaglandin E1, (pH 7 and 300 mOsm/L) (MK medium), and fractionated over a discontinuous Percoll (Pharmacia, Les Ulis, France) density gradient as previously reported, with slight modifications. Briefly, nucleated marrow cells were counted and suspended in Percoll medium to a final density of 1.020 g/cm^3 and at a cell concentration of 10 x 10^6 cells/mL.

Two densities of Percoll, 1.077 and 1.050, were overlaid on a centrifuge tube. Marrow cells suspended in the 1.050 Percoll solution were deposited on top of the 1.050 layer and then centrifuged at 300g for 30 minutes. Cells located at the 1.020/1.050 and 1.050/1.077 interphase were recovered. The first cell fraction was enriched up to 10-fold in recognizable MK, while the second fraction that was used to set up cultures was enriched in immature cells.

MK cultures. Cultures were performed from the marrow of HIV-infected and of normal donors using the cell fraction with a density of 1.050 to 1.077. Cells were cultured in suspension for 12 days in Iscove's medium containing 1% deionized albumin (bovine serum albumin, Cohn fraction V; Sigma Chemicals, St Louis, MO), 10% plasma from patients with aplastic marrow, and 5% conditioned medium from lymphocytes stimulated by phytohemagglutinin as previously described. Flasks were incubated in a fully humidified atmosphere with 5% CO2 at 37°C. The percentage of MK grown was evaluated after labeling of cytosin preparations.
was used in this study. The riboprobe with an anti-von Willebrand factor (vWF) polyclonal antibody (see "S-labeled HIV-1 RNA probe (Dupont NEP 200, Biotechnology Systems Division, Paris, France), antisense of an ENV sequence, background on cell lines and normal MK. The stringency of the hybridization procedure in at least three separate experiments. Results were routinely fresh marrow purified MK because the number of available slides was limited. In contrast, these three antibodies alone or in combination were used for cultured MK.

**Table 1. Patient Population, Clinical Values, and Results of In Situ Hybridization and PCR**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Grade CDC</th>
<th>p24 Ag Serum (pg/mL)</th>
<th>Platelet Count ($\times 10^9$/L)</th>
<th>Fresh Marrow MK In Situ Hybridization</th>
<th>Cultured MK % Obtained</th>
<th>Cultured MK In Situ Hybridization</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>II</td>
<td>0</td>
<td>&lt; 50</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>II</td>
<td>247</td>
<td>&lt; 50</td>
<td>ND</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>II</td>
<td>0</td>
<td>&lt; 50</td>
<td>±</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>II</td>
<td>0</td>
<td>&lt; 50</td>
<td>+</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>II</td>
<td>0</td>
<td>&lt; 50</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>II</td>
<td>200</td>
<td>&lt; 50</td>
<td>+</td>
<td>20</td>
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<td>80</td>
<td>-</td>
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<tr>
<td>8</td>
<td>F</td>
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<td>0</td>
<td>&lt; 50</td>
<td>+</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>II</td>
<td>0</td>
<td>&lt; 50</td>
<td>ND</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>II</td>
<td>650</td>
<td>&lt; 50</td>
<td>ND</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>II</td>
<td>&gt; 1,000</td>
<td>110</td>
<td>ND</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>II</td>
<td>0</td>
<td>100</td>
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<td>+</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>IV-A</td>
<td>820</td>
<td>77</td>
<td>ND</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>IV-C-2</td>
<td>380</td>
<td>&lt; 50</td>
<td>ND</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>IV-C-2</td>
<td>0</td>
<td>&lt; 50</td>
<td>+ (few MK)</td>
<td>4</td>
<td>-</td>
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<tr>
<td>17</td>
<td>M</td>
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<td>120</td>
<td>ND</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>IV-D</td>
<td>0</td>
<td>120</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>IV-C-2</td>
<td>&gt; 1,000</td>
<td>120</td>
<td>ND</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>IV-C-2</td>
<td>ND</td>
<td>&lt; 50</td>
<td>- (few MK)</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>IV-C</td>
<td>800</td>
<td>&lt; 50</td>
<td>ND</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined; +, positive; −, negative; ±, weakly positive.

with an anti-von Willebrand factor (vWF) polyclonal antibody (see below).

