Pathophysiology of HIV related thrombocytopenia: an analysis of 41 patients

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Abstract

Aim—To analyse the pathogenic mechanism of HIV related thrombocytopenia.

Methods—Forty one patients with thrombocytopenia and HIV-1 infection were investigated over two years. Anticardiolipin antibodies were measured using an enzyme linked immunosorbent assay and antiplatelet antibodies were measured using an immunocapture technique. Tests for VDRL, C3 and C4, antinuclear antibodies and rheumatoid factor were also carried out in all patients and 80 control subjects (HIV-1 positive but non-thrombocytopenic). Indium-oxine labelled platelets were transfused in 13 patients. P24 antigen were also measured in 12 bone marrow aspirates.

Results—Antiplatelet antibodies and circulating immune complexes were found exclusively in the thrombocytopenic group; values for antiplatelet antibodies and circulating immune complexes were both higher in homosexual and bisexual patients. Three kinds of pattern were observed using 111 In-labelled platelets: splenic (n = 10); hepatic (n = 2); and destruction of bone marrow in just one case. The two most influential factors in the sequestration pattern were antiplatelet antibodies in the splenic uptake and circulating immune complexes in the hepatic and marrow sequestration. All patients, except three, had decreased platelet recovery. In those patients with a CD4 lymphocyte count of less than 200×10^6 cells/l the recovery was clearly greater (53%) than in patients who had more than 200×10^6 /l (28%). Finally, in seven of the 12 patients who were chosen for immunohistochemical study, p24 antigen was detected in the megakaryocytes, verifying that HIV-1 infects such cells.

Conclusions—The pathogenic mechanism of HIV related thrombocytopenia is probably multifaceted. Antiplatelet antibodies and circulating immune complexes would cause peripheral destruction in the spleen, liver, and bone marrow, in that order; and, on the other hand, there would be an ineffective immune thrombopoiesis and direct infection of the megakaryocytes which could cause a change in the function and maturity of these cells.

Thrombocytopenia is one of the earliest haematological disorders which appears in the evolution of HIV-1 infection. Its association with this infection seems nowadays unquestionable; but its pathophysiological mechanism has not yet been completely defined.

Studies have dealt with the mechanism through which HIV-1 might induce the synthesis of antibodies against the platelets (Ris J, *et al.* Abstract presented at the XXth Congress of Internal Medicine, Stockholm, 1990.)¹⁻³ The polyclonal activation of B lymphocytes could contribute to the synthesis of antibodies and the formation of circulating immune complexes that could be bound on to the platelet membrane.⁴ Experimentally, this mechanism has been related to the thrombocytopenia produced by certain retroviruses.

The presence of circulating immune complexes in HIV-1 positive patients was found in 88% of cases.⁵ The detection of retroviral antigens in some of the circulating immune complexes proves that the immune system reacts against the virus.⁶ Furthermore, Yu *et al.*⁶ have suggested that immunoglobulin might be directed against the anti-HIV antibodies. There are, however, other unresolved issues: among them, the kind of patterns of platelet sequestration in these patients compared with those patients with classic idiopathic thrombocytopenic purpura (ITP), and whether HIV-1 has a more direct association with thrombocytopenia.

Methods

The study was carried out between 1988 and 1991 at the Internal Medicine Department of "La Paz" Hospital in Madrid (Spain). Forty one patients who met the following criteria were selected: HIV-1 seropositive; a platelet count of less than 100×10^9 /l; and an absence of myelodysplasia, aplastic anaemia, hypersplenism, drug intake, and other viruses.

Thirty two patients were men; nine women. Thirty two were intravenous drug misusers, five were homosexual men, three were heterosexual women and one was a man of unknown risk. At the time of diagnosis, seven patients belonged to the Centers for Disease Control⁷ (CDC) group IV-C1 (AIDS), three to group IV-C2, one to group IV-A, and the other 30 patients to group IV-E. Only those patients with thrombocytopenia alone were included in group IV-E, as we have recommended in previous publications.^{8,9}

Anticardiolipin antibodies (ACA) were measured by enzyme linked immunosorbent

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assay (ELISA)¹⁰: in our hospital, the test is considered positive if IgG is > 5 U/ml or IgM is >3.2 U/ml, or both. Circulating immune complexes were measured using the Ortho Serum ELISA Test System (C1q-IgG),⁵ regarding as positive those values above 35 µg/ml. Antiplatelet antibodies were analysed by the immunocapture technique (Capture-P. Immucor Inc, Norcross, USA), modified in accordance with procedures published by Jui *et al*¹¹ and Rachel *et al*.¹² The VDRL test and measurement of complement C3 and C4, antinuclear antibodies, and rheumatoid factor were also done. All these variables were also analysed in a control group of 80 HIV-1 infected, but non-thrombocytopenic patients.

