Platelet and Coagulation Defects Associated with HIV-1-Infection

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Platelet Defects

HIV-1 seropositive individuals: homosexuals (1), intravenous narcotic addicts (2), hemophiliacs (3), and their heterosexual partners (4) develop chronic immunologic thrombocytopenic purpura (ITP). The thrombocytopenia can present early in the course of HIV-1-infection as well as in association with AIDS. The early onset disease (emphasized in this review) is clinically indistinguishable from classic autoimmune thrombocytopenic purpura (ATP) seen predominantly in females, with respect to increased megakaryocytes in the bone marrow, peripheral destruction of antibody-coated platelets and/or impaired platelet production, negative antinuclear antibody, and response to prednisone, i.v. γ globulin and/or splenectomy. The disease is different from classic ATP with respect to the markedly increased platelet-associated IgG, C3C4, the presence of circulating immune complexes and the male predominance.

Historical Descriptions

Homosexual ITP (HSITP)

Shortly after the first recognition of Kaposi’s sarcoma in sexually-active homosexuals at the Bellevue Hospital Oncology Clinic of New York University Medical Center (5), an “epidemic” of thrombocytopenia was recognized in the same cohort of patients at the same institution in November 1980. The syndrome was clinically indistinguishable from classic ATP, which is seen predominantly in females (11). Eleven severe cases were first described with a mean platelet count of 16,000 ± 3,000/µL, as well as two mild cases. All patients had increased megakaryocytes in the bone marrow, no splenomegaly, negative antinuclear antibody titers, and no clinical disorders known to cause thrombocytopenia. Other findings included: decreased helper/suppressor T-cell ratios, elevated platelet-bound IgG, elevated circulating immune complexes, lymphopenia, and elevated γ globulin. Several patients also presented with hypergammaglobulinemia, elevated circulating immune complexes, lymphopenia, decreased helper/suppressor T-cell ratios and positive serology for HIV-1.

Narcotic Addict ITP (NITP)

In November 1982 (2 years later), a similar epidemic of chronic ITP was noted in 70 intravenous (IV) narcotic addicts treated at Bellevue Hospital (female to male ratio: 1 to 3), with mean platelet counts of 53,000 ± 4,000 (range 13,000 to 140,000/µL) (2). These patients were or had been chronic intravenous abusers of heroin, cocaine, or both for a mean duration of 10 years. Thirty-three patients had stopped taking IV drugs for an average of 21 months, indicating that the thrombocytopenia was not caused by acute exposure to narcotics. Other possible causes of thrombocytopenia such as classic ATP, hypersplenism, chronic active hepatitis, or AIDS had been ruled out. Elevated platelet-bound IgG, C3C4 and serum circulating immune complexes were also noted.

Hemophiliac ITP (HITP)

In 1983, Ratnoff et al. (3) reported on the development of ITP in five patients with hemophilia (AHF deficiency) who were multiply-transfused with lyophilized AHF concentrates for 5 to 9 years with a mean platelet count of 34,600 ± 29,000 (range 8,000 to 82,000/µL). This syndrome was clinically indistinguishable from classic ATP. All five had elevated platelet-bound IgG levels. These patients also presented with hypergammaglobulinemia, elevated circulating immune complexes, lymphopenia, decreased helper/suppressor T-cell ratios and positive serology for HIV-1.

Heterosexually Transmitted HIV-1-Related ITP

In 1987 six cases of HIV-1-related ITP were recognized in “non-risk” individuals who had acquired the disorder through heterosexual contact (4). One such patient was a 64-year-old white woman whose contact was her husband who had received an HIV-1-contaminated blood transfusion for a coronary bypass. The patient developed thrombocytopenia approximately 2 years after resumption of sexual intercourse. Thus, HIV-1-related ITP can be disguised as classic ATP, and is therefore part of the differential diagnosis of unexplained thrombocytopenia. A careful social-sexual history is mandatory for diagnosing patients.

Thrombotic Thrombocytopenic Purpura (TTP)

In 1988, eight cases of TTP associated with HIV-1 infection were reported (6-8). All 8 cases had been recognized since 1985, relatively late in the development of the HIV-1 epidemic. Five cases reported from NYU Medical Center (7) from 1985 to 1987 represented one third of all cases of TTP diagnosed at the institution. When this rate of inci-
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dence, 5 of 15 patients, was compared with the incidence of patients hospitalized with AIDS during the same period (3560 of 171,210), the difference was significant by chi square analysis, indicating that the apparent association is not caused by ascertainment bias.

