Mouse Models for Immune-Mediated Platelet Destruction or Immune Thrombocytopenia (ITP)

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Immune thrombocytopenia (ITP) is a debilitating, life-threatening autoimmune disorder affecting more than 4 in every 100,000 adults annually, stemming from the production of antiplatelet antibody resulting in accelerated platelet destruction and thrombocytopenia. Numerous animal models of ITP have been developed that contributed to the basic understanding of the underlying mechanisms of ITP onset, progression, and maintenance. Rodent models that develop ITP spontaneously, or by passive transfer of an antiplatelet sera or antibody, play an instrumental role in the investigation of ITP mechanisms responsible for the breakdown of tolerance in human ITP; in studies of the immunopathology underlying the progression of platelet destruction, and in elucidation of the mechanisms of therapeutic amelioration of ITP by existing and new therapeutic modalities. This unit captures the protocols for the implementation and readout of passive antibody transfer mouse models of ITP, established by the infusion of a commercially-available monoclonal rat anti-mouse CD41 platelet antibody.

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INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by a low platelet count in the absence of bone marrow-related or other abnormalities (Robson, 1949; Lo and Deane, 2014). ITP is a clotting disorder with patients suffering from an increased tendency to bleed. This can affect the skin, which results in increased tendency to bruise in the form of purpura (hence the earlier term for ITP: idiopathic thrombocytopenic purpura). When the platelet count drops substantially lower, internal bleeding from a variety of mucous membranes can result, and the ensuing ITP can be severe and fatal (Lilleyman, 1997). Both acute and chronic forms of ITP are observed. Acute ITP generally affects children and often resolves spontaneously in several weeks. Chronic ITP, however, mainly affects adults and can last for 6 months or longer. The annual incidence of chronic ITP is estimated at 2-5 adults per 100,000 persons (Schoonen et al., 2009).
The immunopathology of ITP is caused by the breakdown of tolerance and production of antiplatelet autoantibodies that accelerate platelet destruction. The underlying cause of this breakdown is not entirely clear, but most studies in chronic ITP patients highlight roles for the B and T cell compartments, antigen presenting cells, and dysfunction of the regulatory T cell compartment (Trent et al., 1981; Liu et al., 2013; McKenzie et al., 2013; Talaat et al., 2014). The autoimmune antibodies generated, primarily of IgG isotype, are often readily detectable and exhibit immunoreactivity against one or more different platelet glycoproteins (Harrington et al., 1951; Karpatkin et al., 1972; McMillan, 2000b; McFarland, 2002). These include the collagen receptor (glycoproteins Ia/IIa or CD49b/CD29; He et al., 1994), von Willebrand factor and thrombin receptor (glycoproteins Ib/IX or CD42a-d; Kiefel et al., 1991), and fibrinogen receptor (glycoproteins IIb/IIIa or CD41/CD61; Hou et al., 1997). In most cases, platelet autoantigens are either CD41 or CD42 complexes (Kiefel et al., 1991; Hou et al., 1997). Antibody-opsonized platelets passing through either the spleen or liver are recognized—due to the Fc region on the platelet-attached antibody—by specific Fc receptors on mononuclear phagocytes. Subsequently, the platelets are phagocytosed primarily by splenic macrophages and Kupffer macrophages in the liver (Steck, 1969; George and Raskob, 1998; McFarland, 2002). ITP autoantibodies also bind megakaryocytes and interfere with platelet production by a direct effect on their proliferation and maturation and can also induce apoptosis (McMillan et al., 1978; McMillan, 2000a; Chang et al., 2003; McMillan et al., 2004; Yang et al., 2010; Perdomo et al., 2013). The combined destruction of platelets and their reduced production lead to low systemic platelet counts and ITP.