**In situ hybridization.** The protocol used was derived from the work of Harper et al, with a few modifications. Cells were rinsed twice with modified PBS (MK medium) and resuspended at 10⁶ cells/mL. They were cytostriped on glass slides for 1 minute at 700 rpm, air dried, and fixed in 4% paraformaldehyde dissolved in phosphate buffer (pH 7.4) for 3 minutes, as according to Lawrence and Singer. Slides were then stored in 70% ethanol until use. A decS-labeled HIV-1 RNA probe (Dupont NEP 200; Biotechnology Systems Division, Paris, France), antisense of an ENV sequence, was used in this study. The riboprobe (10⁵ cpm) contained in 20 μL of 50% formamide (BRL, Cergy Pontoise, France), 2X SSC (0.6 mol/L NaCl, 0.06 mol/L sodium citrate, 10% dextran sulfate [Pharmacia]), and 10 mmol/L dithiothreitol was hybridized to cells at 52°C for 16 hours. Each batch of the HIV probe was tested for its background on cell lines and normal MK. The stringency of the baths was modified according to these results and in some cases an RNase treatment was added. In some experiments, slides were washed sequentially at 52°C three times in three baths each for 5 minutes: (1) 2X SSC, (2) 2X SSC/50% formamide, (3) 1X SSC in the absence of RNase treatment. However, in most experiments, an RNase digest (5 μg/mL RNase A in 2X SSC) for 10 minutes at 37°C and two additional baths (0.5X SSC and 0.1X SSC at 52°C) were required to get a high specific signal. Slides were then dehydrated through graded ethanol and autoradiographed for 7 to 14 days with nuclear track emulsion NTB 2 (Kodak-Pathe, Marne la Vallée, France) developed and stained with May-Grünwald-Giemsa.

Cells from each patient were subjected to the in situ hybridization procedure in at least three separate experiments. Results were compared with those of negative and positive controls simultaneously processed according to the same protocol of hybridization.

As a positive control, a decS-labeled antisense murine β actin riboprobe (a generous gift from Dr F. Dautry, Villejuif, France) of the same size was used. Controls for nonspecific binding were decS-labeled riboprobes of pBR322 (Dupont NEP 227; Biotechnol-
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10^9/L. No contaminant cells were detected morphologically in these platelet preparations.

Platelet antibody determination. Platelet-associated IgG were detected according to the method described by von dem Borne et al.21 Sera were tested for antiplatelet membrane activity by means of an indirect immunofluorescence test on paraformaldehyde-treated platelets using fluorescein-conjugated F(ab'); antihuman light chains (Dakopatts). A direct test was performed using the above mentioned fluorescein-conjugated antibody.

Detection of anti-HIV antibodies in platelet eluates. Platelet-associated IgG were ether eluted22 and the direct eluates tested for anti-HIV activity in comparison with the last platelet washing samples. Anti-HIV antibodies were detected by means of Western blot immunosassay (Dupont de Nemours or Pasteur Transfusion, Paris, France). Western blots were considered positive if visual examination showed antibody binding to one or more HIV proteins.

RESULTS

Presence of anti-HIV antibodies on platelets from patients with HIV-associated thrombocytopenia. The immunologic analysis of antiplatelet antibodies of most patients included in this study has been published in detail elsewhere.2 By means of indirect immunofluorescence antibodies against normal platelets were found in the sera of 16 of the 17 patients studied (the exception was patient 14) (Table 2). Direct immunofluorescence tests with antihuman Ig on platelets were positive for all patients and, in two cases (patients 2 and 17), antibodies against normal platelets could be eluted. Furthermore, in all 14 patients studied, anti-HIV antibodies were present in the platelet eluates (Table 2), while platelet washes were negative. The specificity of these antibodies was determined by Western blot. As shown in Fig 1 and Table 2, their specificity was essentially for Gp 160 (Fig 1). In contrast, only in a minority of nonthrombocytopenic HIV-infected patients could anti-HIV antibodies be eluted from platelets (data not shown).

These data indicate that platelets from thrombocy-
topenic HIV-infected patients have anti-HIV antibodies on their surface. Therefore, we subsequently tried to determine whether this lineage was infected by HIV.