Thrombotic microangiopathy (TMA) is a heterogeneous disorder that includes both classic thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). Intravascular platelet aggregates are characteristic of the pathologic lesions with sparing of the liver and lung (9) and von Willebrand factor expression is a prominent feature (10). TMA has been subsequently described in over 150 HIV-1 cases (6, 7, 11-14).

Incidence and Prognosis

**HIV-1-ITP.** In the early description of the epidemic, sexually-active homosexual patients presented with ITP in the presence as well as absence of AIDS. Although many patients developed ITP early after HIV-1 infection, at least 11 other ITP patients had not developed AIDS 4-7.5 years (mean 5.2 years) after HIV-1 seropositivity (15). Several patients studied (9 patients) reverted to normal platelet counts 5-27 months (median 10 months) after the diagnosis of thrombocytopenia in the absence of treatment (i.e. splenectomy or steroids). One of these patients presented with a platelet count of 11,000/μL.

Thrombocytopenia can occur without clinical symptoms and increases in incidence with duration of illness and the development of AIDS. For example, the incidence of thrombocytopenia in an asymptomatic group of 26 seropositive homosexual men was 0 percent in 1987 (16). This incidence rose to 8 percent in 59 patients with non-AIDS HIV-1-related symptoms and 30 percent in 20 patients with AIDS. The incidence of thrombocytopenia (<100,000/μL) in 679 HIV-infected i.v. drug abusers was 21-37% (17, 18); 5% with platelet counts <30,000/μL (18). The incidence of thrombocytopenia in 1711 HIV-1-infected patients with hemophilia varied from 5-19% (19, 20). The incidence rose in the first 5 years, with the risk increasing to 50% in older patients 1 year after the development of AIDS (20).

An analysis of 44 HIV-1-ITP patients studied for a period of 844 person-months revealed no greater risk for the development of AIDS than a similar non-thrombocytopenic seropositive cohort over a period of 36 months (21).

**HIV-1-TTP.** While complete remissions are observed with appropriate treatment in HIV-associated TTP, unlike classic TTP the reported long-term survival is poor, with no survivors reported beyond 2 years (11, 13). HIV-associated TTP may differ from classic TTP in its gradual onset, presence of concurrent medical illness, occasional spontaneous improvement and inferior response to treatment (11).

Treatment

The rationale for treatment of HIV-1-ITP has been to use the lowest possible corticosteroid dose capable of raising the platelet count to safe levels, generally >25,000/μL with absence of significant purpura. Prednisone is administered at a dose of 30 to 40 mg/day for 1 to 2 weeks and rapidly tapered to a maintenance dose of 10 to 15 mg/day. The patient is then observed for 10 months, if possible, which is the median time for spontaneous reversion to a normal platelet count in 18% of our patients studied. If the patient has not obtained a safe platelet count by this time, or requires more than 10 to 15 mg of prednisone per day to maintain such a platelet count, splenectomy is performed. In our study of 41 homosexual patients, 35 had a moderate (>50,000/μL) to excellent (>100,000/μL) response while on corticosteroids (30 relapsed following cessation of corticosteroids). An excellent response to splenectomy was noted in 27 of 32 patients (84%) with a mean follow-up time of 14 months (15, 22, 23). Of our original cohort of homosexual patients, 26 of 41 (63%) did not require treatment for their thrombocytopenia. There is no rigorous evidence that corticosteroid treatment or splenectomy contributes to the development of AIDS. Prednisone therapy has been complicated, however, by the development of oral candidiasis as well as activation of latent herpes simplex virus.

The efficacy of azidodeoxythymidine (AZT) in the treatment of ITP in homosexuals is often dramatic (24, 25). There is an exponential rise in platelet count within the first 1 to 2 weeks of AZT treatment (200 mg every 6 h), with the maintenance of normal or higher counts for 7 weeks (duration of study). Hematocrits decreased in an exponential manner during the same period of time. After discontinuation of AZT, the platelet count remained elevated for more than 4 weeks in 3 of 5 patients. These observations on the relatively rapid rise in platelet count following AZT treatment may therefore suggest that HIV-1 infection of bone marrow precursor cells, megakaryocytes or stromal cells may be inhibiting platelet production (see below). Similar results have been reported with the new anti-retroviral drugs: Indinavir, Saquinavir and ritonavir. Twenty-three patients treated without AZT increased their platelet counts from 63,000 to 125,000/μL and decreased their viral load 74 fold during 6 months of observation with this treatment. No correlation was noted with CD4 count (26).

