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Use of mouse models to study the mechanisms and consequences of RBC clearance

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Abstract

Mice provide tractable animal models for studying the pathophysiology of various human disorders. This review discusses the use of mouse models for understanding red-blood-cell (RBC) clearance. These models provide important insights into the pathophysiology of various clinically relevant entities, such as autoimmune haemolytic anaemia, haemolytic transfusion reactions, other complications of RBC transfusions and immunomodulation by Rh immune globulin therapy. Mouse models of both antibody- and non-antibody-mediated RBC clearance are reviewed. Approaches for exploring unanswered questions in transfusion medicine using these models are also discussed.

Keywords

RBC antigens and antibodies; Transfusion reactions; Immunohaematology

Introduction: Mice as a tractable experimental model

Although the best way to understand human disease is to study humans, this is often practically difficult and ethically unacceptable [1]. Mice became an important tool for studying human pathophysiology because of their relative ease of genetic manipulation, small size, relatively low cost, short gestation and fecundity. In addition, resources, such as the Mouse Phenome Database (<http://www.jax.org/phenome>), provide phenotypic and genotypic information on approximately 40 common inbred strains, thereby providing researchers with convenient tools for selecting the appropriate model. The increasing availability of better methods for studying mouse physiology *in vivo* also facilitates their usefulness and dominance as animal models.

Genetics

The complete genome sequences of several common inbred mouse strains are available [2]. Despite the common assertion that ‘mice are not human,’ there appear to be only ~300 unique genes separating mice and humans [3], with ~99% of human genes having a mouse homologue and a highly conserved gene order [1]. Thus, mice and humans are actually quite similar. However, there are differences that must be considered when using mice for preclinical studies of human disease. For example, although mouse models are important for immunologic experimentation *in vivo*, there are key differences in both innate and adaptive immunity (for review, see [4]). In particular, human blood is neutrophil rich (50-70%), whereas mouse blood is lymphocyte rich (75-90%) [5]. As another example, immunoglobulin IgG subclasses and Fc-receptors are somewhat different (for review, see [6] and [7]), complicating crossspecies correlations of these molecules [4]. In addition, mice and humans express toll-like receptor orthologues differently [8]. Mouse complement function is also believed to be ‘weak’ in comparison with humans and other species [9,10]. Finally, transfused allogeneic white-blood-cells are cleared at different rates in mice and humans [11]. To overcome some of these limitations, ‘humanized’ mice, defined as either immunodeficient mice repopulated with human immune cells or mice genetically engineered to express human genes, are useful for studying human cells and biologic processes *in vivo* [12]. Although there are caveats to using ‘humanized’ mice, they provide another promising preclinical model for testing therapeutic approaches.

The genetic variability in any inbred mouse strain is almost negligible, allowing study of disease-causing mutations on an isogenic background [1]. Thus, identifying genetic loci regulating a disease or phenomenon is facilitated by genetic analysis of experimental mouse models of human disease. By crossing one mouse strain expressing the trait of interest with one resistant to that trait, and then genetically mapping the offspring using linkage analysis, one can identify quantitative trait loci associated with the trait of interest. For example, this technique identified the complement factor 5 and Fc-gamma receptor (Fc γ R) IIb genes as susceptibility alleles in a collagen-induced arthritis model [13]. In the future, the ‘Collaborative Cross’ [14], currently being developed by the Complex Trait Consortium, will provide the scientific community with a large ‘outbred’ set of recombinant inbred strains designed for complex trait analysis. In addition to enabling quantitative trait loci studies, this resource will allow for gene-environment interaction analysis and for validating predictive genetics. In the current context, these resources may help shed light on the role of genetic variation in important issues in transfusion medicine; these include the genetic contribution to differences in alloimmunization rates, in the clinical severity of transfusion reactions and in transfused RBC survival *in vivo* after prolonged storage *in vitro*.

Measuring RBC clearance

RBC transfusions are a simple type of adoptive transfer and the simplest type of tissue transplantation, thereby allowing testing of multiple variables. One of the most classical, common and useful experimental approaches in transfusion medicine is the measurement of RBC clearance, alternatively known as a ‘RBC survival study’. RBC clearance studies can be performed both *in vitro* and *in vivo*. Several types of genetically engineered mice, which do or do not express a defined RBC antigen, can be used for modelling allogeneic RBC transfusions and performing RBC survival studies (see Table 1). Mouse strains expressing differing, genetically encoded, blood group antigens could also be used for this purpose. In addition to exploiting genetic differences, the RBCs themselves can be modified or labelled *ex vivo* and then used to measure RBC clearance.

Studies can also be performed *in vitro* using phagocytic cells, which can be either immortalized mouse cell lines (e.g. RAW 264.7 or J774 cells) or primary cultures of cells obtained directly from mice (e.g. peritoneal exudate macrophages, bone marrow-derived macrophages, liver Kupffer cells). Macrophages are incubated *in vitro* with treated or untreated RBCs and phagocytosis is quantified. Phagocytosis can be measured in several different ways; for example, the number of phagocytes with ingested RBCs can be counted in several microscopic fields, along with the total number of phagocytes in those fields. The phagocytic index can then be defined as either the total number of RBCs ingested by 100 phagocytes or the total number of phagocytes with at least one ingested RBC per 100 phagocytes. Phagocytosis can also be quantified by first using hypotonic lysis to remove non-ingested RBCs followed by spectrophotometric measurement of the haemoglobin in a given number of phagocytes [15]. Finally, RBCs can be labelled, and the label used to measure clearance both *in vitro* and *in vivo*.

Radioactive chromium (i.e. ^{51}Cr) is the most common label used for RBC survival studies *in vivo* [16]. This not only allows facile measurement of RBC clearance, but also allows determination of the organ distribution of the cleared RBCs. Flow cytometric approaches offer additional benefits, including not requiring radioactivity, allowing evaluation of whether an antigen is still present on the RBC surface (e.g. the antigen-loss phenomenon [17]), and allowing evaluation of RBC coating by antibodies and/or complement (i.e. the direct antiglobulin test).

Flow cytometric approaches can be either immunological or non-immunological. Immunological flow cytometry survival studies involve either haptening RBCs with moieties such as dinitrophenyl, trinitrophenyl and 4-hydroxy5-iodo-3-nitrophenylacetyl, followed by detecting and counting haptened RBCs using labelled hapten-specific antibodies. However, some haptens induce antibody responses, thereby limiting their use in long-term survival studies [18]. Similar flow cytometric approaches can detect circulating transgenic RBCs (Table 1) using antigen-specific antibodies. By this approach, anti-hen egg lysozyme (HEL) antibodies in wild-type mice induced non-haemolytic antigen loss of HEL expressed on the surface of transfused HEL-transgenic RBCs [17]. By flow cytometry, the incompatible HEL-transgenic RBCs circulated with a normal lifespan, exhibiting selective loss of HEL antigen, but not other antigens. This illustrates an important advantage of flow cytometry: using multiple antibodies with different fluorescent labels provides significantly more information than other methods, such as agglutination or radioactive labelling.

Non-immunologic flow cytometric approaches also exist for tracking circulating RBCs. Lipophilic fluorescent dyes, such as 3,3'-dihexadecyloxycarbocyanine perchlorate (DiO) and chloromethylbenzamido 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), among others, can label RBCs [19]. In addition, RBCs can be labelled with biotin *ex vivo* and detected by flow cytometry using fluorescently labelled streptavidin. Virtually, all mouse RBCs can also be simultaneously biotinylated *in vivo* [16], thereby differentiating those RBCs from subsequently produced new RBCs; this is useful for enriching young or old RBCs and also for determining RBC lifespan. Finally, a recently described approach, which avoids labelling and washing steps pretransfusion, involves transfusing transgenic, green fluorescent protein (GFP)-expressing RBCs [20]; although the recombinant GFP did not alter RBC circulatory lifespan, other rheologic properties were not examined.

Organ sites of RBC clearance and cells involved

In mice, Fc γ R-mediated phagocytosis of IgG-coated RBCs predominantly occurs in the spleen; complement C3b-coated RBCs are rapidly sequestered in the liver. Some C3b-

coated RBCs are phagocytosed, a process mediated by specific complement receptors [e.g. complement receptor 3 (CR3)], whereas other RBCs are modified by a C3b inactivator and released back into the circulation [21]. In addition, experimental splenectomy shows that although the spleen is required for alloimmunization to HEL-transgenic RBCs [22], it is not required for antigen loss of HEL-transgenic RBCs [17].

Mouse models can also be used to study the cells involved in phagocytosis. To investigate the role of macrophages, they can be depleted with liposomal clodronate [23] or gadolinium chloride [24]. A transgenic mouse generated using a CD11b- or CD11c-diphtheria toxin receptor construct also allows for conditional macrophage and/or dendritic cell depletion after diphtheria toxin injection [25,26]. Transgenic mice that express an inducible suicide gene also allow for conditional depletion of cells in the monocyte-macrophage system [27]. In addition, macrophage function can be inhibited with inert colloids, thorotrast, or various lipids [28]. In particular, ethanol decreases complement-dependent phagocytosis [21]. In contrast, macrophage function is enhanced by lipopolysaccharide (LPS); LPS induces 'inside-out' signalling of the CR3 integrin, thereby increasing complement-mediated phagocytosis of sheep RBCs by Kupffer cells [15]. In addition, glucan, a neutral polysaccharide, stimulates phagocytosis, increasing sheep RBC clearance in mice by fourfold [29]. Finally, increased phagocytosis accompanies viral and bacterial infections, in both laboratory animals and humans [30].

