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Adoptive transfer method to study platelet function in mouse models of disease

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Abstract

Platelets play an important role in many physiological and pathological situations. However, the molecular mechanisms by which platelets contribute to health and disease are often ill-defined. One of the limiting factors to these studies is a fast but reliable method to generate animals with platelet-specific signaling defects. We here review recent approaches to establish an adoptive platelet transfer model in mice.

Introduction

Studies in animal models of disease identified a central role for platelets in various physiological and pathological situations, including hemostasis and thrombosis, angiogenesis, vascular integrity, development, immunity, ischemia-reperfusion injury, arthritis, atherosclerotic lesion development, tumor metastasis, and sepsis [1,2]. While studies on the contribution of platelets to developmental processes depend on the availability of genetically modified mice, experimentally induced severe thrombocytopenia is the most frequently used model system to identify whether platelets are critical in a particular patho-physiological process. For example, studies in animals depleted of virtually all circulating platelets provided strong evidence for a critical contribution of these cells to angiogenesis [3,4], vascular integrity [5,6], or tumor metastasis [7,8]. Clarification of the molecular mechanisms by which platelets contribute to health and disease, however, is much more complicated as genetic deletion or chemical inhibition of platelet signaling molecules or vasoactive/ immune-modulatory mediators generally also affects cells of the innate and adaptive immune response as well as the vessel wall. Deletion of genes specifically in the megakaryocyte/platelet-lineage with the loxP/PF4-Cre system has been instrumental to

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overcome this limitation [9]. The use of the Cre-Lox system, however, is limited due to the costs and the time associated with the generation, breeding, and maintenance of these mice. Thus, an alternative, more efficient method to generate mice with platelet-specific signaling defects is required to uncover the molecular mechanisms by which platelets contribute to the above discussed patho-physiological situations.

Genetic, chemical, and antibody-based approaches to induce thrombocytopenia

For many years, scientists have tried to generate mice with very low platelet counts that could be used for adoptive transfer studies with genetically modified or inhibitor-treated platelets (Table 1). Genetic approaches have led to the generation of mice with very low platelet counts. For example, peripheral platelet counts in mice lacking the thrombopoietin receptor c-Mpl are reduced by ~90% compared to controls due to a defect in megakaryocytopoieses [10]. However, the remaining platelets are fully functional and genetic deletion of c-Mpl also affects other hematopoietic progenitor cells. Genetic deficiency in the transcription factor p47 NF-E2 [11,12] strongly impairs thrombopoiesis in mice. The resulting severe thrombocytopenia (mice are virtually free of circulating platelets) leads to perinatal lethality due to excessive hemorrhage. In addition, p47 NF-E2 knockout mice show several red blood cell defects, including anisocytosis and hypochromia. Thus, genetic models of thrombocytopenia are of limited use for adoptive transfer studies. Thrombocytopenia in mice can also be induced by chemotherapeutic agents such as 1,4-butanediol dimethanesulfonate (Busulfan) [13] or Abt-737, a small molecule inhibitor that targets pro-survival Bcl-2 proteins [14,15]. The cytotoxic effects of both compounds, however, are not limited to the megakaryocyte/platelet lineage. Busulfan-treated mice also show marked leukopenia and thus should not be used for studying inflammation in mice. Abt-737 is less cytotoxic to leukocytes, likely due to the fact that these cells express another pro-survival relative, myeloid cell leukemia-1 (Mcl-1), which is insensitive to Abt-737 [16]. While busulfan affects megakaryocyte maturation and platelet generation, Abt-737 causes apoptosis and clearance of circulating platelets and therefore does not allow for the adoptive transfer of donor platelets. Cytotoxic antibodies directed towards platelet-specific antigens do not affect peripheral erythrocyte or leukocyte counts [13,17] and may therefore be considered the only method to completely eliminate circulating platelets without affecting other blood cell populations. However, there are two major problems associated with this method. First, rapid antibody-induced clearance of virtually all circulating platelets can lead to anaphylaxis-like reactions and severe vascular damage in mice [18–20]. These complications are well-documented for antibodies to αIIββ3, the main integrin receptor expressed on platelets. In contrast, antibody targeting of the GPIbα subunit of the von Willebrand receptor complex leads to virtually complete thrombocytopenia without vascular damage in mice. Detailed mechanistic studies demonstrated that anti-GPIbα antibodies induce thrombocytopenia by a unique mechanism that is independent of Fc receptor-mediated clearance of platelets by the reticuloendothelial system. The second major drawback of this method is the fact that thrombocytopenia depends on circulating cytotoxic antibodies. Consequently, transfusion of donor platelets into these thrombocytopenic mice is not possible as long as the antibodies remain in circulation. Thus, successful adoptive

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transfer of platelets requires a method where (1) thrombocytopenia is induced by an anti-GPIbα antibody-like mechanism and (2) circulating antibodies are not cytotoxic towards the transfused platelets.