Intravenous gamma globulin (1 to 2 g/kg for 2 to 5 days) is usually effective in the treatment of HIV-related thrombocytopenia; however, its duration is transient, 7 to 10 days (27-30). Six of eight homosexual patients had a significant rise in their platelet count, as did all of 3 drug abusers and all of 8 hemophiliacs (27). Another group reported a good to excellent initial response in 12 of 17 patients (71%) (28). Patients refractory to prednisone will occasionally respond to gamma globulin given with steroids.

Intravenous anti-D treatment for Rh+ nonsplenectomized patients is also effective in the treatment of the disease. HIV-1-infected children have been reported to have the best results with an effect lasting 21 days in 50% of the responders (31).

Mechanisms of Thrombocytopenia

The mechanism of thrombocytopenia in patients with homosexual, i.v. narcotic addict, and hemophiliac chronic ITP (HSITP, NITP, and HITP, respectively) have been studied extensively by our group (1, 2, 32-39) in patients with early onset HIV-1-infection in the absence of AIDS where the mechanism appears to be increased platelet destruction (40) and compared with those of classic ATP. Myelodysplastic features with hypocellularity have been reported in patients with long-term HIV-infection and hemophilia (median 12.5 yrs from seroconversion) (41). In another study in children, TPO levels were 5 fold elevated in severe thrombocytopenic patients who did not respond to i.v. gamma globulin suggesting marrow failure (42).

Increased Platelet Destruction

Autologous platelet survival studies performed in 13 HSITP patients with a mean platelet count of <33,000/μL demonstrated a platelet survival of less than 1 day (normal survival time 8 to 10 days). Platelet sequestration was exclusively to predominantly splenic in 85 percent of the patients studied. Calculated blood flow and mean transit time were normal, ruling out hypersplenism (43). Autologous platelet survival studies performed in 19 i.v. drug abuser HIV-1-ITP patients with a
mean platelet count of <31,000/µL were < 1 day with no evidence of splenic sequestration (43). In another study mean platelet lifespan in 7 patients was 3 ± 4 h with a marked increase in platelet production but insufficient to maintain a normal platelet count. Five patients with normal platelet counts had a compensated thrombocytolytic state (44). The above data support an increased peripheral platelet destruction mechanism for the pathophysiology. Indeed the rapid improvement following treatment with steroids and/or splenectomy in most early-onset HIV-1-infected patients is strongly suggestive of this pattern. However, others have reported impaired platelet production, as well as decreased platelet survival (see below).

Decreased Platelet Production

Kinetic data on the rapid rise in platelet count (as early as 1 week) following AZT administration suggested that impaired megakaryocyte production, platelet release, or both may also be involved (24, 25). The rapid rise in platelet count following the use of AZT precludes a prior change in anti-platelet Ab levels and suggests that this drug either blocks reticuloendothelial function or prevents HIV-1-induced damage of megakaryocytes, which otherwise results in ineffective thrombopoiesis; or stimulates platelet production. Three platelet kinetic reports suggest that impaired thrombopoiesis may be operative in patients with HSITP. In one study, platelet production was normal or decreased in 7 of 14 patients despite a decreased platelet survival (45). Another group studied platelet survival and turnover in 19 homosexual patients with ITP treated with and without AZT (46). Both cohorts had a decreased platelet survival of about 50 percent of normal. Platelet turnover (production) was significantly decreased (approximately half the normal rate) in the untreated group (13 patients), compared with normal or increased turnover in the treated group (6 patients). Two patients were studied before and after AZT administration. Their rise in platelet count was associated with a three-to-six-fold rise in platelet turnover, with no change in platelet survival. Thus, AZT appears to exert its effect by enhancing platelet production, suggesting that the thrombocytopenia may also be related to impairment of the production or function of megakaryocytes. A third study of 6 patients revealed a combination of shortened autologous platelet survival by 2/3 normal, doubling of splenic platelet sequestration and ineffective delivery of platelets to the peripheral blood despite a 6 fold increase in TPO and a 3 fold increase in megakaryocyte mass (47). These observations are supported by a study of hemopoietic progenitor cells in 15 patients with AIDS or HIV-1 infection, 3 of whom were thrombocytopenic (48). These investigators reported a significant impairment of growth of bone marrow megakaryocyte colony-forming units, as well as granulocyte, erythocyte, and macrophage precursors in comparison with normal controls, which was corrected by deletion of T cells and reversed by readdition of autologous T cells. Because most patients have normal to increased megakaryocytes, it is likely that the impairment is caused by ineffective thrombopoiesis (platelet production and release). The mechanism of inhibition of thrombopoiesis is currently unresolved.

