Antibody-Independent Thrombocytopenia in Lactate Dehydrogenase-Elevating Virus-Infected Mice

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Previously we demonstrated that antibody-mediated thrombocytopenia is strongly enhanced by lactate dehydrogenase-elevating virus (LDV) infection. Here we report that mice infected with LDV develop a moderate thrombocytopenia, even in the absence of immunoglobulins or Fc receptors. A similar decrease of platelet counts was observed after mouse hepatitis virus infection. LDV-induced type I interferon-independent thrombocytopenia was partly suppressed by treatment with clodronate-containing liposomes. Therefore, we conclude that the thrombocytopenia results from increased phagocytosis of nonopsonized platelets by macrophages.

In mice infected with lactate dehydrogenase-elevating virus (LDV), viral replication into a macrophage subpopulation leads to cytolyis of these target cells (11). However, LDV infection also induces a general activation of macrophages (6), resulting in cytokine production (1, 5), increased expression of receptors for opsonized antibodies (6), exacerbated susceptibility of infected mice to endotoxin shock (4), and aggravation of antirynthocyte and antiplatelet antibody pathogenicity (7, 9, 10). In a passive mouse model of antibody-mediated thrombocytopenia, enhancement of phagocytosis of opsonized platelets upon LDV infection leads to a clinical picture with multiple petechiae (9), strongly reminiscent of that observed in patients with virally triggered immune thrombocytopenic purpura (ITP).

Whereas LDV-infected mice that received antiplatelet antibodies developed severe thrombocytopenia, a moderate but significant drop in platelet counts was also observed in infected animals that were not treated with the antiplatelet antibodies. Whether this virally induced thrombocytopenia resulted from the presence in the normal mouse repertoire of immunoglobulins that might opsonize platelets was unknown. The purpose of this work was therefore to determine whether LDV infection could induce an antibody-independent thrombocytopenia and to analyze the mechanisms involved in such a platelet drop.

Female CBA/Ht mice, raised by G. Warnier at the Ludwig Institute for Cancer Research (Brussels, Belgium), were administered by the intraperitoneal route either normal saline (control animals) or approximately 2 × 10^7 50% infectious doses (ID_{50}) of LDV (Riley strain; ATCC, Rockville, MD) in saline. At different time points after treatment, platelets were counted by light microscopy with an improved Neubauer hemacytometer (Marenfeld, Germany) in blood collected from the retro-orbital plexus of anesthetized mice via Unopette pipettes and kits (Unopette micrcollection system; Becton, Dickinson, Franklin Lakes, NJ). As shown in Fig. 1A, LDV administration triggered thrombocytopenia within 1 day. This drop in platelet counts reached a maximum between 2 and 4 days after infection (very significant differences from control animals [P = 0.0002 and <0.0001 at days 2 and 4, respectively]). At 1 week after infection, this transient thrombocytopenia resolved completely. Whereas platelet levels in control animals might vary from one experiment to another, which could be the result of counting method, thrombocytopenia resulting from LDV infection was also consistently observed in C57/BL6 mice, although it was not as pronounced as in CBA animals (Fig. 1B [P = 0.0027]; see also Fig. 3, below). In DBA2 mice, a slight decrease of platelet levels did not reach significance (Fig. 1B [P = 0.1592]). Interestingly, genetic differences have been reported in thrombocytopenia induced by immunization of mice with rat platelets (11). Similarly, in this model CBA and 129/Sv mouse strains were relatively more sensitive than BALB/c or DBA2 animals, indicating the importance of the genetic background in immune-mediated thrombocytopenia.

In addition, we also tested the effect of infection by using another mouse nidovirus, mouse hepatitis virus (MHV). Inoculation of CBA/Ht mice with approximately 1 × 10^4 50% tissue culture infectious doses (TCID_{50}) of MHV A59 grown in NCTC 1469 cells was also followed by a marked thrombocytopenia at 2 and 4 days postinfection (Fig. 1C [P < 0.0001 for days 2 and 4 versus uninfected mice]). Although mice quickly died from infection, precluding measurement of platelet levels at later times, this early induction of thrombocytopenia suggested that the mechanisms are similar to those triggered by LDV.

