Brief report

Full reconstitution of human platelets in humanized mice after macrophage depletion

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Cotransplantation of human fetal thymic tissue and CD34$^+$ fetal liver cells in nonobese diabetic (NOD)/severe combined immunodeficiency (SCID) or NOD/SCID/$\gamma c^{-/-}$ mice results in the development of multilineage human hematopoietic cells. In this study, we show that these humanized mice had extremely low levels of human platelets. The presence of human megakaryocytes at a normal concentration in the bone marrow suggests that human megakaryocytic differentiation occurred efficiently in these mice. Rapid increase in human platelets in blood to levels comparable with those of human peripheral blood mononuclear cells (PBMCs) after macrophage depletion indicates that mouse macrophages are responsible for the poor human platelet reconstitution in humanized mice. In support of this possibility, human platelets were rapidly rejected after infusion into untreated mice, but persisted in macrophage-depleted mice. These findings indicate that inhibition or depletion of recipient mouse macrophages may provide a useful means for evaluating human thrombopoiesis and platelet function in vivo using immunodeficient mice. (*Blood*. 2012;120(8):1713-1716)

Introduction

Platelets are derived from megakaryocytes and play an essential role in normal hemostasis and thrombotic disorders.[1] Platelet transfusion is an effective means of preventing or treating bleeding, and an important supportive measure for patients undergoing transplantation or cytoreductive therapies.[2] To satisfy the excessive demand for platelets, considerable effort has been made to develop new approaches to platelet production.[3] Although immunodeficient mice have been widely used to study human lymphohematopoietic cell function in vivo,[4] there has been no report of achieving adequate human platelet reconstitution in these mice.[5-7]

We have shown that cotransplantation of human fetal thymic tissue and CD34$^+$ cells led to durable high levels of human hematopoietic reconstitution in nonobese diabetic (NOD)/severe combined immunodeficiency (SCID) or NOD/SCID/$\gamma c^{-/-}$ (NSG) mice.[8-10] In this study, we show that human platelets were extremely low in these humanized mice, and failed human platelet reconstitution was caused by macrophage-mediated xenorejection, but not by defect in human megakaryocyte differentiation or platelet biogenesis.

Methods

Humanized mice were made by cotransplantation of human fetal thymic tissue (under the renal capsule) and CD34$^+$ cells (intravenously), and human hematopoietic cell reconstitution was assessed by flow cytometry as previously described.[9,11] The presence of human megakaryocytes in bone marrow of humanized mice was determined by flow cytometry and immunohistochemistry. Macrophage depletion in mice was performed by intravenous injection of liposome-encapsulated clodronate.[11,12] In platelet clearance assay, freshly prepared human platelets were intravenously injected into immunodeficient mice, and surviving platelets were detected using anti–human CD42a by flow cytometry. Protocols involving the use of human tissues and animals were approved by the Human Research Committee and Subcommittee on Research Animal Care of Columbia University Medical Center. Detailed description of all materials and methods is provided in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Results and discussion

Poor human platelet reconstitution in humanized mice

Blood collected from humanized NSG and NOD/SCID mice between 9 and 18 weeks after receiving human CD34$^+$ cells or CD34$^+$ cells plus human thymic tissue was analyzed for human platelet reconstitution. Consistent with our previous results,[8-10] humanized mice had high levels of human CD45$^+$ cells in peripheral blood mononuclear cells (PBMCs; Figure 1A-B), including CD3$^+$ T cells and CD19$^+$ B cells (data not shown). The levels of human CD45$^+$ cell chimerism were significantly greater in NSG mice than in NOD/SCID mice, with an average of 83% and 45%, respectively ($P < .001$). However, the blood levels of human platelets in these mice were low, with an average of 1.6% and 1.2% for NSG and NOD/SCID mice, respectively (Figure 1A-B).

Presence of human megakaryocytes in humanized mice

Platelets are formed by megakaryocytes in the bone marrow and are subsequently released into the bloodstream. Megakaryocytes account for a very small fraction (less than 1%) of bone marrow cells.[13,14] To determine whether the poor human platelet reconstitution in humanized mice was caused by retarded human megakaryocyte differentiation, we assayed human megakaryocytes in bone marrow. Human CD45$^+$CD61$^+$ megakaryocytes were readily