Corticosteroids and often intravenous immunoglobulin (IVIg) are standard first-line treatments for acute and chronic ITP and are used to quickly restore platelet counts with clinical response rates of up to 70% (Godeau et al., 1993; Zimmer et al., 2004). Response rates increase to 80% to 90% with thrombopoietin (TPO) mimetics that increase new platelet production (Kuter, 2009). For relapsing patients, splenectomy is the only treatment option with the potential to completely resolve chronic ITP (Palandri et al., 2014), but a substantial proportion of patients still relapse following splenectomy (Mikhael et al., 2009). A number of approaches are thus under investigation in the search for additional treatment options for chronic ITP. Cyclosporine, anti-Rh(D) antibodies, anti-CD40L, Fc-blocking monoclonal antibodies, rituximab (anti-CD20 antibody targeting B cells), alemtuzumab (anti-CD52 targeting T cells), and other approaches have been evaluated (Killick et al., 1997; Willis et al., 2001; Nomura et al., 2003; Kuwana et al., 2004; Suvajdzic et al., 2006; Godeau et al., 2008; Patel et al., 2008; Pels, 2011; Franchini et al., 2012; Patel et al., 2012). A spleen tyrosine kinase (SYK) inhibitor, fostamatinib, is currently being evaluated in a Phase 3 trial for use in chronic ITP (ClinicalTrials.gov Identifier: NCT02076412).

Animal models of ITP, primarily mouse models, have contributed substantially to the understanding of acute and chronic ITP and remain to be heavily utilized in research aimed at elucidating the mechanisms of onset and at evaluating novel therapeutic modalities. Neschadim and Branch (2015) provide a detailed overview of the various ITP models developed, their ability to mimic the human condition, and utility in ITP research. Passive antibody transfer models of ITP have been used extensively by our laboratory and others to understand the mechanisms of action of IVIg. IVIg works well in mouse models and is usually administered to mice at the therapeutic dose used in humans (1 g/kg or 2 g/kg; Godeau et al., 1993) without any titration of its activity to account for use in different species. Herein, we provide detailed protocols for ITP mouse models based on the passive transfer of antiplatelet antibodies that are utilized in our laboratory in the search of alternative treatment options for ITP.
From among several passive antibody transfer ITP models developed, we and most other laboratories utilize the rat anti-mouse CD41-based (anti-glycoprotein Iib, clone MWReg30, rat IgG1:λ antibody) model, which is based on a self-antigen that is highly relevant to the human disorder. The infusion of the anti-CD41 antibody leads to a rapid-onset ITP with clear involvement of the phagocytic monocytes in platelet destruction. The passive antibody transfer-induced ITP model also offers a degree of tunability, allowing for the severity and persistence of the ITP to be controlled by adjusting the dosage and frequency of the antiplatelet antibody administered. Repeat administration of the anti-CD41 antibody, in particular with a dose-escalation regimen, maintains a prolonged thrombocytopenia that closely resembles chronic human ITP (Katsman et al., 2010). Without a dose-escalation regimen, a recovery of platelets is often observed, mediated by increased platelet production as a compensatory bone marrow response to the induced thrombocytopenia (Katsman et al., 2010). The antibody dose-escalation model, developed in our laboratory, results in a prolonged platelet nadir, likely by increasing the rate of platelet destruction and by also affecting the megakaryocyte compartment and compensatory platelet production (Katsman et al., 2010). We use both the dose-escalation (Basic Protocol) and constant-dose (Alternate Protocol) models to facilitate drug development efforts on small molecule-based therapeutics as replacement for IVIg in ITP (Purohit et al., 2013, 2014).