Antibody-mediated RBC clearance

Mouse models have been used for decades to study antibody-mediated mechanisms of RBC clearance. We will first review the general considerations for immune RBC clearance and then discuss mouse models of alloantibody-mediated clearance, followed by those describing auto-antibody-mediated clearance.

General considerations

After IgG antibodies bind to RBCs, the exposed Fc regions initiate phagocytosis by binding to Fc γ R on the macrophage surface [31]. This induces a complex signalling cascade, initiated by Fc γ R clustering and tyrosine phosphorylation of the immunoreceptor tyrosine-activation motif in the cytoplasmic domain of activating Fc γ R. This cascade results in cytoskeletal rearrangement and ingestion of antibody-coated RBCs into phagosomes, where acidification and proteolysis release haemoglobin and haeme; the latter is metabolized by haeme-oxygenase, producing biliverdin, carbon monoxide and iron (for a review of Fc γ R signalling, see Ref. [31]). Mouse models were critical in determining the pathways involved in this process. For example, the roles of the tyrosine kinase, Syk and other Src family tyrosine kinases in Fc γ R-mediated phagocytosis were demonstrated in the respective knockout (KO) mice (reviewed in [32]). Pharmacologic inhibitors of this pathway were also evaluated in mice. For example, a Syk inhibitor ameliorated both autoimmune haemolytic anaemia (AIHA) and autoimmune thrombocytopenic purpura (ITP) in mouse models; this was also efficacious in humans with chronic refractory ITP [33], highlighting the relevance of mouse models for preclinical studies of novel therapies.

Various mechanisms also regulate phagocytosis to control whether a RBC is internalized or not. For example, a threshold level of Fc γ R clustering is required to initiate phagocytosis [31], implicating antibody coating density and antigen density in RBC internalization. In addition, an inhibitory receptor, Fc γ RIIb, with a cytoplasmic domain immunoreceptor tyrosine-inhibitory motif, recruits phosphatases that inhibit the signalling required for phagocytosis. Therefore, the ratio between inhibitory and activating Fc γ R affects phagocytosis. The IgG subclass opsonizing RBCs also affects phagocytosis by differentially binding to activating and inhibitory Fc γ R. Mice have four Fc γ R types (Fc γ RI, IIB, III and

IV) [7]; as examples, the high-affinity Fc γ RI preferentially binds IgG2a, medium-affinity Fc γ RIV binds IgG2a and IgG2b and low-affinity Fc γ RIIb and Fc γ RIII bind IgG1, IgG2a and IgG2b (note: mice lack Fc γ RIIa) [34]. In addition, IgG is glycosylated at asparagine 297 in the Fc region. Deletion of this N-glycan abolishes Fc γ R binding, and variations in terminal monosaccharide expression (i.e. sialic acid or fucose) affect IgG-Fc γ R interactions [34]. For example, terminal sialylation promotes an anti-inflammatory effect dependent on Fc γ RIIb function, potentially explaining some beneficial effects of intravenous immunoglobulin [35]. Finally, exogenous factors influence phagocytosis. For example, pro-inflammatory cytokines (e.g. tumour necrosis factor- α), complement fragment C5a and LPS enhance Fc γ R expression, thus enhancing phagocytosis [34]. Mouse models are ideal for studying these issues because of the availability of KO mice (e.g. of Fc γ -chain, Fc γ RIIb, or C5) and of monoclonal antibodies of chosen specificity, isotype, subclass and glycosylation. For example, Fc γ -chain KO mice, lacking Fc γ RI and Fc γ RIII, are resistant to haemolytic anaemia induced by anti-mouse RBC antibodies [36].

Complement

Before proceeding to alloantibody- and autoantibody-induced RBC clearance models, the role of the complement system will be discussed. Complement-mediated RBC destruction causes intravascular and/or extravascular haemolysis. IgM antibodies, in particular, activate the classical complement pathway to completion, producing intravascular haemolysis [37,38]. Antibodies that only fix complement to the C3 stage produce extravascular haemolysis by RBC phagocytosis in the liver and spleen [37,38]. Complement opsonization is primarily because of C3b (generated by the classic, alternative, or lectin pathways) and C3bi (the factor I cleavage product of C3b) [39]. At least one receptor recognizes C3b (i.e. CR1), and at least three receptors recognize C3bi (CR2, CR3 and CR4) [39]. In addition, the recently described CR1g receptor, only expressed on macrophages, recognizes both C3b- and C3bi-opsonized particles [40].

The CR3 integrin (also known as CD11b/CD18, Mo-1, $\alpha_M\beta_2$, or Mac-1) mediates phagocytosis of complementopsonized RBCs [41]. CR3 requires prior activation (i.e. 'inside-out' signalling) to initiate phagocytosis [42]. Although RBC-binding IgG antibodies may or may not fix complement, complement-fixing IgG antibodies can enhance RBC clearance because of synergism between Fc γ R- and complement-mediated signalling [43]. To evaluate the role of complement in RBC clearance in mice, various KO strains are available (e.g. C3 KO, C4 KO, C5 KO, or CR3 KO). In addition, cobra venom factor massively activates complement and, therefore, can deplete mice of complement. Novel therapeutics can also be evaluated using these models; for example, CR1-based inhibitors prevent complement-mediated immune haemolysis [44].

Alloantibody-mediated RBC clearance

In contrast to human RBCs, major histocompatibility complex (MHC) antigens are abundantly expressed on mouse RBCs. Despite MHC differences between FVB, C57BL and BALB/c mice, there are no 'naturally occurring' anti-MHC antibodies that cause clearance of 'incompatible' RBCs [45,46]. Although there are approximately 10 non-MHC mouse blood group systems [45], and although transfused, incompatible mouse RBCs are cleared rapidly [46,47], these polymorphisms have neither been identified at the nucleotide or amino acid level nor exploited extensively. In addition, to date, there is no mouse model of haemolytic disease of the newborn (HDN). Nonetheless, female mice from one inbred strain mated with males from a different strain develop MHC-specific anti-RBC alloantibodies [48]. Although there are no differences in litter size [49], this approach could be explored further. Indeed, recent technological advances in Doppler echocardiography allow

measurement of mouse foetal blood velocity [50]. In addition, mouse HDN models could be constructed using the strains described below.

Table 1 summarizes genetically engineered mice that have been, or could be, used to study alloantibody-mediated RBC clearance. Because mouse monoclonal antibodies can be generated by immunizing mice with the antigen of interest (e.g. the MIMA-19 mouse monoclonal anti-human Fy^a was generated using human Fy^b-transgenic mice immunized with Fy^a [51]), these mouse models provide well-controlled systems for testing hypotheses regarding alloantibody-mediated RBC clearance.

hGPA-transgenic model

Human glycophorin A (hGPA) is the most abundant human RBC membrane glycoprotein. The development of hGPA-transgenic mice, expressing hGPA on mouse RBCs [52], led to a mouse model for studying haemolytic transfusion reactions. Thus, non-hGPA-transgenic recipients could be actively immunized to hGPA or passively immunized by infusing IgG or IgM anti-hGPA antibodies. Passive immunization with IgG1 or IgG3 anti-hGPA monoclonal antibodies induces rapid clearance of transfused, incompatible, hGPA-transgenic RBCs [53]. Transfusions into Fc γ -chain and/or complement C3 KO mice show that complement and activating Fc γ Rs are important for clearing incompatible RBCs. IgG-mediated clearance of transfused RBCs induces cytokine storm in this model [19]. In addition, passive immunization with an IgM anti-hGPA monoclonal antibody produces complement-dependent intravascular haemolysis of transfused incompatible RBCs, accompanied by gross haemoglobinuria [53]. Finally, when used to study alloimmunization, this approach suggests that CD4⁺CD25⁺ regulatory T cells are important in regulating the immune response [54].

Fy^b-transgenic model

In a similar model using human Fy^b-transgenic mice [55], wild-type mice transfused with Fy^b-transgenic RBCs developed IgM, and then IgG, alloantibodies to human Fy^b, Fy3 and Fy6. In addition, transfused incompatible Fy^b-transgenic RBCs had decreased survival in alloimmunized mice. Thus, transfusion of antigen-positive RBCs provides a mouse model of alloimmunization, useful for studying alloimmunization mechanisms and the consequences of RBC clearance.

HEL-transgenic model

Surprisingly, transfusion of HEL-transgenic RBCs into wild-type recipients passively immunized with anti-HEL antibodies leads to disappearance of the HEL antigen from the RBC surface rather than RBC clearance, a phenomenon termed 'antigen loss' [17,56]. Antigen loss requires the presence of activating receptors, but not the spleen. In addition, HEL-transgenic mice were used to study alloimmunization, which is spleen dependent [22]. Finally, this model led to the discovery that inflammation provoked by certain molecules [e.g. poly(I:C)], but not others (e.g. LPS), enhances alloimmunization to transfused HEL-transgenic RBCs [57,58].