An unlikely possibility is the direct infection of megakaryocytes with HIV-1 (49, 50). Megakaryocytes have CD4 (51, 52) as well as CXCR4 (53, 54), known receptors for HIV-1gp120 and various megakaryocyte lines are infectable with HIV-1 (52, 55). However reports of HIV-1 infection of wild-type human megakaryocytes reveal little to no infectivity (1-5% of cells) compared to T cells or macrophages with questionable clinical significance (49).

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There is now abundant evidence from several laboratories that CD34+ stem/precursor hematopoietic cells are incapable of being infected in vivo or in vitro with HIV-1 (56-65). It is therefore logical to suggest that HIV-1-infected stromal cells may be responsible for the well-documented impaired megakaryocytopoiesis of HIV-1-infected patients (48, 58, 65, 66). This could be via induction of inhibitory cytokines. This is supported by impaired CFU-MK in 15 HIV-1-seropositive patients compared to control bone marrows (47, 48), the morphological abnormalities detected in the MK of HIV-1-ITP patients (67) decreased MK precursors (48, 58, 65, 66), and impaired MK survival due to increased apoptosis (66). Several cytokines have been shown to inhibit MK growth and differentiation: TGFβ-1 (68-72), PF-4 (73), TNFα (74, 75), α and γ IFN (76) and IL-4 (77). Of interest is the observation that HIV-1 tat protein, released by infected cells stimulate macrophage production of TGFβ-1 (72), one of the most potent negative regulators of hematopoiesis, resulting in a dose-dependent inhibition of CFU-MK. An alternative and/or additional mechanism could be autoimmune destruction of MK’s or their precursor by anti-platelet/MK Ab.

These apparently contradictory conclusions regarding decreased production vs increased destruction of platelets can be reconciled, perhaps by the observations of Nagean and coworkers who performed platelet kinetic studies on HIV-1-ITP patients with and without AIDS. They concluded that patients with AIDS are more likely to have decreased platelet production, whereas patients with early onset HIV-1 infection are more likely to have increased peripheral destruction of platelets (40).

Increased Platelet Consumption

TTP is associated with an absent VWF-cleaving Zn metalloprotease of the ADAMTS13 family (a disintegrin and metalloprotease) in the familial form (78) and the presence of an IgG inhibitor of the protease in the non-familial form (79, 80). In HUS (81) and in bone marrow transplant associated TMA normal protease levels are detected (82). Absent VWF-cleaving protease and associated inhibitor have also been documented in a case report of HIV-associated TTP (83). The availability of a standardized VWF-cleaving protease assay may allow for improved classification and management of TMA and elucidate the precise role of HIV in pathogenesis. In light of its proposed mechanism, TTP may be viewed similarly to ITP, as a manifestation of the clinical spectrum of HIV-related autoimmunity.

Immunologic Measurements of Platelet Antibody and Immune Complexes

Immunologic measurements of platelet associated IgG, C3C4 and serum circulating immune complexes (CIC), determined by the PEG method in homosexual ITP (HSITP), narcotic addict ITP (NITP) and hemophilic ITP (HITP) reveal strikingly elevated values compared to the values found in classic ATP patients, i.e. 2.6-3.8 fold greater IgG, 2.2-3.9 fold greater C3C4, 2.4-7.3 fold greater PEG-IC. Intermediate elevations of these measurements were noted in non-thrombocytopenic HIV-1-seropositive individuals of the same cohorts (2, 32, 38). These data suggested that circulating immune complexes may be responsible for the elevated platelet levels and that no relationship existed between the platelet count and the platelet associated IgG or C3C4. This proved to be the case. Isolated CIC were shown to bind to normal platelets in a concentration-dependent manner; indeed the level of serum CIC correlated with platelet bound IgG, r = 0.5, p < 0.05, n = 17. However, unlike the situation with classic ATP, there was no inverse relationship between platelet count and platelet associated IgG, r = -0.2, p > 0.1, n = 16 (similar results were reported by others); nor was the platelet
However we have detected F(ab’)2 binding in HIV-1 seropositive i.v. normal control groups (39).