### BASIC PROTOCOL

#### ESCALATING-DOSE ANTI-MOUSE CD41 (ANTI-GLYCOPROTEIN IIB) PASSIVE TRANSFER ITP MODEL

The rat anti-mouse CD41 dose-escalation passive antibody transfer mouse model is a highly stringent animal model of ITP that closely mimics the human disorder. Platelet numbers, measured daily, quickly reach a prolonged nadir and remain low throughout the time course of the model. This passive ITP model recapitulates the destruction of platelets by phagocytic monocytes and negative effects on megakaryopoiesis, thus affecting both the circulating platelets and the production of new platelets. The model is based on a rat anti-mouse CD41 antibody (anti-glycoprotein IIb, clone MWReg30, rat IgG1:λ) that is administered daily to each mouse at a dose that is escalated gradually to achieve sustained platelet destruction throughout the time course of the model. Each day, a small sample of blood is collected from each mouse and preserved, and platelets are quantified with the use of a flow cytometer operated in a kinetic, counting mode. The absolute number of platelets for each animal is calculated from the measurements by an empirical standardization using platelet enumeration with a standard hematology analyzer. Alternatively, a hematology analyzer can be used instead of a flow cytometer. The treatment can be administered to select groups of animals either prophylactically (e.g., Day 0) or when the platelet nadir is established (e.g., Day 2) to evaluate therapeutic effects. A single untreated group of animals is typically used to establish the platelet baseline. IVIg-treated animals can be used as a positive control group.

### Materials

- Female inbred Balb/c or C57BL/6 mice, 6 to 8 weeks of age (e.g., Charles River Laboratories, Jackson Laboratories)
- Rat monoclonal anti-mouse glycoprotein IIb antibody, unconjugated and fluorescein isothiocyanate (FITC)-conjugated (CD41; clone MWReg30, rat IgG1, k; e.g., BD Biosciences)
- PBS, pH 7.2 (with calcium and magnesium; see APPENDIX 2A)
- Treatment solutions (optional)
  - 10% citrate-phosphate-dextrose-adenine (CPDA) in PBS (see recipe)
  - Vaseline or glycerin
  - 10% or 100 mg/ml human IVIg (Gammagard S/D; e.g., Baxter Healthcare)
1.5-ml microcentrifuge tubes
50-ml conical polypropylene tubes with sawed-off tip (for use as conical tube animal restrainer)
25-G (5/8 in.) and 27-G (1/2 in.) needles with syringes
P20 (0.5 to 20 μl) pipet tips
Gauze
5-ml round-bottom tubes
Flow cytometer (e.g., BD Biosciences, FACSCalibur)
Hematology analyzer (e.g., Beckman Coulter, cat. no. LH750)

1. Utilize Balb/c or C57BL/6 mice for this experiment and assign into groups of 3 to 5 mice per group or a size needed to achieve the necessary statistical power depending on the design of the experiment. Include an anti-CD41-untreated group as a negative control to establish the baseline level of platelets daily. Include a positive control group treated with IVIg on Day 2. Keep mice under a natural light/dark cycle, at 22 ± 4°C, and feed with standard diet and water ad libitum.

*Mice of strains other than Balb/c and C57BL/6 can be used with this protocol (Katsman et al., 2010; Leontyev et al., 2012).*

*Mice should be allowed to acclimate following transfer. Do not use animals that are old.*

2. Prepare the anti-CD41 antibody solution for daily injection by steriley diluting the unconjugated antibody stock in PBS to the necessary final concentration.

   a. For Days 0 and 1, make up the antibody at a final concentration of 7 μg/ml (1.4 μg per 200 μl, or a 68 μg/kg dose).
   b. For subsequent days, increase the dose by 34 μg/kg each day; for Day 2, use final concentration of 10.5 μg/ml (2.1 μg per 200 μl, or a 102 μg/kg dose).
   c. For Day 3, use final concentration of 14 μg/ml (2.8 μg per 200 μl, or a 136 μg/kg dose).
   d. For Day 4, use final concentration of 17.5 μg/ml (3.5 μg per 200 μl, or a 170 μg/kg dose).
   e. For Day 5, use final concentration of 21 μg/ml (4.2 μg per 200 μl, or a 204 μg/kg dose).

   *The anti-CD41 solutions for injection should be freshly made from the concentrated antibody stock each day.*

3. Prepare any treatment solutions for injection as necessary on the day(s) of their injection, based on the design of the experiment.

4. On each day, prefill 1.5-ml microcentrifuge tubes (1 per mouse) with 1 ml of 10% CPDA in PBS.

5. Collect 10 μl of blood daily by saphenous vein bleeding from each mouse.

   a. Use the conical animal restrainer by allowing the mouse to enter it and then capturing and restraining one of its rear legs by gently pinching its upper thigh and stretching it out.