The transfusion of 111 Indium-oxine (111In) labelled platelets was carried out on 13 patients. All these patients had platelet counts of less than $10 \times 10^9/l$ and none of them was receiving any other treatment. To obtain a greater volume of platelets, and with the purpose of obtaining better results with this test, the studies were carried out with 111In labelled platelets from ABO and HLA-DR compatible donors, who were hepatitis B surface antigen (HBsAg) and HIV negative. Once the platelets had been labelled, they were administered intravenously. During the first hour blood samples were taken to detect the activity of 111In and to calculate the percentage of recovery. For an average adult the percentages oscillate between 55 and 72%.^{13,14} After 24 hours, scintigraphic images were obtained using a gamma camera. We investigated three areas: the spleen; the liver; and the thorax-sternum (bone marrow activity).¹⁵

Finally, to detect HIV-1 infection in megakaryocytes, specific monoclonal antibodies were used against p24 antigen (Chemicom, USA). The study was carried out on 12 biopsy specimens of bone marrow fixed in B5 and paraffin wax, using the avidin biotin alkaline phosphatase technique. The technique had already been applied to samples of lymph nodes from six patients with AIDS (the presence of p24 antigen in endothelial cells, macrophages, and B lymphocytes was observed). To avoid the possibility of false positive results, the same technique was also applied to six samples of bone marrow from HIV negative patients belonging to no behavioural risk group, and here the test did not give any positive result.

The data processing and analysis were carried out on a IBM compatible computer. The graphics were produced with the Harvard Graphics program (Software Publishing Corporation, Mountain View, California USA). The database used was from the SIGMA statistical program (Horus Hardware SA, Madrid, Spain), with which a descriptive study and a comparative analysis of the different variables was performed.

Statistical analysis was performed using Student's *t* test and χ^2 analysis, with Yates's correction. Values of $p < 0.05$ were regarded as significant.

Results

The results of the different immunological variables relating thrombocytopenic and non-thrombocytopenic patients are shown in table 1. Positive circulating immune complexes were present in 64.1% of the thrombocytopenic patients and in 17.7% of the non-thrombocytopenic patients; $p < 0.05$. Antiplatelet antibodies and circulating immune complexes were found exclusively in the thrombocytopenic group. There were no significant differences between the two groups with the rest of the immunological data. The antiplatelet antibody and circulating immune complex values in the different risk groups are shown in table 2. The most significant difference ($p < 0.05$) was the circulating immune complex percentage in homosexual men (80%; 4/5) compared with the rest of the risk groups (23.52%; 8/34). There were no measurable differences between the values of anticardiolipin IgG and IgM antibodies in either group (table 3).

Table 4 gives details of the patients who were subjected to a gamma camera study to detect the areas of greatest platelet destruction. Three main patterns were observed: (1) splenic ($n = 10$); one patient (case 10) also showed increased hepatic uptake; (2) hepatic ($n = 2$) (cases 9 and 11); both showed simultaneous increases in uptake in bone marrow;

Table 1 Immunological variables in thrombocytopenic and non-thrombocytopenic patients

	Thrombocytopenic ($n = 41$)	Non-thrombocytopenic ($n = 80$)	<i>p</i> value
RF	17.85% (5/28)	9.52% (4/42)	0.7259
ANA	0% (0/28)	0% (0/42)	
VDRL	12.5% (5/40)	36.58% (15/41)	0.4376
ACA IgG	44.73% (17/38)	72.41% (42/58)	0.5891
ACA IgM	18.42% (7/38)	43.10% (25/58)	0.4027
C3 (mg/dl)	96.63	98.16	0.8813
C4 (mg/dl)	38.95	33.94	0.6603
CIC	30.76% (12/39)	0% (0/45)	0.0000*
ApAb	56.41% (22/39)	0% (0/38)	0.0000*

RF (+) = Rheumatoid factor; ANA = antinuclear antibodies; ACA = anticardiolipin antibodies; CIC = circulating immune complexes; ApAb = antiplatelet antibodies.

* Significant differences.

Table 2 Percentage of antiplatelet antibodies and circulating immune complexes

	Homosexual and bisexual men	Drug misusers	Others
Circulating immune complexes*	80%	23%	33%
Antiplatelet antibodies	80%	48%	100%

*The difference was significant ($p < 0.05$) between drug misusers and homosexual or bisexual patients.