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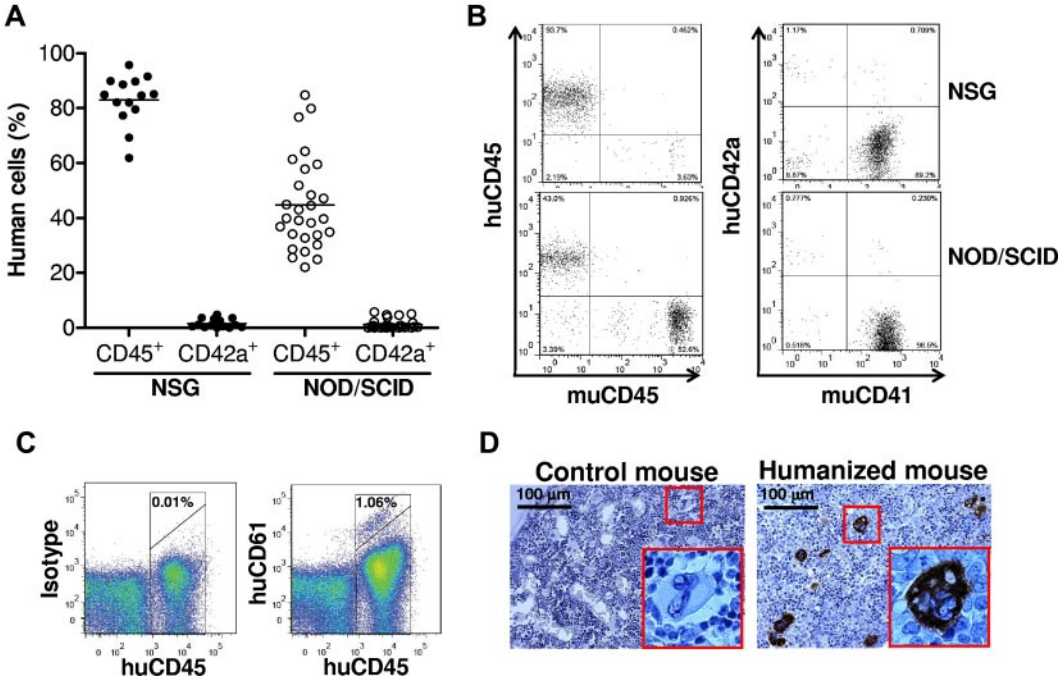

Figure 1. Human CD45$^+$ PBMC and platelet reconstitution in humanized mice. Sublethally irradiated NOD/SCID/$\gamma c^{-/-}$ (NSG; n = 14) and NOD/SCID (n = 28) mice were transplanted with human CD34$^+$ fetal liver cells alone or along with human fetal thymic tissue, and the levels of human CD45$^+$ PBMCs and platelets in blood were analyzed between 9 and 18 weeks after human cell/tissue transplantation. (A) Percentage of human CD45$^+$ cells in PBMCs and of human CD42a$^+$ platelets in peripheral blood. Each symbol represents an individual mouse. (B) Flow cytometry profiles of humanized mouse PBMCs stained with anti–human CD45 and anti–mouse CD45, and platelets stained with anti–human platelet marker CD42a and anti–mouse platelet marker CD41. (C) Representative flow cytometry profiles of bone marrow cells from humanized mice stained with anti–human CD45 and CD61 (right), or with isotype control monoclonal antibodies (left). (D) Immunohistologic staining of bone marrow sections with anti–human CD61. Shown are samples from representative control (left) and humanized (right) mice.

detectable in the bone marrow (ranging from 0.86% to 1.45%) in humanized mice (Figure 1C). Furthermore, immunohistologic staining of bone marrow tissue sections confirmed the presence of human CD61$^+$ morphologically mature megakaryocytes in humanized mice (Figure 1D). These data indicate that the mouse environment is capable of supporting efficient megakaryocyte differentiation, suggesting that the poor human platelet reconstitution may result from rapid clearance of human platelets in these mice. This is in accordance with a recent study[15] showing that immunodeficient knockin mice expressing human thrombopoietin, a major growth factor in megakaryocyte development,[16] have no advantage in human platelet reconstitution after CD34$^+$ cell transplantation.

Mouse macrophages mediate rapid rejection of human platelets in NOD/SCID mice

Macrophages are professional phagocytic cells that are essential to host innate defense against infection.[17] We have recently shown that macrophages mediate robust rejection of human red blood cells (RBCs), but not nucleated hematopoietic cells in NOD/SCID and NSG mice.[11] To determine whether human platelets are also susceptible to rejection by mouse macrophages, we compared the survival of human platelets in NOD/SCID mice with or without macrophage depletion. It has been shown that injection of a large number of human RBCs (as "decoy cells") may delay rejection of subsequently infused human RBCs, probably because of transient saturation of the recipient mouse phagocytic system.[18] Such transient saturation of the recipient phagocytic system would also occur in mice receiving large numbers of human platelets if human platelets are susceptible to phagocytosis by recipient macrophages. To determine an adequate number of human platelets to infuse, we performed a pilot experiment and found that human platelets were readily detectable in blood circulation in the first 2 days in NOD/SCID mice receiving 4×10^8 human platelets, but almost undetectable in those receiving 1.5×10^7 human platelets (data not shown). Based on these data, we decided to infuse 1.5×10^7 human platelets in the experiment assessing the effect of macrophage depletion on human platelet survival. Compared with the control mice treated with PBS-liposomes, in which human platelets were rapidly cleared and became almost undetectable by 10 minutes after infusion, human platelet survival was significantly prolonged in mice that were depleted of macrophages by clodronate-liposomes ($P < .001$; Figure 2A). These results indicate that human platelets are highly susceptible to rejection by recipient macrophages in NOD/SCID mice.