Detection of HIV transcripts in MK enriched from BM. We used the polymerase chain reaction (PCR) technique to determine whether platelets were infected by HIV. This technique has the advantage of being very sensitive and avoids the need for BM aspiration because it can be performed on platelet RNA. It was possible to detect HIV transcripts in RNA extracted from purified platelets. However, the T-cell receptor β chain RNA was also present by PCR in these RNA, meaning that our platelet preparations were not pure enough to give unequivocal results. Therefore, we focused our study on MK. We investigated the presence of HIV proteins or messenger RNA (mRNA) in fresh marrow MK from 10 HIV-infected ITP patients by immunofluorescence and in situ hybridization. A discontinuous Percoll gradient was prepared to obtain a fraction enriched in MK (density ≤ 1.050), and MK were specifically identified by staining with an anti-vWF antibody.

The percentage of MK in the 1.050 fraction varied from 0.5% to 4% according to the sample. Two patients were not entirely evaluable because the number of MK was low (less than 20 per slide).

Expression of HIV proteins (p 24) was studied by immunofluorescence using an MoAb. MK identified by indirect immunofluorescence against vWF (Fig 2B, D, and F) were positive for the p24 HIV protein in 3 of 10 cases. In one patient, significant staining was observed in MK (Fig 2A and C) as compared with irrelevant antibody (Fig 2E).

In Fig 2C and D, positive and negative MK were present side by side. Positive MK represented about 25% of the MK. In the two other patients, staining was weak but above background fluorescence observed either with an irrelevant antibody or with the same MoAb on normal MK. A strong immunofluorescence labeling was observed in the HIV-infected U 937 cell line (data not shown). No correlation was observed between MK labeling with the anti-p24 antibody and the level of plasma p24 in the patients.

Presence of HIV RNA on fresh marrow MK was studied by in situ hybridization technique. Megakaryocytes from five (patients 4, 6, 7, 8, and 13) of eight evaluable patients with marked thrombocytopenia were positive with the HIV probe as illustrated in Fig 3A and B, while the three other patients were negative. Among the positive patients, only a fraction of their MK gave a strong signal (> 300 grains/MK) as shown in Fig 3A, in which we could see a negative and a positive MK side by side.

Specificity of in situ hybridization was assessed using several controls. As negative controls, the MK-enriched preparations from HIV-infected patients were hybridized with either a PBR 322 or λ phage or the β globin chain probes. Using these probes in separated experiments less than 50 silver grains were observed on MK (Fig 3C). All counts of the silver grains were performed visually by two separate investigators. However, when the HIV probe was applied to MK-enriched preparations from HIV-negative ITP patients, a higher nonspecific hybridization was ob-
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Fig 3. Detection of HIV transcripts by in situ hybridization on fresh marrow MK from HIV-infected ITP patients. MK were purified from marrow by a discontinuous Percoll density gradient and were submitted to in situ hybridization using a 35S riboprobe antisense of a Gag sequence of HIV. Exposure time of autoradiographs was 10 days. (A) A positive and a negative MK (arrow) from patient 6. (B) The positive MK from patient 6 shown in (A) observed at a higher magnification to better visualize the silver grains. (C) Negative control, hybridization of MK from patient 6 with λ phage. (D) Negative control, MK from HIV-negative ITP patient hybridized with the HIV probe. The background with this probe is higher than with the λ phage probe (C).

served than with the previous probes (mean number of silver grains, 150; compare Figs 3C and D). Therefore, results of the in situ hybridization with the HIV probe on MK from HIV-infected patients were compared with those from HIV-negative subjects. In addition, a λ phage probe and a β actin probe were always used as a negative and positive internal controls, respectively, in all experiments.

Distribution of HIV RNA-positive MK was studied by counting visually the number of silver grains per cell. High numbers of silver grains per cell could be counted visually because the average size of MK was from fourfold to eightfold larger than that of any other cell type, although this quantitation was quite tedious. As shown in Fig 4A and B, the positive fraction that represented about 30% of the MK population was quite variable from patient to patient. Another fraction had a number of silver grains identical to that seen in the controls (up to 200 grains). Some MK showed an intermediate number of grains (from 200 to 300), suggesting low-level expression of HIV transcripts. In one patient (patient 3), a small number of MK (<10%) showed this pattern of hybridization, while the other MK had a similar number of grains as the HIV-negative MK (Fig 4). It is noteworthy that three of the five cases positive by the in situ hybridization technique were also positive by immunofluorescence (patients 4, 7, and 13).