   *The conical animal restrainer can be made from 50-ml conical polypropylene tubes by sawing off the tip of the tube to allow for ventilation.*

   b. Swab the skin with small amount of Vaseline or glycerin to flatten and remove hair, find the vein, and then use a 25-G needle to gently puncture the vein.
   c. Collect blood using a P20 pipet tip immediately and directly into a 1.5-ml microcentrifuge tube prefilled with 10% CPDA in PBS.
**d. Apply a gauze and gentle pressure for as long as needed for the wound to clot.**

*Blood collection must happen quickly to avoid any clotting from happening. Blood must be free-flowing when collected. Do not collect already clotted blood.*

*Using alternate legs for blood collection each day minimizes scarring and facilitates better healing of the punctured area. However, switching hands is necessary to accomplish this.*

*As ITP develops, clotting will take a progressively longer time to achieve. Ensure hemostasis is achieved (the bleeding has completely stopped) before returning the animal back into its housing unit.*

6. Administer daily injection of anti-CD41 by i.p. injection of 200 μl of the diluted antibody solution to each mouse (except the negative, untreated control mice) following the completion of blood collections (using 27-G needles).

7. Administer any treatment 2 hr following the blood collections and the anti-CD41 injections (using 27-G needles).

8. Administer IVIg by i.p. injection as a positive control, if desired, on Day 0 (for testing prophylaxis effect), 2 hr before or following the blood collections and anti-CD41 injections. For testing amelioration of ITP, administer IVIg or other potential treatment under study on Day 2 when platelet nadir is reached (or at another suitable time point), 2 hr following the blood collections and anti-CD41 injections. Sterilely inject 200 μl or 400 μl IVIg solution per mouse for a dose of 1 or 2 g/kg, respectively (using 27-G needles).

*Alternatively, purified human IgG (Sigma-Aldrich) can be used.*

9. To analyze collected blood, mix the collected blood samples in the 1.5-ml microcentrifuge tubes, and dilute 10-fold by transferring out 50 μl into a 5-ml round-bottom tube containing 0.45 ml CPDA in PBS for flow cytometry.

*Final blood dilution of 1000-fold is achieved.*

10. Set up the flow cytometer prior to the blood sample analyses for platelet enumeration.

*The correct gate can be established by staining platelets from a mouse blood sample with the anti-CD41-FITC-conjugated antibody and analyzing it on a calibrated flow cytometer. The gate on the forward scatter and side scatter dot plot can be preset by backgating on the fluorescent FITC-positive platelets.*

11. Analyze each sample on a calibrated flow cytometer by acquiring the diluted blood sample and measuring the number of platelets passing through the preset forward scatter/side scatter gate over a period of 2 min. Record platelet counts, and convert into absolute counts by using an empirical conversion factor.

*Absolute platelet counts per liter can be established by using an empirical conversion factor, which can be established by comparing the flow cytometer-enumerated counts of a single sample with the same sample analyzed by a hematology analyzer, such as a Beckman Coulter LH750.*

*Alternatively, a hematology analyzer can be used in place of flow cytometry to enumerate platelets. The typical conversion, which has to be determined for each particular flow cytometer and flow rate, is in the range of 0.02–0.04 × 10^9.*

12. Continue the treatment and analysis cycle for as many days as desired.

*A typical ITP model time course is 5 to 7 days (from Day 0 to Day 4 through 6).*

*Figure 15.30.1 depicts sample ITP model results obtained with this protocol.*
Figure 15.30.1 Dose-escalation anti-CD41 passive antibody transfer model of mouse immune thrombocytopenia (ITP). Balb/c animals were either left untreated (baseline platelet measurement), treated with a daily escalating-dose of the anti-CD41 antibody only, or treated with the anti-CD41 antibody and IVIg on Day 0 (prophylactic or prevention model) or Day 2 (treatment or ITP model).