A novel, antibody-based method for the adoptive transfer of platelets

Both requirements can be met when thrombocytopenia is induced by antibodies against human IL4Rα in transgenic mice expressing a chimeric hIL4Rα/GPIbα protein instead of GPIbα on the platelet surface [17]. In these mice, the extracellular domain of GPIbα is replaced by the extracellular domain of hIL4R [21]. Infusion of anti-hIL4R antibodies into these animals leads to rapid, severe thrombocytopenia without signs of an anaphylaxis-like response, suggesting that these antibodies induce platelet clearance by a mechanism similar to that described for anti-GPIbα antibodies. Importantly, transfusion of WT platelets lead to a dose-dependent increase of the peripheral platelet count in thrombocytopenic hIL4Rα/GPIbα mice, and the transfused platelets showed normal hemostatic function when tested in various models of thrombosis and inflammation [17]. To validate the power of this approach, platelets with defects in immunoreceptor tyrosine-based activation motif (ITAM) or G protein-coupled receptor (GPCR) signaling were tested for their ability to maintain vascular integrity in inflammation. To eliminate signaling via the main GPCR receptors, mouse platelets were isolated from *Par4-/-* mice treated with clopidogrel (an irreversible inhibitor of P2Y12), followed by incubation with aspirin to irreversibly prevent cyclooxygenase-mediated thromboxane A2 generation and platelet activation by thromboxane receptors. Before transfusion into thrombocytopenic hIL4Rα/GPIbα-tg mice, these cells were washed to remove free inhibitor. It is important to remember that aspirin is an anti-inflammatory drug and expression of both Par4 and P2Y12 is not specific to platelets. Thus, the adoptive transfer model provided a unique and powerful tool to generate mice with platelet-specific defects in signaling by multiple GPCR receptors. A similar approach was used to eliminate function in both ITAM receptors expressed on the platelet surface. Platelets isolated from mice deficient in C-type lectin-2 were treated with inhibitors to GPVI, washed and infused into a different set of thrombocytopenic hIL4Rα/GPIbα-tg mice [22]. Using this approach, we were able to show that platelet ITAM but not GPCR signaling is critical for vascular integrity at sites of inflammation [17].

Conclusions

There are several key advantages of this novel adoptive platelet transfer method over previously used approaches to inhibit specific platelet functions. First and foremost, it allows for the very rapid generation of mice with platelet-specific signaling defects, as induction of virtually complete thrombocytopenia and platelet transfusion into hIL4Rα/GPIbα-tg mice can be achieved within a few hours. In comparison, the generation of platelet-specific knockout mice requires many months. Second, the adoptive transfer system facilitates the combined use of genetic and pharmacologic approaches to loss of function studies. It is important to point out, however, that pharmacologic inhibition only works for compounds that irreversibly inhibit a specific signaling molecule, as reversible inhibitors would be washed out. Third, adoptive transfer allows us to set the peripheral platelet count in mice before challenge, thereby permitting a greater sensitivity for platelet defects. This is

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particularly important when few circulating platelets are sufficient to prevent a phenotype, such as shown for vascular integrity at sites of inflammation [5]. Without lowering the peripheral platelet count, it seems unlikely that a partial reduction in a critical platelet function would lead to a detectable phenotype. Lastly, it is well-documented that genetic deletion of genes can lead to the upregulation of compensatory mechanisms in mice [23]. In addition, as outlined above, even platelet-specific deletion of genes can lead to marked vascular changes as these cells control angiogenesis and vascular integrity during development [24,25]. The rapid replacement of endogenous platelets with donor platelets, achieved by adoptive transfer, eliminates both of these limitations. In summary, this novel method of adoptive platelet transfer provides a powerful means for deciphering the unique ways by which platelets contribute to health and disease.

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Table 1

Common approaches to induce thrombocytopenia in mice

While a genetic approach leads to a very defined reduction in the peripheral platelet count in mice, thrombocytopenia induced by chemicals or antibodies varies with the amount of these compounds that are given to the animal (indicated by “<”). Note that antibodies to hIL4Rα only induce thrombocytopenia in transgenic mice expressing a chimeric hIL4Rα/GPIbα protein instead of GPIbα [17].

APPROACH	Genetic		Chemical		Antibody (directed against)			
	p47 NFE2$^{-/-}$	**cMpl$^{-/-}$**	**Busulfan**	**ABT-737**	**sera**	**αIIbβ3**	**GPIbα**	***hIL4Rα***
thrombocytopenia	100%	~90%	<90%	<100%	<100%	<100%	<100%	***<100%***
platelet-specific	no	no	dose-dependent	no	yes	yes	yes	***yes***
adverse side effects	bleeding	no	no	no	no	shock hemorrhage	no	***no***
amendable to adoptive transfer	yes	yes	yes	no	no	no	no	***yes***