Table 3 Percentage of anticardiolipin antibodies in thrombocytopenic and non-thrombocytopenic patients

Anticardiolipin antibodies	Thrombocytopenic	Non- thrombocytopenic
IgG	44.74%	72.41%
IgM	18.42%	43.11%

The differences were not significant.

Table 4 Distribution of Indium labelled platelets in 13 patients with HIV related thrombocytopenia

Case No	Clinical bleeding	Antiplatelet antibodies	Circulating immune complexes	Spleen	Liver	Bone marrow	Ratio	Recovery (platelets)
1	No	+	—	77.1*	9.71*	13.19*	7.94	24
2	No	+	+	67.92	10.84	21.24	6.27	
3	Yes	+	+	69.78	12.27	17.95	5.69	60
4	No	+	—	76.32	10.02	13.66	7.62	
5	No	+	+	63.43	11.59	24.98	5.47	26
6	Yes	—	—	77.12	7.77	15.11	9.93	13
7	Yes	—	+	55.09	15.82	22.09	3.48	22
8	No	—	+	63.48	13.58	22.94	4.67	30
9	No	—	+	38.53	24.48	36.99	1.57	34
10	Yes	—	+	58.29	17.14	24.57	3.4	65
11	No	—	—	41.05	21.89	37.06	1.88	
12	No	+	—	79.77	5.02	15.21	15.89	65
13	No	—	—	22.02	26.63	51.35	0.83	14

(Ratio): spleen/liver ratio.

* Percentage of activity in spleen, liver, and bone marrow.

Table 5 Spleen: liver ratio* in patients with HIV related thrombocytopenia

Variable	Ratio	p value
Drug misusers:	4.87	
Other risk groups	7.13	NS
Antiplatelet antibodies (+)	8.14	
Antiplatelet antibodies (—)	4.16	< 0.05
Circulating immune complexes (+)	4.13	
Circulating immune complexes (—)	8.65	< 0.05
CD4 < 200:	4.57	
CD4 > 200	6.26	NS
<50 × 10 ⁹ /l platelets:	4.46	
>50 × 10 ⁹ /l platelets	7.79	NS
Clinical bleeding	5.62	
No symptoms	5.79	NS

*Spleen/liver ratio of distribution of Indium labelled platelets; ApAb = antiplatelet antibodies; CIC = circulating immune complexes; CD4 = lymphocytes/mm³. NS = not significant.

(3) bone marrow pattern (n = 1) (case 13, who also showed increased hepatic uptake). The ratio between hepatic and splenic uptake decreased in three cases, remained stable in three, and increased in seven (in case 12 this even reached 15.89). Platelet recovery decreased in all except three cases (7/10).

Patients with antiplatelet antibodies titres had greater splenic uptake than those negative for antiplatelet antibodies, although in these patients hepatic and bone marrow uptakes were greater (p < 0.05). For circulating immune complexes, it was exactly the opposite pattern (table 5). There were no differences, however, between patients from different risk groups; between patients who did or did not experience clinical bleeding; between those patients who had more or less than 50 × 10⁹/l platelets, nor even between those who had more or less than 200 × 10⁶ lymphocytes CD4/l. The recovery was 54.01% in those who had a CD4 lymphocyte count of less than 200 × 10⁶/l, compared with 27.71% for those who had more than 200 × 10⁶/l (p < 0.01).

P24 antigen was found in the membrane and cytoplasm of megakaryocytes and cells of the myeloid-lymphoid series in seven of 12 cases studied.

Discussion

Important immunological changes occur in HIV infection. Okenhändler (Abstract presented to Sixth International Conference on AIDS, San Francisco, 1990) and Karpatkin¹⁶ have suggested that anti-idiotypic antibodies

anti-HIV antibodies or antibodies to unknown platelet antigens might be responsible.

We compared the findings in thrombocytopenic patients with those found in HIV-1 positive but non-thrombocytopenic patients. Antiplatelet antibodies and circulating immune complexes were exclusive to the thrombocytopenic group (56 and 31%, respectively). The rest of the analysed variables did not show any significant differences between the thrombocytopenic and the non-thrombocytopenic patients.

The presence of antiplatelet antibodies could be related to a secondary immune alteration caused by an increase in the polyclonal activity of B lymphocytes caused by HIV. This would stimulate the production of cross-reacting antibodies,¹⁷ autoantibodies, and antibodies as opposed to viral adsorbed antigens or any other unknown antigens. The discovery of circulating immune complexes in HIV related thrombocytopenia coincides with what has been described in classic secondary thrombocytopenias (drugs or other viral infections).¹⁸ Antiplatelet antibodies and circulating immune complexes were more common in the homosexual and bisexual men than in the drug misusers, though circulating immune complexes were significantly greater (p < 0.05) in the former group (80%) than in the drug misuser group (23%). Our findings seem to confirm Karpatkin's findings¹⁹⁻²¹ concerning the connection between infection with the virus and different pathogenic mechanisms. However, there is no obvious explanation for the higher amounts of circulating immune complexes in homosexual men.