CD47 is a ligand of SIRPα, an immune inhibitory receptor on macrophages.[19,20] It has been demonstrated that CD47-SIRPα pathway prevents normal hematopoietic cells and platelets from phagocytosis by self-macrophages,[19,21,22] and that the lack of efficient cross-reaction between donor CD47 and recipient SIRPα causes rejection of xenogeneic hematopoietic cells by macrophages.[23,24] However, we found that xenogeneic human platelets were significantly more rapidly rejected than allogeneic CD47-deficient mouse platelets in NOD/SCID mice (supplemental Figure 1), implicating that the vigorous rejection of human platelets by mouse phagocytic cells in humanized mice can be mediated by mechanisms other than a failure of CD47-SIRPα interaction. This possibility is supported by previous studies showing that human CD47 is capable of interacting with NOD mouse SIRPα.[25]

Full restoration of human platelets in humanized mice after macrophage depletion

To determine whether the poor human platelet reconstitution in humanized mice was because of rejection by recipient macrophages, we compared the levels of human platelets versus human

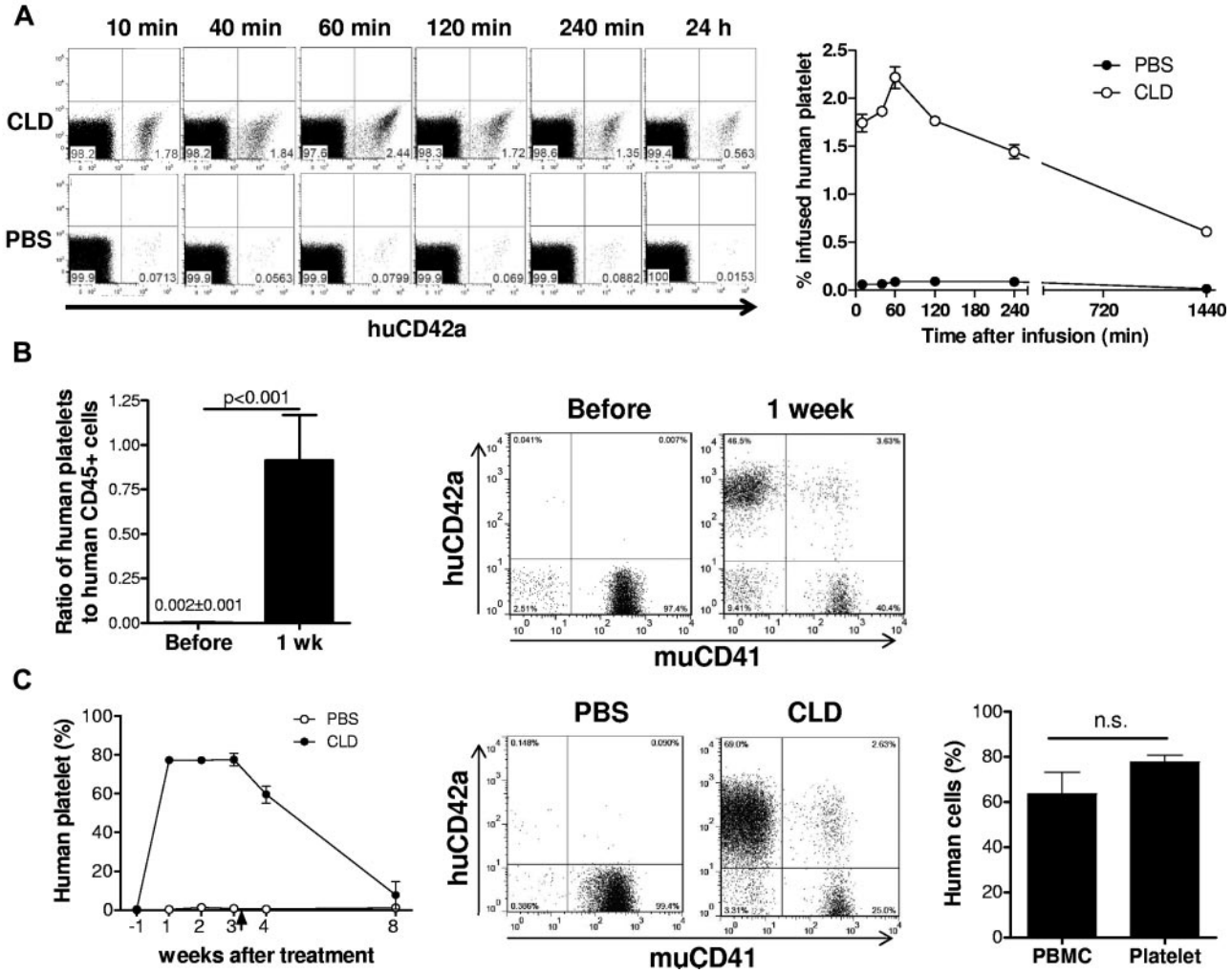

Figure 2. Full restoration of human platelets in humanized mice after mouse macrophage depletion. (A) Human platelets (1.5×10^7) were intravenously injected into macrophage-depleted (CLD; n = 3) or control (PBS; n = 3) NSG mice. Blood was collected at the indicated time points and the percentages of injected human platelets were analyzed by flow cytometry. Shown are representative flow cytometric profiles (left) and kinetic levels (mean ± SEM; right) of huCD42a$^+$ human platelets at the indicated time points after platelet transfusion. Macrophage depletion was performed by intravenous injection of clodronate-liposomes (CLD) at day 3 (100 μL) and day 1 (50 μL) with respect to human platelet injection (day 0); control mice were treated with PBS-liposomes at same volumes and schedule. (B) Blood was collected from 12-week humanized NOD/SCID mice (n = 5) before (Before) and 1 week after (1 week) injection of clodronate-liposomes, and the levels of human CD42a$^+$ platelet and human CD45$^+$ PBMC chimerism were determined by flow cytometry. Shown are ratios (mean ± SEM) of human CD42a$^+$ platelet chimerism to CD45$^+$ PBMC chimerism (left) and flow cytometry profiles showing human platelet chimerism (right) before and 1 week after treatment with CLD. (C) Thirteen-week humanized NOD/SCID mice were treated with CLD (n = 4) or PBS-liposomes (n = 3; 100 μL at days 0, 2, 7, 12, 17, and 22). Blood was collected 1 week before and at several time points after treatment, and the levels of human platelets and human PBMCs were determined by flow cytometry. Shown are levels (mean ± SEMs) of human platelet chimerism at the indicated times (left), representative flow cytometry profiles at week 3 after treatment (middle), and percentages (mean ± SEM) of human platelet versus CD45$^+$ PBMC chimerism at week 3 after treatment (right). NS indicates not significant.