CONSTANT-DOSE ANTI-MOUSE CD41 (ANTI-GLYCOPROTEIN IIb) PASSIVE TRANSFER ITP MODEL

The rat anti-mouse CD41 constant-dose passive antibody transfer mouse model can be used as an alternative to the more stringent dose-escalation animal model of ITP (see Basic Protocol). Platelet numbers, measured daily, still quickly reach a nadir but recovery of platelet levels, albeit incomplete, is observed starting around Day 3 or Day 4 (Katsman et al., 2010). This is owing to the compensatory production of new platelets by megakaryopoiesis. The model is based on the same rat anti-mouse CD41 antibody (anti-glycoprotein IIb, clone MWReg30, rat IgG1:λ) that is administered daily to each mouse at a dose that is kept constant to achieve sustained platelet destruction throughout the time course of the model. Each day, a small sample of blood is collected from each mouse and preserved, and platelets are quantified with the use of a flow cytometer operated in a kinetic, counting mode. The absolute number of platelets for each animal is calculated from the measurements by an empirical standardization. The treatment can be administered to select groups of animals either prophylactically (e.g., Day 0) or when the platelet nadir is established (e.g., Day 2) to evaluate therapeutic effects. A single untreated group of animals is typically used to establish the platelet baseline. IVIg-treated animals can be used as a positive control group. This reduced-stringency model is particularly useful for a preliminary evaluation of therapeutic test articles, which can be administered prophylactically (on Day 0); but, this model is not as useful in the evaluation of chronic ITP due to the compensatory production of platelets that occurs (Katsman et al., 2010). To avoid interference by spontaneous recovery of platelets around Day 3/4 in this model, treatment experiments using IVIg or other treatment modalities are best administered on Day 1, when platelet numbers have substantially decreased but have not quite reached nadir.
Materials

Female inbred Balb/c or C57BL/6 mice, 6 to 8 weeks of age (e.g., Charles River Laboratories, Jackson Laboratories)
Rat monoclonal anti-mouse glycoprotein IIb antibody, unconjugated and fluorescein isothiocyanate (FITC)-conjugated (CD41; clone MWReg30, rat IgG1, k; e.g., BD Biosciences)
PBS, pH 7.2 (with calcium and magnesium, see APPENDIX 2A)
Treatment solutions (optional)
10% citrate-phosphate-dextrose-adenine (CPDA) in PBS (see recipe)
Vaseline or glycerin
10% or 100 mg/ml human IVIg (Gammagard S/D; e.g., Baxter Healthcare)

1.5-ml microcentrifuge tubes
50-ml conical polypropylene tubes with sawed-off tip (for use as conical tube animal restrainer)
25-G (5/8 in.) and 27-G (1/2 in.) needles with syringes
P20 (0.5 to 20 μl) pipet tips
Gauze
5-ml round-bottom tubes
Flow cytometer (e.g., BD Biosciences, FACSCalibur)
Hematology analyzer (e.g., Beckman Coulter, cat. no. LH750)

1. Utilize Balb/c or C57BL/6 animals for this experiment and assign into groups of 3 to 5 mice per group or a size needed to achieve the necessary statistical power depending on the design of the experiment. Include an anti-CD41-untreated group as a negative control to establish the baseline level of platelets daily. Include a positive control group treated with IVIg on Day 2. Keep mice under a natural light/dark cycle, at 22 ± 4°C, and feed with standard diet and water ad libitum.

Mice of strains other than Balb/c and C57BL/6 can be used with this protocol (Katsman et al., 2010; Leontyev et al., 2012).

Mice should be allowed to acclimate following transfer. Do not use animals that are old.

2. Prepare the anti-CD41 antibody solution for daily injection by sterilely diluting the unconjugated antibody stock in PBS to the necessary final concentration. For Days 0 through 5, make up the antibody at a final concentration of 7 μg/ml (1.4 μg per 200 μl or a 68 μg/kg dose).

The anti-CD41 solutions for injection should be freshly made from the concentrated antibody stock each day.