Serum anti-platelet binding has been reported employing an indirect semiquantitative platelet suppression immunofluorescence test in homosexual patients, with impaired binding to Glanzmann’s thrombasthenia platelets [devoid of platelet GPIIb/IIIa (84)]. However, these authors did not prove F(ab’)2 binding. A second group, although unable to elute anti-platelet Ab from HIV-1-ITP narcotic addict patients were able to detect plasma anti-platelet Ib/IX and IIb/IIIa Ab utilizing the MAIPA technique on normal platelets preincubated with patient plasma in 43% of 45 patients (85). Our group has been unable to detect serum F(ab’)2 binding in the homosexual cohort of HIV-1-ITP patients. However we have detected F(ab’)2 binding in HIV-1 seropositive i.v. drug abusers and hemophiliacs (2, 32). The reasons for these differences are likely to be the presence of anti-idiotype blocking Ab (see below).

Composition of Immune Complexes

Anti-F(ab’)2 Antibodies

Studies designed to analyze platelets and immune complexes for viral antigens suggested the presence of anti-antibodies. Fixed washed platelets or eluates of HSITP patients revealed absence of EBV, cytomegalovirus (CMV), herpes simplex virus (HSV), or adenovirus (ADE) viral antigens on their platelets, despite the presence of viral antibodies for these antigens in their sera. Similar findings were noted in fixed immune complexes of these patients. Viral antibodies against EBV, CMV, HSV, and rubella virus were noted in these complexes, and correlated with their presence in sera. No such correlation was noted with preparations from healthy control subjects whose immune complexes were negative in the presence of positive antiviral serum titers (39).

Following this lead, anti-F(ab’)2 antibodies were sought and found in 9 of 12 HSITP and 6 of 6 i.v. drug abusers, which correlated with immune complex levels, r = 0.83, p < 0.01, n = 16. In contrast, anti-F(ab’)2 antibodies were not detectable in 6 patients with classic ATP against F(ab’)2 fragments of subjects from autologous, homologous ATP or normal control groups (39).

Anti-HIV-1gp120 and Anti-idiotype Antibodies

Analysis of serum PEG-CIC as well as platelet acid eluates of HIV-1-ITP, HSITP and NITP patients has revealed the presence of anti-HIV-1gp120 Ab and its anti-idiotype (33, 86). HIV-1 antigen or proviral DNA was not detectable. Approximately 50% of eluted platelet IgG contained anti-HIV-1gp120 Ab (33).

Anti-GPIIIa Ab In Vitro and In Vivo

PEG-IC’s of HIV-1-ITP patients were purified on protein A columns, dissociated in acid, separated on an acidified G200 column and affinity-purified on anti-IgG and anti-IgM columns. Both IgG and IgM reactivity was found against HIV-1gp120, HIV-1gp24 CD4 receptor, and human platelets. Only the IgM reacted with purified Fe fragments, indicating rheumatoid factor (RF) reactivity. The IgG anti-platelet reactivity was shown to be specific for platelets by demonstrating reactivity with F(ab’)2 fragments (34) (Fig. 1). Anti-platelet IgG reactivity was affinity purified on fixed platelets and the eluate incubated with platelet lysates containing [125I]-labeled surface platelet membrane. The immune complex precipitate was then analyzed by SDS-PAGE in the presence and absence of reducing agent. An ~100 Kd predominant band was noted, which paradoxically increased its mw upon reduction demonstrating the physical characteristics of platelet GPIIa. This was proven with specific MoAb’s against platelet GPIIa, simultaneously incubated with platelet lysate (Fig 2) (34). Further studies revealed specificity for amino acids 49-66 on GPIIIa, with an inverse curvilinear relationship between patient anti-platelet IgG serum (as well as anti-GPIIIa49-66) and platelet count (Fig. 3). The in vivo pathophysiologic relevance of these in vitro observations was substantiated by demonstrating that ~25 μg of affinity-purified anti-platelet IgG1 of HIV-1-ITP PEG-IC’s induced dramatic thrombocytopenia in recipient mice (which have 83% homology with human GPIIIa and Fc receptors for human IgG1). In vivo specificity was substantiated by demonstrating prevention or reversal of the anti-GPIIIa-induced thrombocytopenia with an albumin conjugate of GPIIIa49-66 (Fig. 4) (37).