CD45$^+$ cells in the blood of humanized NOD/SCID mice immediately before and 1 week after macrophage depletion with clodronate-liposomes. Consistent with our previous studies,[11] treatment with clodronate-liposomes did not significantly affect the levels of human CD45$^+$ cells in PBMCs (ranged from 28% to 80%) in these mice. Macrophage depletion, however, restored platelet reconstitution to levels comparable with those of human CD45$^+$ cells in blood within 1 week. The ratio of human platelets to CD45$^+$ cell chimerism increased approximately 500-fold from a pretreatment level of 0.002 to approximately 1 at week 1 after treatment (Figure 2B). We also followed the kinetics of human platelet chimerism in humanized NOD/SCID mice that received chronic treatment with clodronate-liposomes (or PBS-liposomes as controls) for 22 days. The levels of human platelet chimerism in the blood of clodronate-liposome–treated humanized mice increased rapidly from $< 1\%$ to approximately 80% within 1 week after starting the treatment regimen, remained stable during the period of macrophage depletion, and declined after withdrawal of the treatment (Figure 2C). However, treatment with PBS-liposomes did not affect human platelet reconstitution in humanized mice (Figure 2C). The levels of human platelet chimerism in clodronate-treated mice were similar to the levels of human CD45$^+$ cell chimerism during the course of treatment, indicating that macrophage depletion fully restored human platelet reconstitution (Figure 2C). These data indicate that the mouse hematopoietic microenvironment is capable of supporting human megakaryocyte maturation and platelet biogenesis, and that the poor human platelet reconstitution in humanized mice was mainly because of rejection by mouse macrophages.

This study demonstrates that poor human platelet reconstitution in human CD34$^+$ cell-grafted mice is mainly caused by macrophage-mediated rejection, but not by insufficient human thrombopoiesis as previously suggested. Full restoration of human platelets in

macrophage-depleted mice indicates that macrophage depletion may provide a means to study human megakaryocyte differentiation, thrombopoiesis, and platelet function in immunodeficient mice.

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Authorship

Contribution: Z.H. designed and performed experiments, analyzed data, and wrote the paper; and Y.-G.Y. conceived the research project, designed experiments, analyzed data, and wrote the paper.

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