Other models

The HEL-Ovalbumin-Duffy (HOD)-transgenic RBC model is useful for studying alloimmunization because the well-characterized HEL and ovalbumin antigens are tethered to the membrane by Duffy, a multipass transmembrane protein. Transfusion of 14-day stored, leukoreduced HOD RBCs enhances alloimmunization to the HEL antigen when compared to transfusion of freshly collected leukoreduced RBCs [59].

H-transferase transgenic mice express the carbohydrate blood group H antigen on RBCs and have decreased expression of the α -gal xenoantigen [60]. Transfusion of H-transgenic RBCs could potentially be used to model Bombay O incompatible transfusion reactions. Alternatively, α -gal KO mice could be used to model antibody-mediated RBC clearance to a carbohydrate antigen [61], in which the KO mice would be transfused with incompatible, α -gal expressing, wild-type RBCs. Mouse Duffy KO mice could similarly be used to model clearance of wild-type RBCs with KO mice as transfusion recipients [62].

Autoantibody-mediated RBC clearance

Studies using monoclonal antibodies from mice with AIHA provided important insights regarding the roles of antibody isotypes, IgG subclasses, complement and Fc γ Rs in antibody-mediated RBC clearance. Several mouse strains develop AIHA, which has been studied most with New Zealand Black (NZB) mice. Nearly all NZB mice develop RBC autoantibodies and AIHA by 6 months of age [63]. Band 3, the RBC anion exchanger, is the dominant autoantigen; however, deletion of Band 3 in NZB mice only shifts the dominant autoantigen, presumably to glycophorin [64]. Many pathogenic anti-RBC monoclonal autoantibodies were described; IgG subclass switch variants of one of these, 34-3C, were used to assess the roles of IgG subclass, Fc γ Rs, and complement C3 in AIHA *in vivo*. IgG2a and IgG2b autoantibodies, which interact with Fc γ RIII and Fc γ RIV and activate complement, are most potent at inducing AIHA [65].

Although some anti-RBC autoantibodies can induce AIHA in both wild-type and C3 KO mice [66], complement may still influence haemolysis. For example, in addition to the synergism between CR3 and Fc γ Rs in promoting phagocytosis, C5a signalling via its receptor (i.e. C5aR) upregulates activating Fc γ R expression, thereby exacerbating IgG-mediated AIHA [67]. Complement regulatory proteins on the RBC surface, such as decay-accelerating factor (CD55), also modulate RBC clearance in mouse AIHA by preventing excessive antibody-mediated complement activation [68].

Non-obese diabetic (NOD) mice are another AIHA model; in addition to developing autoimmune insulin-dependent diabetes, they also develop AIHA with age [69]. Interestingly, NZB and NOD mice have reduced expression of the inhibitory Fc γ RIIb because of a promoter polymorphism [34]. Interleukin-2 KO mice on a BALB/c background also develop severe AIHA causing death by 5 weeks of age [70]; cytokine dysregulation is implicated as a cause of disease in this model [71].

A classical mouse AIHA model involves repeated immunization with rat RBCs [72]. In addition to eliciting rat-specific xenoantibodies, anti-mouse RBC autoantibodies eventually appear. In this model, CD4⁺CD25⁺ regulatory T cells prevent autoantibody formation [73]. In addition, implanting hybridomas derived from these mice induce AIHA in naïve mice [74]. In an alternative model, levodopa, a drug for Parkinson's disease, induces AIHA in humans and in mice [75]. In summary, AIHA occurs in mice 'naturally' (e.g. NZB and NOD strains) or after administration of xenogeneic RBCs or a drug. AIHA can also be studied with greater control by implanting anti-RBC hybridomas or by passive immunization with anti-RBC monoclonal autoantibodies.

Mouse models that simplify genetic and pathogenic factors are important for answering questions regarding the initiation of autoimmune disease [76]. An elegant model involves mice transgenic for immunoglobulin genes comprising the 4C8 anti-RBC autoantibody [77]. In this case, almost all B cells are deleted from the periphery; however, B-1 cells in the peritoneum, which are sequestered from RBCs, remain [77]. Oral LPS administration induces B-1 cell expansion and AIHA, whereas a pathogen-free environment prevents AIHA [76]. Thus, infection may be one factor that incites the induction of AIHA.

Non-antibody-mediated RBC clearance

There are multiple non-antibody-mediated causes of RBC clearance. Although antibodies may participate in some models described below, their role is not as clearly defined as in those described above.

Senescence

A complete understanding of why RBCs have a finite lifespan, after which they are cleared by the monocyte-macrophage system, is still not available. Mouse RBCs have a ~55 day lifespan [20]. Because mature RBCs cannot synthesize new proteins, changes in membrane proteins or phospholipids are probably involved in RBC senescence. Haemoglobin degradation with crosslinking to Band 3, a major RBC membrane-spanning protein, thereby generating a senescent antigen recognized by IgG antibodies, may be a mechanism for clearing senescent RBCs [78-80]. In addition, glycosylation differs between young and old RBCs, with decreased sialylation of older RBCs, suggesting that desialylation is involved in senescent RBC clearance [81]. Indeed, desialylated RBCs are rapidly cleared *in vivo* [82,83] and bind macrophages *in vitro* [81]. Desialylated RBCs may be cleared by asialoglycoprotein receptor-mediated phagocytosis (this lectin recognizes D-galactose exposed by desialylation) [81] and/or Fc γ R-mediated phagocytosis because of increased autoantibody binding to senescent RBCs [84]. Other mechanisms for clearing senescent RBCs probably exist, and phosphatidylserine-mediated mechanisms, described below, may also be involved.

Phosphatidylserine (PS) exposure

Cell membranes contain asymmetrically arranged phospholipids, with PS and phosphatidylethanolamine predominantly found on the inner leaflet and phosphatidylcholine and sphingomyelin on the outer leaflet. This asymmetry is maintained by flipase, an ATP-dependent aminophospholipid translocase. Loss of asymmetry in nucleated cells is a marker of apoptosis, leading to recognition and ingestion by macrophages [85,86]. Macrophages interact with PS by a specific PS receptor or through bridging proteins (e.g. annexin I, milk fat globule EGF-factor 8, growth arrest specific gene 6 protein, β 2-glycoprotein 1, protein S, thrombospondin and Del-1) that link apoptotic cells to a cognate receptor [86]. PS exposure on the outer leaflet of RBC membranes increases with RBC ageing [87] and oxidative damage [85] and is a signal for splenic and liver clearance [88]. This suggests a role for PS exposure in RBC senescence and RBC apoptosis, the latter termed 'eryptosis' [85]. Mechanisms inducing eryptosis include increased cytosolic calcium, producing cell shrinkage by calcium-dependent activation of potassium ('Gardos') channels and by calcium-dependent activation of scramblase, thereby exposing PS [85]. These processes are regulated by prostaglandins, platelet activating factor and ceramide. In addition, eryptosis can be modelled by incubating RBCs with a calcium ionophore and calcium, exposing PS [85]. In contrast, when exposed to a calcium ionophore without calcium, RBCs retain normal membrane asymmetry, thereby serving as an appropriate negative control.

Interestingly, murine (and human) sickle cell disease (SCD) RBCs express PS, potentially because of higher ATP consumption, increased reactive oxygen species production and flipase dysfunction [89]. Increased PS exposure results in rapid RBC clearance [90]; thus, PS exposure during SCD crises may exacerbate haemolysis [91]. In addition, uraemia and inflammation increase RBC PS expression and endothelial cell adhesion, which may explain the increased thrombosis risk in these patients [92]. Finally, iron-deficient RBCs have increased cytosolic calcium, increased PS expression and decreased survival [93].

Oxidative damage

Oxidative damage is implicated in damaging RBCs in glucose 6-phosphate dehydrogenase (G6PD) deficiency [94]. Mutant mice with low G6PD levels are available for modelling this common human disorder [95]. Oxidation-induced haemolysis of G6PD-deficient RBCs can also be modelled in mice by phenylhydrazine treatment, which causes oxidative damage and precipitates denatured haemoglobin (i.e. Heinz bodies), leading to RBC ingestion by spleen and liver macrophages [96]. Although the receptor(s) responsible for clearing these oxidatively damaged RBCs is unknown, clearance is inhibited by scavenger receptor ligands, such as oxidized low-density lipoprotein, liposome-encapsulated PS, polyinosinic acid and fucoidan; however, there may be different scavenger receptor systems in the liver and spleen [97]. Using scavenger receptor class A KO mice, this receptor was found to be a minor participant in phagocytosis of oxidatively damaged RBCs [97]. Studies *in vitro* suggest that lectin-like macrophage receptors recognizing galactosyl residues on these damaged RBCs, as well as Fc γ Rs, are also involved in their ingestion [94,98]. Mouse models are also useful in evaluating methods for preventing this clearance; for example, glucocorticoids effectively reduce clearance of oxidatively damaged RBCs *in vivo* [99].