The presence of this immunodominant autoimmune GPIIIa49-66 epitope appears to be unique, since classic ATP appears to have multiple epitopes within platelet membrane receptors, GPIIb/IIIa and GP Ib.

![Antigen binding diagram](image_url)
The observations in HIV-1-ITP could be related to cross-reactivity with HIV-1 antigens. This is suggested from the work of Bettaieb et al who noted cross-reactivity with HIV-1gp120 and platelet GPIIIa (87); our laboratory which found anti-HIV-1gp120 on platelets of HIV-1-ITP patients (33); Gonzalez-Conejero et al. who noted cross-reactivity with HIV-1gp120 of serum-platelet reactive eluates with GPIIb/IIIa and GPIb/IX in 20% of 45 drug abusers (85); and Chia et al. (88) who described cross-reactive HIV-1p24 and HIV-1gp120 Ab’s in alkaline and acid eluates of serum Ab’s bound to an HIV sepharose 4B affinity column. Indeed 48% of alkaline-eluted Ab bound to platelets as well as p24.

**Rheumatoid Factor Antibodies**

Elevated CD5+ B cells have been reported in patients and mice with autoimmune diseases as well as in HIV-1-ITP (89). CD5+ B cells generally produce low affinity antibodies, which are predominantly RF of the IgM class against Fc of IgG (90). Because PEG-IC’s of these patients contain high concentrations of IgM, RF-IgG complexes were sought within their serum PEG-IC’s. Purified PEG-IC IgM was shown to react with purified PEG-IC IgG of these patients, relocating ~50% of the IgG preincubated with IgM to the Vo (void volume) region of a G200 gel filtration column (34). Thus PEG-IC’s of HIV-1-ITP patients...
Fig. 4  In vivo induction of thrombocytopenia in mice injected with affinity-purified anti-platelet IgG of HIV-1-ITP patients and its reversal with GPIIIa-(49-66).

(A) Thirty micrograms of anti-platelet IgG of patients 6 and 11 or purified control human IgG was injected i.p. into eight experimental and six control mice, respectively. (B) Twelve mice were injected i.p. with 25 μg of anti-platelet IgG of patient 11, as above. Seven of these mice were simultaneously injected in the opposite flank with 226 nmol of GPIIIa-(49-66)-albumin conjugate, whereas five were simultaneously injected with the irrelevant scrambled GPIIIa-albumin conjugate (CGGGARVLEDPR) at the same concentration. (C) Ten mice were injected i.p. with 50 μg of anti-platelet IgG of patients 10 and 11, as above. At 2 h, six of these mice were injected with GPIIIa-(49-66)-albumin conjugate, whereas four were injected with the irrelevant-scrambled GPIIIa-albumin conjugate.
contain RF-IgG complexes as well as anti-HIV-1gp120-anti-idiotype complexes.

Anti-GPIIIa Ab and Anti-idiotype Ab

Serum from HSITP and NITP patients have little anti-platelet Ab as well as anti-GPIIIa49-66 Ab compared to purified serum IgG suggesting the presence of blocking Ab. Indeed no inverse correlation was noted between platelet associated IgG and platelet count. This appeared to be different from our findings in hemophiliac HIV-1 seropositive thrombocytopenic patients (HITP) as well as 15 classic ATP patients, in which serum anti-platelet Ab as well as F(ab)2 fragment binding was easily detectable. Indeed 6/6 HITP patients had elutable anti-platelet Ab from their platelets and an inverse relationship was noted between platelet count and platelet bount IgG, \( r = -0.8, p < 0.001, n = 28 \) (34). Similar results were obtained with classic ATP patients, similarly studied (34). To investigate this difference in detectable serum anti-GPIIIa49-66 Ab in HSITP and NITP patients, crude serum, purified serum IgG and PEG-IC IgG were isolated and studied for anti-GPIIIa49-66 reactivity. This revealed ~150 fold greater Ab reactivity in purified serum IgG and ~4000 fold greater reactivity in PEG-IC IgG (Fig. 5). This was explained by the presence of anti-idiotype Ab2 (both IgG and IgM) sequestered within the PEG-IC.