When marrow cells of the 1.050 fraction other than MK were re-examined, no positive hybridization was observed with the HIV probe. In the 1.077 cellular fraction, few lymphocyte-like cells (<0.1%) hybridized, while granulocytic precursor cells were negative.

HIV transcripts and proteins were not detected in MK grown in vitro. In a second set of experiments, we investigated the presence of HIV in MK grown in vitro in a liquid culture technique that has the advantage of providing a large number of MK from a BM sample. As previously shown, these MK are not mature MK that survived in our culture conditions; they derived from the in vitro differentiation of MK progenitors similar to colony-forming units-MK (CFU-MK).

All 21 patients were studied by this technique. Table 1 shows that the percentage of MK obtained in culture was highly variable from patient to patient (undetectable to 80%). This great variability is also observed with normal marrow samples. No morphologically recognizable MK could be grown in vitro from samples from two of the seven patients with AIDS and their percentage was low in the remaining five. In contrast, most cultures of HIV-infected AIDS-free patients with ITP showed a percentage of differentiating MK similar to normal marrow cultures, although the absolute number of cells was always reduced.

Immunofluorescence for HIV proteins (anti-p24, p18, and GP 120 antibodies used alone or in combination) was negative in all MK cultures, whereas the same antibodies clearly labeled the HIV-infected U 937 cell line (data not shown).

In situ hybridization was performed in all cultures in which sufficient numbers of MK could be recovered (Table 1). When the β actin probe was used, all cells were positively labeled after 1 week of exposure (Fig 5A). In contrast, no significant hybridization was observed with the HIV probe even after a long exposure period (over 3 weeks) (Fig 5B), as compared with normal noninfected cultured MK (Fig 5C). The U937 HIV-infected cells were used as a positive control for the HIV probe in these experiments.
HIV-negative ITP

Patient NO4

Patient NO6

Patient NO8

Fig 4. Histogram representation of in situ hybridization on fresh marrow MK. MK were studied as above. The number of silver grains on more than 40 MK per patient were counted. (A) and (B) represent two independent experiments. MK were classified according to the number of silver grains counted visually by two separate investigators. Differences were slight (about 10%). However, when the number of silver grains exceeded 500 per MK, the precise number could not be determined and these MK were enumerated in a single class (over 500). In experiment (A), the number of silver grains in MK from patients 4, 6, 7, and 8 was counted and compared with the negative control, ie, the number of silver grains in MK from an HIV-negative patient with ITP, hybridized with the same probe. In experiment (B), the same experiment was performed with MK from patients 3 and 13 and compared with the same control. When the λ phage probe was used as an internal negative control, nonspecific hybridization was always lower (less than 50 silver grains) than that found with the HIV probe on noninfected MK, even with high stringent washes.

DISCUSSION

HIV-related ITP has similar features to idiopathic ITP and appears to be related to the deposition of immune complexes or to the presence of antibodies against platelet glycoproteins. The relationship between HIV infection and immune platelet destruction remains unclear. However, the possible infection of MK by HIV should be taken into account because it could impair marrow compensation of platelet destruction and favor platelet destruction either by expression of viral proteins or by exposure of cryptic antigens on the platelet surface. Correction of thrombocytopenia by zidovudine in HIV-infected patients and the recent discovery by Zucker-Franklin and Cao of HIV-infected MK in patients with AIDS support this hypothesis.

We, therefore, investigated whether HIV transcripts or proteins could be detected in fresh or cultured MK of HIV-infected ITP patients. HIV infection of fresh MK but not of cultured MK could be shown. This difference may be an indication of the cellular stage at which HIV infects hematopoietic cells.

HIV transcripts were found on fresh MK by in situ hybridization. However, our results differ slightly from those of Zucker-Franklin and Cao because HIV transcripts were not detected in all our patients and only a fraction of the MK in a given patient clearly express HIV transcripts. These discrepancies are probably related to differences in the patient populations studied, ie, the majority in our study were AIDS-free, whereas all those in that of Zucker-Franklin and Cao had AIDS. Moreover, we detected small amounts of HIV proteins in three cases by means of immunofluorescence. Further studies will be required to show whether it is related to an integration of HIV in MK genome or to an uptake directly or via the Fc receptors and immune complexes. This first hypothesis seems more likely because Zucker-Franklin et al have
showed that HIV may be trapped in the demarcation membranes of MK and platelets.