Oxidative damage of membrane lipids also occurs in several haematological disorders associated with decreased RBC survival [100]. One product of lipid peroxidation, malonyldialdehyde, itself crosslinks membrane lipids and proteins, leading to decreased RBC deformability and increased RBC clearance [100]. This provides yet another useful mouse model for studying the effects of oxidative damage on RBCs.

Xenogeneic RBCs

Transfusions of xenogeneic RBCs have been used for studying various aspects of RBC clearance. For example, sheep RBCs were extensively used to model the immunomodulation seen by using Rh immune globulin in humans to prevent HDN [101]. The mechanisms for clearing xenogeneic RBCs are complex, involving multiple pathways. For example, because of glycosylation differences, surface carbohydrates may be involved in clearing xenogeneic RBCs [102]. In addition, varying glycosylation can activate complement by the alternative and lectin pathways [10]. The presence of 'naturally occurring' xenoantibodies is also mechanistically relevant [44]. Finally, size may matter when xenogeneic RBCs are transfused into mice; for example, human RBCs are twice the diameter of mouse RBCs and may have difficulty navigating narrow murine capillaries.

SCD and thalassaemia

A complete review of mouse models of SCD and thalassaemia is beyond the scope of this article. These diseases exhibit decreased RBC survival because of defects in haemoglobin synthesis. Many mouse SCD models were developed, each with advantages and disadvantages [103,104]. Nonetheless, these SCD models have been instrumental in improving our understanding of vascular [105] and pulmonary complications [106] and for evaluating potential treatments [107]. Similarly, multiple mouse thalassaemia models, with various human mutations, are available [108-116].

CD47

In addition to pro-phagocytic 'eat me' signals provided to phagocytes by PS exposure, antibody Fc domain clustering and complement activation, there are also anti-phagocytic 'don't eat me' signals. For example, CD47, a ubiquitously expressed integrin-associated protein, functions as a marker of self on RBC surfaces by inhibiting phagocytosis through binding to signal regulatory protein- α (SIRP α) on macrophages [117]. CD47 attenuates phagocytosis of IgG and/or complement-opsonized RBCs via immunoreceptor tyrosine-

inhibitory motif-mediated signalling [118]; however, CD47 is not involved in scavenger receptor-mediated phagocytosis [119]. The density of CD47 expression also regulates phagocytosis, because RBCs heterozygous for CD47 are ingested at levels intermediate between RBCs homozygous or null for CD47 [120]. In addition, CD47-deficient NOD mice have accelerated development of AIHA, suggesting that CD47-SIRP α interactions participate in regulating autoimmunity [121]. CD47 levels also decrease during refrigerated RBC storage *in vitro* [122], potentially increasing the clearance of transfused stored RBCs. CD47 density on human RBCs is similar to that seen on murine RBCs [123]; however, decreased CD47 expression on human RBCs is not associated with decreased RBC survival [124]. There is also decreased avidity of human CD47 for SIRP α when compared to mouse CD47, along with differences at cell-cell contact areas, suggesting an alternative role(s) for CD47 on human RBCs [123].

RBC storage

In general, the mechanisms by which RBC damage during refrigerated storage *in vitro* (i.e. the ‘RBC storage lesion’) leads to increased clearance *in vivo* are not yet clear. In addition, controversy remains regarding whether RBC transfusions after prolonged storage (i.e. near to outdate) have adverse consequences in human patients [125]. Questions were also raised regarding the applicability of rodent RBC storage models to the human setting because of a rat RBC storage system in which RBC survival rapidly declined *in vivo* as storage time increased *in vitro* [126]. However, more recently, a mouse RBC storage model was established that may be more relevant [20]. In this case, mouse RBCs stored in CPDA-1 for up to 2 weeks exhibited a 24-h RBC survival of ~64%, which was comparable to human RBCs stored in CPDA-1 for 5 weeks. In addition, in the mouse storage model, there was little increase in PS positive RBCs and only minor changes in RBC CD47 expression, which were similar to stored human RBCs. This model may be a useful platform for studying the mechanisms involved, and the consequences of, transfusing stored RBCs.

Conclusions

Mice are the most widely used species for constructing animal models of human disease. Many mouse models are available for studying various issues in transfusion medicine, in general, and mechanisms of haemolysis, in particular. Some examples of unanswered questions that can be studied with these models are shown in Table 2. To date, mouse experiments provided many important lessons regarding both efferent immunity (e.g. antibody-Fc γ R interactions, the role of antibody isotype and IgG subclass, the mechanisms of complement activation and its control, and the mechanisms and consequences of eryptosis), afferent immunity (e.g. factors regulating antibody response, such as inflammation), innate immunity (e.g. cytokine storm from transfusion of stored RBCs) and RBC physiology. The future holds the potential for many studies that will then translate into improved practices in human transfusion medicine.

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References

1. Taft RA, Davissson M, Wiles MV. Know thy mouse. Trends Genet. 2006; 22:649–653. [PubMed: 17007958]

2. Church DM, Goodstadt L, Hillier LW, Zody MC, Goldstein S, She X, Bult CJ, Agarwala R, Cherry JL, DiCuccio M, Hlavina W, Kapustin Y, Meric P, Maglott D, Birtle Z, Marques AC, Graves T, Zhou S, Teague B, Potamousis K, Churas C, Place M, Herschleb J, Runnheim R, Forrest D, Amos-Landgraf J, Schwartz DC, Cheng Z, Lindblad-Toh K, Eichler EE, Ponting CP. Lineage-specific biology revealed by a finished genome assembly of the mouse. *PLoS Biol.* 2009; 7:e1000112. [PubMed: 19468303]
3. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, Antonarakis SE, Attwood J, Baertsch R, Bailey J, Barlow K, Beck S, Berry E, Birren B, Bloom T, Bork P, Botcherby M, Bray N, Brent MR, Brown DG, Brown SD, Bult C, Burton J, Butler J, Campbell RD, Carninci P, Cawley S, Chiaromonte F, Chinwalla AT, Church DM, Clamp M, Clee C, Collins FS, Cook LL, Copley RR, Coulson A, Couronne O, Cuff J, Curwen V, Cutts T, Daly M, David R, Davies J, Delehaunty KD, Deri J, Dermitzakis ET, Dewey C, Dickens NJ, Diekhans M, Dodge S, Dubchak I, Dunn DM, Eddy SR, Elnitski L, Emes RD, Eswara P, Eyraas E, Felsenfeld A, Fewell GA, Flicek P, Foley K, Frankel WN, Fulton LA, Fulton RS, Furey TS, Gage D, Gibbs RA, Glusman G, Gnerre S, Goldman N, Goodstadt L, Grafham D, Graves TA, Green ED, Gregory S, Guigo R, Guyer M, Hardison RC, Haussler D, Hayashizaki Y, Hillier LW, Hinrichs A, Hlavina W, Holzer T, Hsu F, Hua A, Hubbard T, Hunt A, Jackson I, Jaffe DB, Johnson LS, Jones M, Jones TA, Joy A, Kamal M, Karlsson EK, Karolchik D, Kasprzyk A, Kawai J, Keibler E, Kells C, Kent WJ, Kirby A, Kolbe DL, Korfi I, Kucherlapati RS, Kulbokas EJ, Kulp D, Landers T, Leger JP, Leonard S, Letunic I, Levine R, Li J, Li M, Lloyd C, Lucas S, Ma B, Maglott DR, Mardis ER, Matthews L, Mauceli E, Mayer JH, McCarthy M, McCombie WR, McLaren S, McLay K, McPherson JD, Meldrim J, Meredith B, Mesirov JP, Miller W, Miner TL, Mongin E, Montgomery KT, Morgan M, Mott R, Mullikin JC, Muzny DM, Nash WE, Nelson JO, Nhan MN, Nicol R, Ning Z, Nusbaum C, O'Connor MJ, Okazaki Y, Oliver K, Overton-Larty E, Pachter L, Parra G, Pepin KH, Peterson J, Pevzner P, Plumb R, Pohl CS, Poliakov A, Ponce TC, Ponting CP, Potter S, Quail M, Reymond A, Roe BA, Roskin KM, Rubin EM, Rust AG, Santos R, Sapozhnikov V, Schultz B, Schultz J, Schwartz MS, Schwartz S, Scott C, Seaman S, Searle S, Sharpe T, Sheridan A, Shownkeen R, Sims S, Singer JB, Slater G, Smit A, Smith DR, Spencer B, Stabenau A, Stange-Thomann N, Sugnet C, Suyama M, Tesler G, Thompson J, Torrents D, Trevaskis E, Tromp J, Ucla C, Ureta-Vidal A, Vinson JP, Von Niederhausern AC, Wade CM, Wall M, Weber RJ, Weiss RB, Wendl MC, West AP, Wetterstrand K, Wheeler R, Whelan S, Wierzbowski J, Willey D, Williams S, Wilson RK, Winter E, Worley KC, Wyman D, Yang S, Yang SP, Zdobnov EM, Zody MC, Lander ES. Initial sequencing and comparative analysis of the mouse genome. *Nature.* 2002; 420:520–562. [PubMed: 12466850]
4. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol.* 2004; 172:2731–2738. [PubMed: 14978070]
5. Doering DC, Borowicz JL, Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin Pathol.* 2003; 3:3. [PubMed: 12971830]
6. Gessner J, Heiken H, Tamm A, Schmidt R. The IgG Fc receptor family. *Ann Hematol.* 1998; 76:231–248. [PubMed: 9692811]
7. Nimmerjahn F, Ravetch J. Fc receptors: old friends and new family members. *Immunity.* 2006; 24:19–28. [PubMed: 16413920]
8. Rehli M. Of mice and men: species variations of Toll-like receptor expression. *Trends Immunol.* 2002; 23:375–378. [PubMed: 12133792]
9. Ong GL, Mattes MJ. Mouse strains with typical mammalian levels of complement activity. *J Immunol Methods.* 1989; 125:147–158. [PubMed: 2607149]
10. Ish C, Ong GL, Desai N, Mattes MJ. The specificity of alternative complement pathway-mediated lysis of erythrocytes: a survey of complement and target cells from 25 species. *Scand J Immunol.* 1993; 38:113–122. [PubMed: 8346410]
11. Goodarzi M, Lee T, Pallavicini M, Donegan E, Busch M. Unusual kinetics of white cell clearance in transfused mice. *Transfusion.* 1995; 35:145–149. [PubMed: 7825210]
12. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol.* 2007; 7:118–130. [PubMed: 17259968]