The IgM anti-idiotype was further studied since it is predominantly a blocking Ab, as demonstrated by specificity for F(ab')2, fragments of anti-GPIIIa49-66, and inhibition with peptide GPIIIa49-66, not with a control peptide. The IgM anti-idiotype Ab is not polyreactive. In a group of HIV-1-infected patients (non-thrombocytopenic as well as thrombocytopenic) a positive correlation was noted between platelet count and anti-idiotype IgM \( (r = 0.7, p = 0.0001, n = 22) \). The in vivo relevance of the IgM anti-idiotype Ab was tested by inducing thrombocytopenia (~30% baseline) in mice with anti-GPIIIa-(49-66). This revealed ~150 fold greater Ab reactivity in purified serum IgG and ~4000 fold greater reactivity in PEG-IC IgG (Fig. 5). This was explained by the presence of anti-idiotype Ab2 (both IgG and IgM) sequestered within the PEG-IC.

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These observations along with the presence of platelet reactive idiotype-anti-idiotype complexes (i.e., HIV-1gp120) as well as the presence of RF offer an explanation for the markedly elevated immune complex/Ig on platelets of HIV-1-ITP patients. They also offer an explanation for the presence of IgG on platelets of HIV-1-infected patients with normal platelet counts – namely the presence of sufficient anti-idiotype Ab to neutralize the thrombocytopenia.
A recent report that recognized the presence of platelet fragments within the serum PEG-IC’s stimulated a search for antibody-dependent platelet lysis. This was found to occur in vitro with gel-filtered platelets (Fig. 7 and 8) as well as in vivo in association with the induction of thrombocytopenia. The same results were noted with F(ab’)_2 fragments as well as intact IgG in complement-deficient mice, indicating the absence of complement-induced lysis. This new pathophysiologic mechanism of platelet destruction was shown to be induced via peroxide generation from a platelet-associated NADPH oxidase pathway. In vitro platelet lysis could be blocked by scavengers of reactive oxygen species (catalase, superoxide dismutase, diphenyleneniodonium) (Fig. 9) and platelet lysis did not occur in NADPH oxidase-deficient mice (p47phox-/-). Thrombocytopenia was significantly improved compared to its induction in wild-type mice by 60% (Fig. 10).

**Coagulation Abnormalities**

A number of coagulation abnormalities have been described in HIV disease including acquired deficiency states of the physiologic anticoagulants: protein C (91), protein S (92) and heparin cofactor II (93). Of these, protein S deficiency is the most consistently observed and is present in up to 73% of HIV-infected men (92, 94, 95). Most commonly there is reduction in free protein S and coordinate reduction in functional protein S activity. C4bBP levels are normal indicating that reduced protein S is not a consequence of a shift to the bound form, which is typically seen in inflammatory states. There is no correlation of free protein S deficiency with clinical thrombosis in HIV-infected men (96). Protein S is reduced in numerous other conditions including pregnancy, use of oral contraceptives, DIC, acute thrombosis and liver disease. The clinical significance of acquired protein S deficiency associated with HIV disease is unclear. High levels of plasma von Willebrand factor have been reported in HIV disease and might be indicative of activated endothelium (97).
Anticardiolipin antibodies (aCL) and lupus anticoagulants (LA) are frequent incidental laboratory findings in HIV-infected patients. The presence of LA is an established risk factor for both venous and arterial thrombosis in patients with SLE and is detected by a prolonged partial thromboplastin time (aPTT) that fails to correct with mixing, or alternatively by prolongation of the Kaolin clotting time, diluted tissue thromboplastin time or dilute Russell viper venom time. Elevated aCL are found in 20-70% of HIV patients (98) and are predominantly IgG (99). LA activity is prevalent in HIV-infected patients and consists mainly of IgM antibody (100, 101). Despite the high prevalence of aCL and LA in HIV-infected patients, the clinical manifestations of the classic antiphospholipid antibody syndrome (APS) such as stroke or TIA, recurrent venous thrombosis, cutaneous lesions and miscarriage or the manifestations of catastrophic APS are distinctly unusual. Only a single case report links APS to HIV infection (102). Anti-beta-2-glycoprotein I (β2GPI) and anti-prothrombin antibodies are seen less commonly in HIV infected patients than in APS/SLE or primary APS patients. The biological false positive test for syphilis is rarely detected in HIV-infected patients (103). This antiphospholipid antibody specificity might explain the lack of clinically evident thrombosis in the HIV-infected population. It is suggested that elevated aCL in HIV results from pan-B cell stimulation/hypergammaglobulinemia or alternatively could result from infection. The presence of aCL and lupus anticoagulant activity do not correlate with the clinical features of HIV including medication use, infections or malignancy or CD4 T-cell count nor with the development of thrombosis (96). LAs are not generally considered to pose a bleeding risk, however associated thrombocytopenia (104), platelet dysfunction or hypoprothrombinemia (105) may result in unexpected bleeding particularly after surgery and prophylactic transfusion of platelets and plasma may be appropriate in select cases.