3. Prepare any treatment solutions for injection as necessary on the day(s) of their injection based on the design of the experiment.

4. On each day, prefill 1.5-ml microcentrifuge tubes (1 per mouse) with 1 ml of 10% CPDA in PBS.

5. Collect 10 μl of blood daily by saphenous vein bleeding from each mouse.

a. Use the conical animal restrainer by allowing the mouse to enter it and then capturing and restraining one of its rear legs by gently pinching its upper thigh and stretching it out.

The conical animal restrainer can be made from 50-ml conical polypropylene tubes by sawing off the tip of the tube to allow for ventilation.
b. Swab the skin with a small amount of Vaseline or glycerin to flatten and remove hair, find the vein, and then use a 25-G needle to gently puncture the vein.

c. Collect blood using a 200-μl P20 pipet tip immediately and directly into a 1.5-ml microcentrifuge tube prefilled with 10% CPDA in PBS.

d. Apply a gauze and gentle pressure for as long as needed for the wound to clot.

*Blood collection must happen quickly to avoid any clotting from happening. Blood must be free-flowing when collected. Do not collect already clotted blood.*

*Using alternate legs for blood collection each day minimizes scarring and facilitates better healing of the punctured area. However, switching hands is necessary to accomplish this.*

*As ITP develops, clotting will take a progressively longer time to achieve. Ensure hemostasis is achieved (the bleeding has completely stopped) before returning the animal back into its housing unit.*

6. Administer daily injection of anti-CD41 by i.p. injection of 200 μl of the diluted antibody solution to each mouse (except the negative, untreated control mice) following the completion of blood collections (using 27-G needles).

7. Administer any treatment 2 hr following the blood collections and anti-CD41 injections (using 27-G needles).

8. Administer IVIg by i.p. injection as a positive control, if desired, on Day 0 (for testing prophylaxis effect), 2 hr before or following the blood collections and anti-CD41 injections. For testing amelioration of ITP, administer IVIg or other potential treatment under study on Day 1 when platelet counts are dropping but have not yet reached nadir, 2 hr following the blood collections and anti-CD41 injections, to avoid interference of the interpretation of the results by spontaneous recovery of platelets. Sterilely inject 200 μl or 400 μl IVIg solution per mouse for a dose of 1 or 2 g/kg, respectively (using 27-G needles).

*Alternatively, purified human IgG (Sigma-Aldrich) can be used.*

9. To analyze collected blood mix the collected blood samples in the 1.5-ml microcentrifuge tubes and dilute 10-fold by transferring out 50 μl into a 5-ml round-bottom tube containing 0.45 ml CPDA in PBS for flow cytometry.

*Final blood dilution of 1000-fold is achieved.*

10. Set up the flow cytometer prior to the blood sample analyses for platelet enumeration.

*The correct gate can be established by staining platelets from a mouse blood sample with the anti-CD41-FITC-conjugated antibody and analyzing it on a calibrated flow cytometer. The gate on the forward scatter and side scatter dot plot can be preset by backgating on the fluorescent FITC-positive platelets.*

11. Analyze each sample on a calibrated flow cytometer by acquiring the diluted blood sample and measuring the number of platelets passing through the preset forward scatter/side scatter gate over a period of 2 min. Record platelet counts, and convert into absolute counts by using an empirical conversion factor.

*Absolute platelet counts per liter can be established by using an empirical conversion factor, which can be established by comparing the flow cytometer enumerated counts of a single sample with the same sample analyzed by a hematology analyzer, such as a Beckman Coulter LH750. Alternatively, a hematology analyzer can be used in place of flow cytometry to enumerate platelets. The typical conversion, which has to be determined for each particular flow cytometer and flow rate, is in the range of 0.02–0.04 × 10⁹.*

12. Continue the treatment and analysis cycle up to Day 3.
Figure 15.30.2  Constant-dose anti-CD41 passive antibody transfer model of mouse immune thrombocytopenia (ITP). Balb/c animals were either left untreated (baseline platelet measurement), treated with a daily constant-dose of the anti-CD41 antibody only, or treated with the anti-CD41 antibody and IVIg on Day 0 (prophylactic or prevention model). IVIg was given 2 hr prior to blood collection followed immediately by anti-CD41 administration or on Day 1 (treatment or ITP model). IVIg was given 2 hr following blood collection. Spontaneous recovery of platelets (bone marrow-compensated thrombopoiesis) is seen at Day 4 in the mice that were only given the anti-platelet antibody, anti-CD41.