LDV infection has been reported to enhance phagocytosis of antibody-opsonized erythrocytes and platelets (7, 9). To determine whether LDV-induced thrombocytopenia in animals that did not receive antiplatelet antibodies also resulted from increased platelet phagocytosis, CBA mice were treated with 200 μl clodronate-containing liposomes, prepared as described previously (9), prior to LDV inoculation. This treatment completely abrogated...
antibody-mediated platelet destruction in LDV-infected mice (9) and did not modify platelet counts in uninfected animals (2). As shown in Fig. 2A, infection alone again triggered a significant thrombocytopenia \( (P < 0.0001) \) that was not modified by the administration of control liposomes containing phosphate-buffered saline (PBS). In contrast, treatment with clodronate-containing liposomes significantly prevented the development of thrombocytopenia (significant difference between treatment with PBS-containing versus clodronate-containing liposomes, \( P = 0.0005 \)). However, this prevention was not complete, as platelet levels in LDV-infected mice treated with clodronate-containing liposomes were still lower than those in uninfected animals \( (P = 0.0003) \). It is difficult to determine whether this residual thrombocytopenia in treated mice resulted from an incomplete depletion of phagocytosing macrophages or from alternative mechanisms responsible for the decrease in platelet counts. Because type I interferons produced after LDV infection may modulate macrophage activity (5), thrombocytopenia was measured in alpha interferon (IFN-α) receptor knockouts, originally derived by M. Aguet on a 129/Sv background (8). Mice unable to respond to type I interferons developed significant thrombocytopenia \( (P = 0.0007) \) that was not statistically different from that of their normal counterparts \( (P = 0.2188) \) (Fig. 2B), suggesting that type I interferons are not involved.

Very early thrombocytopenia induction is unlikely to result from de novo production of antiplatelet antibodies triggered by viral infection. However, as LDV infection increases the phagocytosis of platelets opsonized with antibodies (10), it can be speculated that the thrombocytopenia observed after LDV inoculation of mice that did not receive antibody treatment corresponded to the destruction of platelets bound by natural antibodies present in the immune repertoire of normal animals. Therefore, we compared this decrease of platelet counts in animals deficient for immunoglobulins (IFN-α receptor knockouts), originally derived by M. Aguet on a 129/Sv background (8). Mice unable to respond to type I interferons developed significant thrombocytopenia \( (P = 0.0007) \) that was not statistically different from that of their normal counterparts \( (P = 0.2188) \) (Fig. 2B), suggesting that type I interferons are not involved.

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FIG 1 Spontaneous thrombocytopenia triggered by viral infection. (A) Platelet levels were determined at different times after treatment of groups of 5 CBA/Ht mice with NaCl (controls) or LDV. Results shown are means ± standard errors of the means (SEM). (B) Platelet levels were determined at 4 days after LDV infection of groups of 3 to 4 CBA/Ht, DBA2, and C57/BL6 mice. Results shown are means ± SEM. (C) Platelet levels were determined at different times postinfection of groups of 3 CBA/Ht mice with MHV. Results shown are means ± SEM.

FIG 2 Involvement of phagocytosis in the spontaneous thrombocytopenia triggered by LDV infection. (A) Platelet levels were determined 2 days after LDV infection of groups of 4 CBA/Ht mice. Liposome treatment was administered as indicated, 0.5 day before infection. Results shown are means ± standard errors of the means (SEM). (B) Platelet levels were determined 1 day before (control) and 2 days after LDV infection of groups of 3 129/Sv and IFNAR KO mice. Results shown are means ± SEM.

LDV-induced thrombocytopenia was also measured in animals deficient for either FcγRI (generated recently in the laboratory of J. S. Verbeek on a C57/BL6 background [unpublished data]) or for all FcγR, also on a C57/BL6 background. The Fcγ receptor (FcyR)-deficient mouse strain was generated by crossing a FcγRI KO strain (3) with a novel FcγRII, III, IV KO strain that was generated recently in the laboratory of J. S. Verbeek with a single recombination step that removed all three linked genes in
C57BL/6 embryonic stem cells (unpublished data). A significant thrombocytopenia developed after LDV infection in mice deficient for both Fcγ and Fcμ receptors (Fig. 3B and C; P = 0.0020, 0.0006, and 0.0002 for C57/BL6 mice, FcγRI, II, III, IV mice, and FcμR KO mice, respectively). Similar results were obtained with FcγRI and FcγRII, III, IV KO mice (data not shown).

We therefore postulate that macrophages from LDV-infected mice can destroy platelets in vivo independently of antibody opsonization. Whether the platelets are normal or are modified by physiological processes, such as aging, or by the infection itself remains to be determined.

Thus, nidovirus infection of immunocompetent mice leads to a moderate and transient thrombocytopenia that varies between animals with different genetic backgrounds. This thrombocytopenia is mediated largely by phagocytosis and develops independently from antibodies. This model of mouse infection may thus serve to analyze some aspects of human ITP, since this disease is mediated largely by phagocytosis and develops independently from antibodies. This thrombocytopenia developed after LDV infection in mice deficient for both Fcγ and Fcμ receptors (Fig. 3B and C; P = 0.0020, 0.0006, and 0.0002 for C57/BL6 mice, FcγRI, II, III, IV mice, and FcμR KO mice, respectively). Similar results were obtained with FcγRI and FcγRII, III, IV KO mice (data not shown).

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Thus, nidovirus infection of immunocompetent mice leads to a moderate and transient thrombocytopenia that varies between animals with different genetic backgrounds. This thrombocytopenia is mediated largely by phagocytosis and develops independently from antibodies. This model of mouse infection may thus serve to analyze some aspects of human ITP, since this disease frequently develops in children after infection with common viruses (12).

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