**HIV and Thrombosis**

Venous thromboembolic disease (VTE) is uncommon in AIDS patients but has been described in a number of case reports and in retrospective reviews (91, 106–108). Thrombosis at unusual sites including...
retinal vein (109) and sagittal sinus (110) have been reported which is also a characteristic feature of thrombophilic states. The majority of VTE is associated with advanced HIV (CD4 count < 200), underlying AIDS-related malignancy or opportunistic infection, while idiopathic DVT is less common. It is not known whether the incidence of VTE differs in HIV patients as compared to the general population since this has not been studied in a prospective case control manner. Given the diagnostic challenge of VTE and the protean clinical manifestations of HIV disease itself, the incidence of DVT may be underestimated in this patient population. Further studies will be required to determine the thrombosis risk attributable to HIV and related co-morbidities, as well as the optimal strategies for DVT treatment and prophylaxis and the utility of thrombophilia screening.

HIV and Hemophilia

AIDS is the leading cause of death in hemophilia. There has not been documented HIV seroconversion since 1986 (111). ITP in HIV-infected hemophiliacs may further worsen the risk of bleeding. The treatment of hemophilia is complicated by the development of high titer factor VIII inhibitors, which may resolve with advanced HIV disease due to loss humoral immunity (112).

A number of reports have suggested that protease inhibitors have the potential to cause increased bleeding in HIV-infected hemophiliacs (113). Increase in frequency of bleeding episodes, unusual sites of bleeding and increased factor concentrate requirement have all been noted (114). Ritonavir has received particular attention in this regard, but all the currently available protease inhibitors are linked to increased bleeding. The mechanism of bleeding in the majority of cases was not established, although platelet dysfunction was identified as a cause of bleeding in two hemophiliacs (115). Mucosal bleeding has also been reported in non-hemophiliacs treated with protease inhibitors indicating that the effect is not related to factor VIII per se (114, 116).

Fig. 9  Generation of Peroxide and Effect of Peroxide Inhibitors, Catalase, Diphenyleneiodonium (DPI) and Superoxide Dismutase (SOD) with Platelet Particle Formation Induced by Anti-GPIIIa49-66 Ab or Thrombin. A. Gel filtered platelets were preincubated with inhibitor or buffer for 15 min prior to the addition of rabbit anti-GPIIIa49-66 Ab or control IgG. C refers to control non-immune IgG (similar results with control plus highest inhibitor concentration employed), IS refers to rabbit anti-GPIIIa49-66 immune serum. Bars labelled 2, 3, 4 after bar 1 refer to doubling concentrations of inhibitor (50 u/ml catalase, 5 nM/ml DPI and 12.5 u/ml SOD), n = 4, SEM is given. B. Generation of peroxide by anti-GPIIIa49-66 at 1 h (panels A, B, C) and 4 h (panels D, E, F). Platelets were loaded with the intracellular dye, DCFH-DA and then treated with buffer (panels A and D), control IgG (panels B and E) or human anti-GPIIIa49-66 (panels C and F). C. Effect of peroxide inhibitors on thrombin-induced platelet particle formation. Gel-filtered platelets were preincubated with inhibitors, as in AA and then treated with 1 u/ml thrombin for 4 h. Bars refer to doubling concentrations, n = 4.

Fig. 10  Effect of Anti-GPIIIa49-66 Ab on Platelet Count and Platelet Particle Formation in Control C57BL/6 and p47phox(-/-) Mice. Control C57BL/6 or p47phox(-/-) mice were injected i.p. with 25 ug of intact anti-GPIIIa49-66 or 16 ug of its F(ab’)2 fragment and platelet count and % platelet particles monitored at 2 and 4 h. A. Platelet count. B. % platelet particle. N = 6-8 for each group, SEM is given.
References


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