In contrast, HIV transcripts and proteins were not detected by in situ hybridization or immunolabeling in MK differentiating in vitro from the CFU-MK, even in the cases in which fresh MK were infected. In addition, the absolute number of MK in culture was diminished in comparison with that obtained with normal marrows. However, the proportion and morphology of MK obtained in vitro from the marrow of non-AIDS patients did not differ from those of normal subjects. These results confirm that ITP in asymptomatic HIV-infected patients is not due to a major defect in megakaryocytopoiesis. In contrast, in AIDS, thrombocytopenia may be related to defective megakaryocytopoiesis, as previously described, because no MK could be grown in vitro from the marrow of some AIDS patients.

Several explanations for the differences between MK obtained in vivo and in vitro can be forwarded. The first explanation is that MK progenitors, CFU-MK, are not infected by HIV and that the virus infects MK during their terminal differentiation in the marrow. It is not yet clear whether hematopoietic progenitors are infected by HIV. Some reports have shown that these cells are infected by HIV, but these results are all based on indirect evidences. Donahue et al.²⁰ have shown that anti-Gp 120 antibodies inhibit the growth of hematopoietic progenitors (burst-forming unit-erythroid [BFU-E] and granulocyte-macrophage colony-forming unit [CFU-GM]) from HIV-infected patients but not from normal subjects. In addition, it has been shown that normal purified progenitors can be infected by HIV in vitro, although, after culture, HIV was only detected in macrophage-like cells.²¹ In contrast, more recently, Laer et al.²⁷ and Molina et al.²⁸ have shown that HIV genome is not detected by PCR in CD34⁺ marrow cells or in hematopoietic progenitor-derived colonies from HIV-infected patients, respectively.²⁷,²⁸ Therefore, there is no evidence that CFU-MK are infected by HIV and, we cannot, thus, exclude the possibility that MK are infected during terminal differentiation. This hypothesis is strengthened by the presence of CD4, the cellular receptor for Gp 120, on mature MK because it has been recently reported that a fraction of MK strongly express the CD4 molecule.²¹ In addition the presence of CD4 on the majority of leukemic cell lines with an MK phenotype (HEL, MEG 01, CMK) (personal results) is consistent with the presence of low amount of CD4 on MK. However, absence of CD4 on MK would not totally exclude infection by HIV because infection of CD4-negative cells has been recently described.²⁹

The second hypothesis is that CFU-MK are infected, but are unable to differentiate in vitro. It has been reported that hematopoietic progenitor cells from HIV-infected patients
are decreased in number\textsuperscript{24,25}, the reasons remain unclear but it cannot be excluded that infection of these progenitors by HIV leads to defective differentiation in vitro. A last hypothesis remains that MK are not truly infected but are able to uptake the virus. In this case, the absence of virus in the liquid culture may explain why cultured MK do not express HIV transcripts.

The mechanisms by which a possible MK infection by HIV may favor the immune destruction of platelets remain unknown. It is noteworthy that anti-HIV antibodies were eluted from the platelet surface of all the patients studied here. Karpatkin et al\textsuperscript{9} assumed that such eluted anti-HIV antibodies belonged to immune complexes and were fixed to the platelet surface by Fc receptors. However, we provide evidence that viral proteins are expressed by MK. Therefore, viral antigens may be present on the platelets of some patients, but at a low antigenic density, rendering their detection extremely difficult but leading to platelet destruction by anti-HIV antibodies. In the patients studied here, antiplatelet antibodies were also detected in the serum. In most cases, they are probably directed against components of the GPIIb/IIIa complex because they do not bind to thrombasthenic type I platelets.\textsuperscript{7} The alternative hypothesis is that some anti-HIV antibodies are cross-reactive with platelet determinants. Indeed, we have recently observed the presence of such antibodies in one patient (unpublished result). Infection of MK by HIV may thus favor the emergence of cross-reactive antibodies between HIV and platelets by unmasking some cryptic antigens on the platelet surface.

In conclusion, this study shows that infection of MK occurs in patients with ITP. Further investigations will be required to determine the precise relationships between HIV infection of MK and pathogenesis of these thrombocytopenia.

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