Usually, by Day 4 spontaneous recovery of platelets begins to significantly interfere with confident interpretation of the results. Thus, a typical ITP model time course is 4 to 5 days (from Day 0 to Day 3 or 4).

Figure 15.30.2 depicts sample ITP model results obtained with this protocol.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2A.

Citrate-phosphate-dextrose-adenine (CPDA), 10%, in PBS

Prepare 10% CPDA in PBS by combining 5 ml citrate-phosphate-dextrose-adenine solution (Sigma-Aldrich) with 45 ml PBS (pH 7.2; see APPENDIX 2A). The solution can be stored at room temperature short-term, but refrigerate for prolonged storage.

COMMENTARY

Background Information

The uptake and destruction of autoantibody-opsonized platelets by phagocytic monocytes is the hallmark of chronic immune thrombocytopenia (ITP). When antibody-opsonized platelets pass through the spleen or liver they are taken up by mononuclear phagocytes that recognize the Fc portion of the antiplatelet antibody due to expression of specific Fc receptors (Steck, 1969; George and Raskob, 1998; McFarland, 2002). These platelets are thus recognized, become attached to, and are phagocytosed in the spleen by the splenic macrophages and
in the liver by Kupffer macrophages (Aster and Keene, 1969; Najean et al., 1997). With the identification of autoimmune antibodies directed against the various platelet self-antigens—including the collagen receptor (glycoproteins Ia/IIa or CD49b/CD29; He et al., 1994), von Willebrand factor and thrombin receptor (glycoproteins Ib/IX or CD42a-d; Kiefel et al., 1991), and fibrinogen receptor (glycoproteins IIb/IIIa or CD41/CD61; Hou et al., 1997)—passive antibody transfer animal models of ITP quickly emerged that relied on the transfer of autoantibody specificity. Early models utilized the transfer of autoreactive sera or purified autoreactive IgG followed by the development of more reproducible models that relied on the infusion of autoreactive monoclonal antibodies. The passive antibody transfer ITP models in mice and other species closely recapitulate the mechanisms of platelet opsonization by autoantibody and destruction by phagocytes that lead to the establishment of chronic ITP. Passive transfer ITP models can also be highly useful in mimicking the mechanisms of autoantibody effects on the megakaryocytic compartment and inhibition of the de novo platelet production, such as our anti-CD41 dose-escalation mouse model of ITP. As such, the passive ITP models can be highly useful in evaluating treatment modalities for chronic ITP and the underlying mechanisms of action of these agents. The short time course, quantitative readout, and relative ease of implementation of this model allow the results of treatment to be rapidly observed and measured, as well as accurately compared to the efficacy of other treatments. Despite the utility and popularity of the passive transfer ITP models, studies of the onset of ITP and the mechanisms leading to the immunological breakdown of tolerance to platelet antigens are best conducted in other models, such as the NZW × BXSB F1 mouse model of ITP—the first mouse model to be developed that recapitulates spontaneous chronic ITP (Oyaizu et al., 1988; Mizutani et al., 1990). Neschedim and Branch (2015) provide an in-depth overview of the broad variety of animal models of ITP and their respective utility in capturing the different mechanistic and pathological aspects of this disorder.