Finally, anticardiolipin antibodies, IgG, and IgM do not seem to be related to the pathogenesis of thrombocytopenia, in contrast to the findings of Harris¹⁰ concerning classic ITP. Like Intrator *et al*,²² we have not found any such association.

Thrombocytopenia is probably produced by a combination of marrow destruction, with subsequent ineffective production of, and a decrease in, the number of circulating platelets due to the sequestration and destruction caused by some of the peripheral organs of the mononuclear phagocytic system.²³

Kinetic studies of ¹¹¹In in patients with classic ITP show three kinds of pattern:

destruction in the spleen, the liver, and bone marrow.^{15,24} These patterns were also observed in our study. The significant increase in the destructive activity of the spleen in HIV positive patients was interesting and was considerably greater than in patients with classic ITP. This could explain the excellent results obtained by removing the spleen of these patients, although opinions vary as to the correlation between the results of the ¹¹¹In labelled platelet studies and the therapeutic response.^{13,15}

Nearly all of the patients had a low platelet recovery, which shows a rapid decrease in transfused platelets as a result of peripheral destruction.¹⁵ Measurable differences ($p < 0.01$) were only found in patients with less than 200×10^6 CD4/lymphocytes who showed a recovery of 53% (practically normal) compared with 28% (decreased) in the others. In fact, patients from the IV-E group, with a better immunological state, showed, during the whole study, a lower platelet count than those from the IV-C1 group, with a low CD4 count and a possible macrophagic disorder, which might explain why peripheral destruction was not so great in these patients.

There were no differences in clinical bleeding or platelet count among the behavioural risk groups, but we found important differences in the presence of antiplatelet antibodies or circulating immune complexes. There was an increase in splenic uptake for antiplatelet antibody positive patients and in hepatic and marrow uptake for circulating immune complex positive patients. The exact cause for the presence of circulating immune complexes or antiplatelet antibodies which influences the kind of sequestration pattern is not known. A possible explanation could be the existence of a different platelet "affinity" in the different sequestration levels. Liver and bone marrow, with cells containing receptors for IgM and C3, probably have a more important role in the elimination of platelets in patients whose platelets have circulating immune complexes bound on to the surface,

while the antibody coated platelets would be more likely to be phagocytosed by the macrophages of the spleen as happens in classic ITP.

The importance of the bone marrow as a sequestration area is possibly greater than has been found in kinetic platelet studies.¹⁵ On the other hand, some authors suggest that HIV infected megakaryocytes could inhibit platelet production.²⁵ There is also evidence of megakaryocytic growth being affected by this viral infection, as well as morphological changes produced in such cells.²⁶ But these studies were done in vitro. As far as we are aware there is no in vivo evidence for this as yet.

To detect the presence of p24 antigen, and therefore HIV, in the bone marrow we used a monoclonal antibody technique that has previously been used to detect such antigens in the lymphocytes of the lymph nodes or other cells—for example in the intestine.²⁷ Although only 12 patients were studied, we managed to prove that megakaryocytes were infected with HIV-1, by detecting p24 antigen in cytoplasm and the membrane. There was no direct correlation between the presence of p24 antigen in blood and the presence or absence of antiplatelet antibodies or circulating immune complexes.

Infection of the megakaryocytes could produce, in some patients, ineffective autoimmune thrombopoiesis similar to that which occurs in classic ITP,²⁸ or it could produce a maturative and functional change as a result of direct infection by the virus. The primary mechanism of the virus could be the following: HIV-1 induces the production of thrombopoiesis inhibitors or penetrates megakaryocytes through a receptor that has as yet not been identified. This receptor could be CD4,²⁸ another unidentified antigen, or could even be introduced by the circulating immune complexes and used as a "key" through the Fc receptor. The last mechanism has been seen in other virus infections,¹⁶ but obviously it is not unique as not all the patients showing p24 antigen in megakaryocytes were positive for circulating immune complexes.

In conclusion, HIV-1 related thrombocytopenia could have a multifaceted pathogenic mechanism (figure). Antiplatelet antibodies and circulating immune complexes could cause the consequent peripheral destruction in the spleen, liver, and bone marrow in that order; and, on the other hand, there would be ineffective immune thrombopoiesis and direct infection of the megakaryocytes, resulting in a change in the function and maturity of these cells.

Pathogenic hypothesis of HIV related thrombocytopenia.

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