13. Johansson AC, Sundler M, Kjellen P, Johannesson M, Cook A, Lindqvist AK, Nakken B, Bolstad AI, Jonsson R, Alarcon-Riquelme M, Holmdahl R. Genetic control of collagen-induced arthritis in a cross with NOD and C57BL/10 mice is dependent on gene regions encoding complement factor 5 and FcγRIIb and is not associated with loci controlling diabetes. *Eur J Immunol.* 2001; 31:1847–1856. [PubMed: 11433381]
14. Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, Belknap JK, Bennett B, Berrettini W, Bleich A, Bogue M, Broman KW, Buck KJ, Buckler E, Burmeister M, Chesler EJ, Cheverud JM, Clapcote S, Cook MN, Cox RD, Crabbe JC, Crusio WE, Darvasi A, Deschepper CF, Doerge RW, Farber CR, Forejt J, Gaile D, Garlow SJ, Geiger H, Gershenfeld H, Gordon T, Gu J, Gu W, de Haan G, Hayes NL, Heller C, Himmelbauer H, Hitzemann R, Hunter K, Hsu HC, Iraqi FA, Ivandic B, Jacob HJ, Jansen RC, Jepsen KJ, Johnson DK, Johnson TE, Kempermann G, Kendziorski C, Kotb M, Kooy RF, Llamas B, Lammert F, Lassalle JM, Lowenstein PR, Lu L, Lusk A, Manly KF, Marcucio R, Matthews D, Medrano JF, Miller DR, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Morris DG, Mott R, Nadeau JH, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Page GP, Paigen B, Paigen K, Palmer AA, Pan HJ, Peltonen-Palotie L, Peirce J, Pomp D, Pravenec M, Prows DR, Qi Z, Reeves RH, Roder J, Rosen GD, Schadt EE, Schalkwyk LC, Seltzer Z, Shimomura K, Shou S, Sillanpaa MJ, Siracusa LD, Snoeck HW, Spearow JL, Svenson K, Tarantino LM, Threadgill D, Toth LA, Valdar W, de Villena FP, Warden C, Whatley S, Williams RW, Wiltshire T, Yi N, Zhang D, Zhang M, Zou F. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet.* 2004; 36:1133–1137. [PubMed: 15514660]
15. Steffan AM, Kirn A. C3-mediated phagocytosis induced in murine Kupffer cells by 'in vitro' activation with endotoxin. *Gastroenterol Clin Biol.* 1986; 10:117–121. [PubMed: 3516782]
16. Hoffmann-Fezer G, Maschke H, Zeitler HJ, Gais P, Heger W, Ellwart J, Thierfelder S. Direct *in vivo* biotinylation of erythrocytes as an assay for red cell survival studies. *Ann Hematol.* 1991; 63:214–217. [PubMed: 1932301]
17. JC, Zimring; Hair, GA.; Chadwick, TE.; Deshpande, SS.; Anderson, KM.; Hillyer, CD.; Roback, JD. Nonhemolytic antibody-induced loss of erythrocyte surface antigen. *Blood.* 2005; 106:1105–1112. [PubMed: 15831698]
18. Nakashima I, Takashi M, Nagase F, Kato N. Primary antibody responses to hapten-modified syngeneic cells: exceptional immunogenicity of erythrocytes. *Eur J Immunol.* 1981; 11:946–948. [PubMed: 6799301]
19. Hod EA, Cadwell CM, Liepkalns JS, Zimring JC, Sokol SA, Schirmer DA, Jhang J, Spitalnik SL. Cytokine storm in a mouse model of IgG-mediated hemolytic transfusion reactions. *Blood.* 2008; 112:891–894. [PubMed: 18483395]
20. Gilson CR, Kraus TS, Hod EA, Hendrickson JE, Spitalnik SL, Hillyer CD, Shaz BH, Zimring JC. A novel mouse model of red blood cell storage and posttransfusion *in vivo* survival. *Transfusion.* 2009; 49:1546–1553. [PubMed: 19573176]
21. Messner RP, Meryhew NL, DeMaster EG. Selective, prolonged alteration of complement-mediated immune clearance after acute exposure of mice to ethanol. *Clin Immunol Immunopathol.* 1994; 70:73–80. [PubMed: 8261672]
22. Hendrickson JE, Saakadze N, Cadwell CM, Upton JW, Mocarski ES, Hillyer CD, Zimring JC. The spleen plays a central role in primary humoral alloimmunization to transfused mHEL red blood cells. *Transfusion.* 2009; 49:1678–1684. [PubMed: 19413728]
23. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods.* 1994; 174:83–93. [PubMed: 8083541]
24. Hildebrand F, Hubbard WJ, Choudhry MA, Frink M, Pape HC, Kunkel SL, Chaudry IH. Kupffer cells and their mediators: the culprits in producing distant organ damage after traumahemorrhage. *Am J Pathol.* 2006; 169:784–794. [PubMed: 16936255]
25. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest.* 2005; 115:56–65. [PubMed: 15630444]

26. Probst HC, Tschannen K, Odermatt B, Schwendener R, Zinkernagel RM, Van Den Broek M. Histological analysis of CD11c-DTR/GFP mice after *in vivo* depletion of dendritic cells. *Clin Exp Immunol.* 2005; 141:398–404. [PubMed: 16045728]
27. Burnett SH, Kershner EJ, Zhang J, Zeng L, Straley SC, Kaplan AM, Cohen DA. Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J Leukoc Biol.* 2004; 75:612–623. [PubMed: 14726498]
28. Stuart AE, Cooper GN. Susceptibility of mice to bacterial endotoxin after modification of reticuloendothelial function by simple lipids. *J Pathol Bacteriol.* 1962; 83:245–254. [PubMed: 13917924]
29. Morrow SH, Diluzio NR. The Fate of Foreign Red Cells in Mice with Altered Reticuloendothelial Function. *Proc Soc Exp Biol Med.* 1965; 119:647–652. [PubMed: 14328966]
30. Ringle DA, Herndon BL, Bullis HR Jr. Effects of RES-stimulating lipids and zymosan on shock. *Am J Physiol.* 1966; 210:1041–1047. [PubMed: 5949956]
31. Swanson JA, Hoppe AD. The coordination of signaling during Fc receptor-mediated phagocytosis. *J Leukoc Biol.* 2004; 76:1093–1103. [PubMed: 15466916]
32. Cox D, Greenberg S. Phagocytic signaling strategies: Fc(gamma)receptor-mediated phagocytosis as a model system. *Semin Immunol.* 2001; 13:339–345. [PubMed: 11708889]
33. Podolanczuk A, Lazarus AH, Crow AR, Grossbard E, Bussel JB. Of mice and men: an open-label pilot study for treatment of immune thrombocytopenic purpura by an inhibitor of Syk. *Blood.* 2009; 113:3154–3160. [PubMed: 19096013]
34. Nimmerjahn F. Activating and inhibitory FcγRs in auto-immune disorders. *Springer Semin Immunopathol.* 2006; 28:305–319. [PubMed: 17115158]
35. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science.* 2006; 313:670–673. [PubMed: 16888140]
36. Clynes R, Ravetch JV. Cytotoxic antibodies trigger inflammation through Fc receptors. *Immunity.* 1995; 3:21–26. [PubMed: 7621075]
37. Schreiber AD, Frank MM. Role of antibody and complement in the immune clearance and destruction of erythrocytes. I. *in vivo* effects of IgG and IgM complement-fixing sites. *J Clin Invest.* 1972; 51:575–582. [PubMed: 4536807]
38. Schreiber AD, Frank MM. Role of antibody and complement in the immune clearance and destruction of erythrocytes. II. Molecular nature of IgG and IgM complement-fixing sites and effects of their interaction with serum. *J Clin Invest.* 1972; 51:583–589. [PubMed: 4622104]
39. Brown EJ. Complement receptors and phagocytosis. *Curr Opin Immunol.* 1991; 3:76–82. [PubMed: 1675856]
40. Helmy KY, Katschke KJ Jr, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, Scales SJ, Ghilardi N, van Lookeren Campagne M. CR1g: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell.* 2006; 124:915–927. [PubMed: 16530040]
41. Yan J, Vetvicka V, Xia Y, Hanikyrova M, Mayadas TN, Ross GD. Critical role of Kupffer cell CR3 (CD11b/CD18) in the clearance of IgM-opsonized erythrocytes or soluble beta-glucan. *Immunopharmacology.* 2000; 46:39–54. [PubMed: 10665778]
42. Ehlers MR. CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect.* 2000; 2:289–294. [PubMed: 10758405]
43. Meryhew NL, Runquist OA. A kinetic analysis of immunemediated clearance of erythrocytes. *J Immunol.* 1981; 126:2443–2449. [PubMed: 7229383]
44. Yazdanbakhsh K, Kang S, Tamasauskas D, Sung D, Scaradavou A. Complement receptor 1 inhibitors for prevention of immune-mediated red cell destruction: potential use in transfusion therapy. *Blood.* 2003; 101:5046–5052. [PubMed: 12576307]
45. Stimpfling JH, Reichert AE, Blanchard S. Genetic control of the immune response to the Ea-2 alloantigen system of the mouse. I. The humoral antibody response. *J Immunol.* 1976; 116:1096–1098. [PubMed: 1254962]
46. Moller G. Biologic properties of 19 S and 7 S mouse isoantibodies directed against isoantigens of the H-2 system. *J Immunol.* 1966; 96:430–439. [PubMed: 5932577]
47. Erickson RP, Herzenberg LA, Goor R. Partial Immune Elimination of Homologous Red Blood Cells in Mice. *Transplantation.* 1964; 2:175–182. [PubMed: 14133141]