Critical Parameters

Treatment dosing and scheduling should be optimized for each model. In our hands, optimal IVIg treatment of Balb/c and C57BL/6 mice can be achieved with single doses of 1 g/kg and 2 g/kg, respectively, given on Day 2 (Katsman et al., 2010) using the dose-escalation anti-mouse CD41 passive transfer ITP model or Day 1 using the constant-dose anti-mouse CD41 passive transfer ITP model. While our model is optimized with the use of the rat anti-CD41 antibody (anti-glycoprotein IIb, clone MWRég30, rat IgG1:λ), a number of different passive antibody-transfer ITP models have been established. Early passive transfer ITP models relied on the transfer of autoreactive sera. Antiplatelet serum infused into C57BL/6 or CH3 mice induces ITP (Corash and Levin, 1990), and injection of rabbit purified IgG from an antiplatelet serum into naïve Balb/c or C57BL/6 mice induces ITP (Cox et al., 1991).

Models utilizing the repeated injection of monoclonal antiplatelet antibodies offer a more reproducible approach. Such ITP mouse models have been developed based on the injection of the mouse 6A6 antibody (mouse IgG2A; derived from male NZW × BXSB F1 mice that develop systemic lupus erythematosus [SLE]; Mizutani et al., 1993; Samuelsson et al., 2001), rat anti-mouse CD41 (anti-glycoprotein IIb, clone MWRég30, rat IgG1:λ antibody), or hamster anti-mouse CD61 (anti-integrin β3, clone 2C9.G2) into naïve Balb/c or C57BL/6 mice (Crow et al., 2001; Song et al., 2003; Katsman et al., 2010). The antigenic determinant of the monoclonal antibody 6A6 on platelets is unclear and may cross-react with other self-antigens (Mizutani et al., 1993). The passive antibody ITP models are all characterized by the rapid onset of the ITP and clear involvement of phagocytic monocytes in platelet destruction. However, a constant-dose anti-CD41 antibody transfer ITP model is not ideal for the study of human ITP as following initial thrombocytopenia a spontaneous recovery of platelets is often observed, stemming from compensatory platelet production in response to the thrombocytopenia (Katsman et al., 2010). In contrast, our dose-escalation anti-CD41 antibody transfer ITP model maintains thrombocytopenia by increasing anti-CD41 antibody dosing to combat the increased compensatory production of platelets via the inhibition of the megakaryopoiesis, which yields a prolonged platelet nadir and thus closely recapitulates chronic human ITP (Katsman et al., 2010).

Troubleshooting

Some differences can be observed in the response to treatment in the various mouse models for ITP.
strains used for establishing the ITP model. As examples, our evaluation of the role of the inhibitory Fc receptor (FcγRIIB), as well as analysis of the induction of 23 different cytokines in the passive ITP model in response to treatment with IVIg, revealed distinct strain-related differences (Leontyev et al., 2012; Leontyev et al., 2014). It is best to optimize treatment regimens in the particular strain used in the ITP model and compare treatment responses in the same mouse strain. Comparative evaluation of treatments in more than a single mouse strain is advised.

Anticipated Results

The passive antibody transfer model described in the Basic Protocol is characterized by a rapid-onset ITP, with the platelet levels dropping sharply to a sustained nadir over 2 days. The effect of treatments can be observed very quickly, depending on the agent studied. IVIg administered on Day 2 restores platelet levels within 24 hr, with treatment peaking over the next 24 to 48 hr. Recovery of up to 50% to 60% of normal platelet counts can be observed, but full recovery is not typically seen, likely due to the negative effects of antibody dose-escalation on megakaryopoiesis. The reduced-stringency, constant-dose model described in the Alternate Protocol often exhibits spontaneous platelet recovery starting on Day 4 of the model but can occur as early as Day 3. This can make the interpretation of treatment results more challenging for treatments that take longer to act. The reduced-stringency model is best suited to evaluate agents that act quickly and/or evaluate agents in a prophylactic setting (administered on Day 0). However, it should again be emphasized that the prophylactic approach is not consistent with the human disease where thrombocytopenia is already present prior to treatment.

Time Considerations

The mouse models of ITP described in this unit take 1 week to complete once the mice are acclimated and ready for treatment.

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The views expressed herein do not necessarily represent the view of the federal government of Canada.

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