48. Goodlin RC, Herzenberg LA. Pregnancy Induced Hemagglutinins to Paternal H-2 Antigens in Multiparous Mice. *Transplantation*. 1964; 2:357–361. [PubMed: 14144347]
49. Taylor GM. The level and distribution of antibody in syngeneic and allogeneic mated pregnant mice pre-immunized with H-2 alloantigens. *Immunology*. 1973; 25:703–792. [PubMed: 4586737]
50. Rounioja S, Rasanen J, Glumoff V, Ojaniemi M, Makikallio K, Hallman M. Intra-amniotic lipopolysaccharide leads to fetal cardiac dysfunction. A mouse model for fetal inflammatory response. *Cardiovasc Res*. 2003; 60:156–164. [PubMed: 14522418]
51. Halverson GR, Chaudhuri A, Huang T, Yazdanbakhsh K, Reid ME. Immunization of transgenic mice for production of Mo-Abs directed at polymorphic blood group antigens. *Transfusion*. 2001; 41:1393–1396. [PubMed: 11724984]
52. Auffray I, Marfatia S, de Jong K, Lee G, Huang CH, Paszty C, Tanner MJ, Mohandas N, Chasis JA. Glycophorin A dimerization and band 3 interaction during erythroid membrane biogenesis: *in vivo* studies in human glycophorin A transgenic mice. *Blood*. 2001; 97:2872–2878. [PubMed: 11313283]
53. Schirmer DA, Song SC, Baliff JP, Harbers SO, Clynes RA, Krop-Watorek A, Halverson GR, Czerwinski M, Spitalnik SL. Mouse models of IgG- and IgM-mediated hemolysis. *Blood*. 2007; 109:3099–3107. [PubMed: 17119123]
54. Yu J, Heck S, Yazdanbakhsh K. Prevention of red cell alloimmunization by CD25 regulatory T cells in mouse models. *Am J Hematol*. 2007; 82:691–696. [PubMed: 17492644]
55. Campbell-Lee SA, Liu J, Velliquette RW, Halverson GR, Shirey RS, Chaudhuri A, Reid ME, Ness PM, Baldwin WM. The production of red blood cell alloantibodies in mice transfused with blood from transgenic Fyb-expressing mice. *Transfusion*. 2006; 46:1682–1688. [PubMed: 17002623]
56. Zimring JC, Cadwell CM, Spitalnik SL. Antigen loss from antibody-coated red blood cells. *Transfus Med Rev*. 2009; 23:189–204. [PubMed: 19539874]
57. Hendrickson JE, Roback JD, Hillyer CD, Easley KA, Zimring JC. Discrete Toll-like receptor agonists have differential effects on alloimmunization to transfused red blood cells. *Transfusion*. 2008; 48:1869–1877. [PubMed: 18564394]
58. Zimring JC, Hendrickson JE. The role of inflammation in alloimmunization to antigens on transfused red blood cells. *Curr Opin Hematol*. 2008; 15:631–635. [PubMed: 18832936]
59. Hendrickson JE, Hod EA, Spitalnik SL, Hillyer CD, Zimring JC. Storage of murine red blood cells enhances alloantibody responses to an erythroid-specific model antigen. *Transfusion*. 2010; 50:624–628.
60. Shinkel TA, Chen CG, Salvaris E, Henion TR, Barlow H, Galili U, Pearse MJ, d'Apice AJ. Changes in cell surface glycosylation in alpha1,3-galactosyltransferase knockout and alpha1,2-fucosyltransferase transgenic mice. *Transplantation*. 1997; 64:197–204. [PubMed: 9256173]
61. LaTemple DC, Galili U. Adult and neonatal anti-Gal response in knock-out mice for alpha1,3galactosyltransferase. *Xenotransplantation*. 1998; 5:191–196. [PubMed: 9741457]
62. Dawson TC, Lentsch AB, Wang Z, Cowhig JE, Rot A, Maeda N, Peiper SC. Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). *Blood*. 2000; 96:1681–1684. [PubMed: 10961863]
63. Scott BB, Sadigh S, Stow M, Mageed RA, Andrew EM, Maini RN. Molecular mechanisms resulting in pathogenic anti-mouse erythrocyte antibodies in New Zealand black mice. *Clin Exp Immunol*. 1993; 93:26–33. [PubMed: 8324901]
64. Hall AM, Ward FJ, Shen CR, Rowe C, Bowie L, Devine A, Urbaniak SJ, Elson CJ, Barker RN. Deletion of the dominant autoantigen in NZB mice with autoimmune hemolytic anemia: effects on autoantibody and T-helper responses. *Blood*. 2007; 110:4511–4517. [PubMed: 17785581]
65. Azeredo da Silveira S, Kikuchi S, Fossati-Jimack L, Moll T, Saito T, Verbeek JS, Botto M, Walport MJ, Carroll M, Izui S. Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinity anti-erythrocyte autoantibody. *J Exp Med*. 2002; 195:665–672. [PubMed: 11901193]
66. Sylvestre D, Clynes R, Ma M, Warren H, Carroll MC, Ravetch JV. Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. *J Exp Med*. 1996; 184:2385–2392. [PubMed: 8976192]

67. Kumar V, Ali SR, Konrad S, Zwirner J, Verbeek JS, Schmidt RE, Gessner JE. Cell-derived anaphylatoxins as key mediators of antibody-dependent type II autoimmunity in mice. *J Clin Invest.* 2006; 116:512–520. [PubMed: 16453025]
68. Molina H, Miwa T, Zhou L, Hilliard B, Mastellos D, Maldonado MA, Lambris JD, Song WC. Complement-mediated clearance of erythrocytes: mechanism and delineation of the regulatory roles of Crry and DAF. Decay-accelerating factor. *Blood.* 2002; 100:4544–4549. [PubMed: 12393518]
69. Baxter AG, Mandel TE. Hemolytic anemia in non-obese diabetic mice. *Eur J Immunol.* 1991; 21:2051–2055. [PubMed: 1889456]
70. Sadlack B, Lohler J, Schorle H, Klebb G, Haber H, Sickel E, Noelle RJ, Horak I. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4 + T cells. *Eur J Immunol.* 1995; 25:3053–3059. [PubMed: 7489743]
71. Hoyer KK, Kuswanto WF, Gallo E, Abbas AK. Distinct roles of helper T-cell subsets in a systemic autoimmune disease. *Blood.* 2009; 113:389–395. [PubMed: 18815283]
72. Playfair JH, Marshall-Clarke S. Induction of red cell auto-antibodies in normal mice. *Nat New Biol.* 1973; 243:213–214. [PubMed: 4541395]
73. Mqadmi A, Zheng X, Yazdanbakhsh K. CD4 + CD25 + regulatory T cells control induction of autoimmune hemolytic anemia. *Blood.* 2005; 105:3746–3748. [PubMed: 15637139]
74. Cooke LA, Staines NA, Morgan A, Moorhouse C, Harris G. Haemolytic disease in mice induced by transplantation of hybridoma cells secreting monoclonal anti-erythrocyte autoantibodies. *Immunology.* 1982; 47:569–572. [PubMed: 7129530]
75. Sharon R, Naor D. Experimental model of autoimmune hemolytic anemia induced in mice with levodopa. *Clin Immunol Immunopathol.* 1989; 52:160–172. [PubMed: 2736806]
76. Murakami M, Nakajima K, Yamazaki K, Muraguchi T, Serikawa T, Honjo T. Effects of breeding environments on generation and activation of autoreactive B-1 cells in anti-red blood cell autoantibody transgenic mice. *J Exp Med.* 1997; 185:791–794. [PubMed: 9034157]
77. Okamoto M, Murakami M, Shimizu A, Ozaki S, Tsubata T, Kumagai S, Honjo T. A transgenic model of autoimmune hemolytic anemia. *J Exp Med.* 1992; 175:71–79. [PubMed: 1730928]
78. Low PS, Waugh SM, Zinke K, Drenckhahn D. The role of hemoglobin denaturation and band 3 clustering in red blood cell aging. *Science.* 1985; 227:531–533.
79. Lutz HU, Fasler S, Stammler P, Bussolino F, Arese P. Naturally occurring anti-band 3 antibodies and complement in phagocytosis of oxidatively-stressed and in clearance of senescent red cells. *Blood Cells.* 1988; 14:175–203. [PubMed: 3179455]
80. Kay MM. Generation of senescent cell antigen on old cells initiates IgG binding to a neoantigen. *Cell Mol Biol (Noisy-legrand).* 1993; 39:131–153.
81. Vaysse J, Gattegno L, Bladier D, Aminoff D. Adhesion and erythrophagocytosis of human senescent erythrocytes by autologous monocytes and their inhibition by beta-galactosyl derivatives. *Proc Natl Acad Sci USA.* 1986; 83:1339–1343. [PubMed: 3456592]
82. Aminoff D, Bell WC, Fulton I, Ibgebriksen N. Effect of sialidase on the viability of erythrocytes in circulation. *Am J Hematol.* 1976; 1:419–432. [PubMed: 1008055]
83. Gattegno L, Bladier D, Cornillot P. The role of sialic acid in the determination of survival of rabbit erythrocytes in the circulation. *Carbohydr Res.* 1974; 34:361–369. [PubMed: 4842011]
84. Galili U, Korkesh A, Kahane I, Rachmilewitz EA. Demonstration of a natural antigalactosyl IgG antibody on thalassemic red blood cells. *Blood.* 1983; 61:1258–1264. [PubMed: 6839023]
85. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F. Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem.* 2005; 15:195–202. [PubMed: 15956782]
86. Wu Y, Tibrewal N, Birge RB. Phosphatidylserine recognition by phagocytes: a view to a kill. *Trends Cell Biol.* 2006; 16:189–197. [PubMed: 16529932]
87. Boas FE, Forman L, Beutler E. Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. *Proc Natl Acad Sci USA.* 1998; 95:3077–3081. [PubMed: 9501218]
88. Schroit AJ, Madsen JW, Tanaka Y. *in vivo* recognition and clearance of red blood cells containing phosphatidylserine in their plasma membranes. *J Biol Chem.* 1985; 260:5131–5138. [PubMed: 3988747]

89. Banerjee T, Kuypers FA. Reactive oxygen species and phosphatidylserine externalization in murine sickle red cells. *Br J Haematol.* 2004; 124:391–402. [PubMed: 14717789]
90. de Jong K, Emerson RK, Butler J, Bastacky J, Mohandas N, Kuypers FA. Short survival of phosphatidylserine-exposing red blood cells in murine sickle cell anemia. *Blood.* 2001; 98:1577–1584. [PubMed: 11520810]
91. Chadebech P, Habibi A, Nzouakou R, Bachir D, Meunier-Costes N, Bonin P, Rodet M, Chami B, Galacteros F, Bierling P, Noizat-Pirenne F. Delayed hemolytic transfusion reaction in sickle cell disease patients: evidence of an emerging syndrome with suicidal red blood cell death. *Transfusion.* 2009; 49:1785–1792. [PubMed: 19413729]
92. Bonomini M, Sirolli V, Gizzi F, Di Stante S, Grilli A, Felaco M. Enhanced adherence of human uremic erythrocytes to vascular endothelium: role of phosphatidylserine exposure. *Kidney Int.* 2002; 62:1358–1363. [PubMed: 12234306]
93. Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, Lang F. Enhanced programmed cell death of iron-deficient erythrocytes. *FASEB J.* 2006; 20:368–370. [PubMed: 16371427]
94. Horn S, Bashan N, Moses S, Gopas J. Phagocytosis of phenylhydrazine oxidized and G-6-PD deficient red blood cells: the role of sugars and cell-bound immunoglobulins. *Adv Exp Med Biol.* 1991; 307:285–300. [PubMed: 1805591]
95. Spolarics Z, Condon MR, Siddiqi M, Machiedo GW, Deitch EA. Red blood cell dysfunction in septic glucose-6-phosphate dehydrogenase-deficient mice. *Am J Physiol Heart Circ Physiol.* 2004; 286:H2118–H2126. [PubMed: 14751857]
96. Adachi H. Pathological study on experimental Heinz body anemia: intracellular changes of phagocytized red cells in macrophages of the spleen and liver. *Acta Pathol Jpn.* 1977; 27:657–675. [PubMed: 930587]
97. Terpstra V, van Berkel TJ. Scavenger receptors on liver Kupffer cells mediate the *in vivo* uptake of oxidatively damaged red blood cells in mice. *Blood.* 2000; 95:2157–2163. [PubMed: 10706889]
98. Horn S, Gopas J, Bashan N. A lectin-like receptor on murine macrophage is involved in the recognition and phagocytosis of human red cells oxidized by phenylhydrazine. *Biochem Pharmacol.* 1990; 39:775–780. [PubMed: 2106324]
99. Ganick DJ, Segel GB, Chamberlain J, Hirsch L, Klemperer MR. The effects of splenectomy and glucocorticoids on survival and hepatic uptake of damaged red cells in the mouse. *Am J Hematol.* 1977; 2:365–373. [PubMed: 602926]
100. Jain SK, Mohandas N, Clark MR, Shohet SB. The effect of malonyldialdehyde, a product of lipid peroxidation, on the deformability, dehydration and 51Cr-survival of erythrocytes. *Br J Haematol.* 1983; 53:247–255. [PubMed: 6821655]
101. Karlsson MC, Wernersson S, Diaz de Stahl T, Gustavsson S, Heyman B. Efficient IgG-mediated suppression of primary antibody responses in Fcγ receptor-deficient mice. *Proc Natl Acad Sci USA.* 1999; 96:2244–2249. [PubMed: 10051626]
102. Kyoizumi S, Masuda T. A lectinlike receptor on murine macrophage cell line cells, Mm1: involvement of sialic acid-binding sites in opsonin-independent phagocytosis for xenogeneic red cells. *J Leukoc Biol.* 1985; 37:289–304. [PubMed: 3855439]
103. Nagel RL, Fabry ME. The panoply of animal models for sickle cell anaemia. *Br J Haematol.* 2001; 112:19–25. [PubMed: 11167777]
104. Mancini EA, Hillery CA, Bodian CA, Zhang ZG, Luty GA, Collier BS. Pathology of Berkeley sickle cell mice: similarities and differences with human sickle cell disease. *Blood.* 2006; 107:1651–1658. [PubMed: 16166585]
105. Turhan A, Weiss LA, Mohandas N, Collier BS, Frenette PS. Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. *Proc Natl Acad Sci USA.* 2002; 99:3047–3051. [PubMed: 11880644]
106. Hsu LL, Champion HC, Campbell-Lee SA, Bivalacqua TJ, Mancini EA, Diwan BA, Schimel DM, Cochard AE, Wang X, Schechter AN, Noguchi CT, Gladwin MT. Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability. *Blood.* 2007; 109:3088–3098. [PubMed: 17158223]

107. Turhan A, Jenab P, Bruhns P, Ravetch JV, Coller BS, Frenette PS. Intravenous immune globulin prevents venular vaso-occlusion in sickle cell mice by inhibiting leukocyte adhesion and the interactions between sickle erythrocytes and adherent leukocytes. *Blood*. 2004; 103:2397–2400. [PubMed: 14630831]
108. Ciavatta DJ, Ryan TM, Farmer SC, Townes TM. Mouse model of human beta zero thalassemia: targeted deletion of the mouse beta maj- and beta min-globin genes in embryonic stem cells. *Proc Natl Acad Sci USA*. 1995; 92:9259–9263. [PubMed: 7568113]
109. Jamsai D, Zaibak F, Khongnium W, Vadolas J, Voullaire L, Fowler KJ, Gazeas S, Fucharoen S, Williamson R, Ioannou PA. A humanized mouse model for a common beta0-thalassemia mutation. *Genomics*. 2005; 85:453–461. [PubMed: 15780748]
110. Lewis J, Yang B, Kim R, Sierakowska H, Kole R, Smithies O, Maeda N. A common human beta globin splicing mutation modeled in mice. *Blood*. 1998; 91:2152–2156. [PubMed: 9490703]
111. Rivella S, May C, Chadburn A, Riviere I, Sadelain M. A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. *Blood*. 2003; 101:2932–2939. [PubMed: 12480689]
112. Shehee WR, Oliver P, Smithies O. Lethal thalassemia after insertional disruption of the mouse major adult beta-globin gene. *Proc Natl Acad Sci USA*. 1993; 90:3177–3181. [PubMed: 8475058]
113. Skow LC, Burkhart BA, Johnson FM, Popp RA, Popp DM, Goldberg SZ, Anderson WF, Barnett LB, Lewis SE. A mouse model for beta-thalassemia. *Cell*. 1983; 34:1043–1052. [PubMed: 6313205]
114. Vadolas J, Nefedov M, Wardan H, Mansooriderakshan S, Voullaire L, Jamsai D, Williamson R, Ioannou PA. Humanized beta-thalassemia mouse model containing the common IVSI-110 splicing mutation. *J Biol Chem*. 2006; 281:7399–7405. [PubMed: 16421096]
115. Yang B, Kirby S, Lewis J, Detloff PJ, Maeda N, Smithies O. A mouse model for beta 0-thalassemia. *Proc Natl Acad Sci USA*. 1995; 92:11608–11612. [PubMed: 8524813]
116. Huo Y, McConnell SC, Liu SR, Yang R, Zhang TT, Sun CW, Wu LC, Ryan TM. Humanized Mouse Model of Cooley's Anemia. *J Biol Chem*. 2009; 284:4889–4896. [PubMed: 19098001]
117. Oldenburg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. *Science*. 2000; 288:2051–2054. [PubMed: 10856220]
118. Oldenburg PA, Gresham HD, Lindberg FP. CD47-signal regulatory protein alpha (SIRPalpha) regulates Fcgamma and complement receptor-mediated phagocytosis. *J Exp Med*. 2001; 193:855–862. [PubMed: 11283158]
119. Olsson M, Oldenburg PA. CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcgamma receptor-mediated but not scavenger receptor-mediated recognition by macrophages. *Blood*. 2008; 112:4259–4267. [PubMed: 18779391]
120. Olsson M, Nilsson A, Oldenburg PA. Dose-dependent inhibitory effect of CD47 in macrophage uptake of IgG-opsonized murine erythrocytes. *Biochem Biophys Res Commun*. 2007; 352:193–197. [PubMed: 17112468]
121. Oldenburg PA, Gresham HD, Chen Y, Izui S, Lindberg FP. Lethal autoimmune hemolytic anemia in CD47-deficient non-obese diabetic (NOD) mice. *Blood*. 2002; 99:3500–3504. [PubMed: 11986200]
122. Bessos H, Seghatchian J. Red cell storage lesion: the potential impact of storage-induced CD47 decline on immunomodulation and the survival of leucofiltered red cells. *Transfus Apher Sci*. 2005; 32:227–232. [PubMed: 15784458]
123. Subramanian S, Tsai R, Sen S, Dahl KN, Discher DE. Membrane mobility and clustering of Integrin Associated Protein (IAP, CD47)-major differences between mouse and man and implications for signaling. *Blood Cells Mol Dis*. 2006; 36:364–372. [PubMed: 16697668]
124. Mouro-Chanteloup I, Delaunay J, Gane P, Nicolas V, Johansen M, Brown EJ, Peters LL, Van Kim CL, Cartron JP, Colin Y. Evidence that the red cell skeleton protein 4.2 interacts with the Rh membrane complex member CD47. *Blood*. 2003; 101:338–344. [PubMed: 12393467]
125. Dzik W. Fresh blood for everyone? Balancing availability and quality of stored RBCs *Transfus Med*. 2008; 18:260–265.

126. d'Almeida MS, Jagger J, Duggan M, White M, Ellis C, Chin-Yee IH. A comparison of biochemical and functional alterations of rat and human erythrocytes stored in CPDA-1 for 29 days: implications for animal models of transfusion. *Transfus Med.* 2000; 10:291–303. [PubMed: 11123813]
127. Hendrickson JE, Desmarets M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD, Zimring JC. Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. *Transfusion.* 2006; 46:1526–1536. [PubMed: 16965580]
128. Desmarets M, Cadwell CM, Peterson KR, Neades R, Zimring JC. Minor histocompatibility antigens on transfused leukoreduced units of red blood cells induce bone marrow transplant rejection in a mouse model. *Blood.* 2009; 114:2315–2322. [PubMed: 19525479]
129. Dominici M, Tadjali M, Kepes S, Allay ER, Boyd K, Ney PA, Horwitz E, Persons DA. Transgenic mice with pancellular enhanced green fluorescent protein expression in primitive hematopoietic cells and all blood cell progeny. *Genesis.* 2005; 42:17–22. [PubMed: 15828004]

Table 1

Genetically engineered mice with useful RBC antigens

Mouse line	Tissue distribution	Characteristics	Reference
Duffy (Fy ^b)-transgenic	All cells	Used to model alloimmunization. Mice initially form IgM, and then IgG, anti-Fy ^b antibodies. Mice produce anti-Fy ^b , -Fy3 and -Fy6 antibodies.	[55]
Human glycophorin A (hGPA)-transgenic	RBCs only	Many monoclonal anti-hGPA antibodies available. Incompatible transfusion produces haemolytic transfusion reactions in mice accompanied by cytokine storm.	[19],[52],[53],[54]
Hen egg lysozyme (HEL)-transgenic	All cells	Mice passively immunized with anti-HEL followed by transfusion with HEL-RBCs induced HEL-specific antigen loss. HEL-specific T-cell receptor-transgenic mice enhance alloimmunization to transfused HEL-RBCs. Alloimmunization to HEL-RBCs requires a spleen and is augmented by certain types of inflammation.	[17],[22],[57],[127]
HEL-Ovalbumin-Duffy (HOD)-transgenic	RBCs only	Immunologically useful model due to presence of a well-characterized combination of antigens with specific immunological tools, such as HEL- and ovalbumin-specific T-cell receptor- and HEL-specific B cell receptor-transgenic mice. Refrigerator-stored leukoreduced HOD RBCs are more immunogenic than fresh leukoreduced RBCs.	[128],[59]
H-transferase-transgenic	All cells	Results in decreased expression of the α -Gal xenoantigen and increased expression of the normally cryptic sialylated Tn and Forssman antigens. Can be used to develop model of Bombay O RBCs.	[60]
Duffy antigen/receptor for chemokines (DARC) knockout	All cells	DARC is required for <i>Plasmodium vivax</i> infection of RBCs. Serves as chemokine sink. LPS challenge produces increased inflammation. Can be used to develop a haemolytic transfusion reaction model with the KO mice as recipients of incompatible transfusions from wild-type donors. The reactions in these mice may be more severe when compared to those in wild-type mice.	[62]
alpha 1, 3galactosyltransferase (α -Gal) knockout	All cells	Lack of α -Gal expression, which is universally expressed in mice. Can be used to develop a carbohydrate antigen-based haemolytic transfusion reaction model, with the KO as recipients of incompatible transfusions from wild-type donors.	[61]
Green fluorescent protein (GFP)-transgenic	All cells	Used to develop a mouse blood bank model of RBC storage. Useful for performing RBC survival studies without the need to label or wash RBCs pretransfusion.	[129],[20]

Table 2

Examples of questions amenable to study with mouse models of RBC clearance

Mechanism

Why are subpopulations of incompatible RBCs resistant to IgG- and/or IgM-mediated RBC clearance?

What signaling pathways produce the cytokine storm induced by haemolytic transfusion reactions?

Why are some haemolytic transfusion reactions clinically severe, whereas others are benign?

What host and donor factors influence RBC alloimmunization to allogeneic transfusions?

Can a mouse model of HDN be constructed to study immunization, immunomodulation and pathogenesis?

RBC storage

Can new storage solutions be developed to minimize the RBC storage lesion?

What biomarkers identify RBCs that are more prone to clearance *in vivo* after refrigerated storage *in vitro*?

Why do RBCs from some donors not perform well *in vivo* after refrigerated storage *in vitro*? Is this genetically determined?

Autoimmunity

Why is Band 3 the dominant autoantigen in NZB mice with AIHA?

What is the mechanism(s) for initiating the production of RBC autoantibodies?

Antigen loss

Does antigen loss occur more commonly than is currently believed?

What is the mechanism(s) responsible for antigen loss?

What factors determine whether RBC antigens are specifically removed from the RBC surface or whether the intact RBC is ingested? What are the roles in antigen loss of the antigen, the antibody, the macrophage and combinations thereof?

Treatment

What therapies can reduce antibody-mediated clearance and ameliorate the downstream consequences of this clearance?

Is there evidence that any of the following approaches are beneficial: corticosteroids, intravenous immunoglobulin, other immunosuppressants and complement-inhibitors?

AIHA, autoimmune haemolytic anaemia, HDN, haemolytic disease of the newborn, NZB, New Zealand Black.