HHS Public Access

Author manuscript

Transfusion. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Transfusion. 2015 July ; 55(0): S47–S58. doi:10.1111/trf.13147.

Pathogenesis and mechanisms of antibody-mediated hemolysis

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Abstract

Background—The clinical consequences of antibodies to red blood cells (RBC) have been studied for a century. Most clinically relevant antibodies can be detected by sensitive in vitro assays. Several mechanisms of antibody-mediated hemolysis are well understood. Such hemolysis following transfusion is reliably avoided in a donor/recipient pair, if one individual is negative for the cognate antigen to which the other has the antibody.

Study design and results—Mechanisms of antibody-mediated hemolysis were reviewed based on a presentation at the Strategies to Address Hemolytic Complications of Immune Globulin Infusions Workshop addressing intravenous immunoglobulin (IVIG) and ABO antibodies. The presented topics included the rates of intravascular and extravascular hemolysis; IgM and IgG isoagglutinins; auto- and alloantibodies; antibody specificity; A, B, A,B and A1 antigens; A1 versus A2 phenotypes; monocytes/macrophages, other immune cells and complement; monocyte monolayer assay (MMA); antibody-dependent cell-mediated cytotoxicity (ADCC); and transfusion reactions due to ABO and other antibodies.

Conclusion—Several clinically relevant questions remained unresolved, and diagnostic tools were lacking to routinely and reliably predict the clinical consequences of RBC antibodies. Most hemolytic transfusion reactions associated with IVIG were due to ABO antibodies. Reducing the titers of such antibodies in IVIG may lower the frequency of this kind of adverse event. The only way to stop these events is to have no anti-A or anti-B antibodies in the IVIG products.

> … except for the immunohematologist's work on the erythrocyte, little has appeared in the literature on membrane-bound complement components of other cells.[1]
>
> George Garratty, 1980.

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Conflict of interest disclosure: The author does not have a conflict of interest relevant to this article.

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Authorship contribution: This manuscript represents the author's thoughts on the subject and his interpretation of a set of slides prepared by Dr George Garratty (Supplementary Material) as presented in the transcript of the Strategies to Address Hemolytic Complications of Immune Globulin Infusions Workshop organized by FDA/PPTA/NHLBI in Bethesda on January 28, 2014.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Slide set. George Garratty: Pathogenesis and mechanisms of immune hemolytic anemia. January 2014

Introduction

The immune mediated destruction of circulating red blood cells (RBC) is described by two distinct mechanisms: one is the intravascular destruction of RBC by complement lysis, which is initiated by antibodies that are often, but not exclusively, of IgM class. The second mechanism is extravascular destruction by immune cells, which recognize IgG and complement bound to RBC. If an IVIG product is free of IgM, intravascular hemolysis should be rare. There is, however, evidence that IgG, purposefully in high abundance in IVIG products, can induce intravascular hemolysis.

If IgM is bound to a RBC in the circulation, the complement cascade may become activated and punctures the membrane, causing intravascular hemolysis. The required complement factors are contributed by the patient and not found in IVIG products. Unless large amounts of C3b are generated to combine with C5, complement activation does not proceed to lysis. IgG bound to a RBC can mark the RBC for extravascular destruction, which occurs in the reticuloendothelial system (RES, also known as mononuclear phagocyte system) of primarily the spleen and liver and is effected by macrophages (Fig. 1).[2]

In theory, IgM antibodies are expected to activate complement more readily than IgG antibodies.[3] However, two IgG molecules close together on the RBC surface can activate complement. Hence, in practice the nature and distribution of antigen sites seems to be more important than immunoglobulin class, because the ability of RBC alloantibodies to bind complement is closely related to blood group specificity.[4]

Rate of hemolysis

The clinically important difference between the 2 mechanisms of hemolysis is the maximum rate of RBC destruction. Extravascular hemolysis is limited to 0.25 ml packed RBC/kg/hour by the capacity of the RES. For example in a 70 kg patient, 18 ml of packed RBC can be destroyed in 1 hour and more than 400 ml in 24 hours. Such an amount of cell destruction may impair the ability of the organ systems to cope with the patient's underlying condition. While this hemolysis burdens the system, it is a rather slow process and typically not life threatening. In contrast, intravascular hemolysis can destroy 200 ml of RBC or more in 1 hour. A drop of hemoglobin by 5 g/dl may occur within hours, which can be fatal if not rapidly adjusted by compatible RBC transfusions.

Not many antibodies destroy RBC intravascularly by a complement-mediated mechanism.[5] Only anti-A and anti-B destroy RBC commonly this way; other antibodies, such as anti-Jk^a, -Jk^b, Vel, -PP1Pk (formerly -Tj^a), and -Le^a are capable of causing intravascular lysis on rare occasions, but few others are capable of activating complement efficiently enough to form the membrane attack complex.[5]

IgM- or IgG-mediated intravascular hemolysis can cause anemia at 10 g/dL without any need for transfusion, but transfusion should be considered at 7 g/dl or less. Even if a patient's hemoglobin concentration drops precipitously from 10 g/dl to 5 g/dl, there may not necessarily be an urgent need to transfuse depending on the patient's vital signs. The situation differs drastically if the hemoglobin concentration is 7 g/dl before hemolysis and a

drop to 2 g/dl is imminent; in this scenario transfusion will be needed urgently. Such clinical occurrences are not rare, but because they do not have to be routinely reported, they are not systematically recorded in aggregate form.[6]

In practice, the risk of reaching critically low hemoglobin concentrations will increase as patient blood management (PBM) prompts us to lower the transfusion trigger to transfuse less and patients with 7 g/dl are seen more frequently. Consequently, the clinical response time to an incipient intravascular hemolysis will be shortened in patients with significantly lower hemoglobin concentrations. These considerations exemplify that the topic of this Workshop was very timely[7–12] and may become even more relevant over time. Also, the incidence of immune hemolysis induced by IVIG has increased[13,14] and is of particular concern in smaller patients, especially children.

Monocytes and molecules involved in hemolysis

Cell-mediated hemolysis requires recognition of RBC and binding to monocytes or macrophages, which control extravascular hemolysis. Several proteins are involved in marking RBCs for recognition by macrophages (Table 1).[15] The Fc-receptor is critical in binding RBC coated with IgG. Accumulation of activated complement proteins on the RBC surface, so effective in causing antibody-mediated intravascular hemolysis, also contributes to cell-mediated hemolysis as the 2 complement proteins C3b and iC3b are recognized by macrophages and facilitate RBC binding.

Once a macrophage has bound a RBC, it has 3 ways to effect hemolysis of the RBC (Fig. 1): (i) the RBC is engulfed and destroyed inside the macrophage (MΦ); (ii) the RBC is fragmented as the macrophage nibbles fragments off the RBC membrane, leaving the rest of the RBC to eventually detach and float in the blood, where it can be visualized on a smear as a spherocyte, which may have shortened survival; and (iii) the RBC stays outside of the macrophage and is lysed by antibody-dependent cell-mediated cytotoxicity (ADCC),[16,17] in which the macrophage secretes toxic substances lytic for the attached RBC.

All 3 pathways begin with the adherence of RBC to macrophages. The Monocyte Monolayer Assay (MMA) can be applied in clinical diagnostics[8,18–22] and measures adherence or phagocytosis or both, but not ADCC. A few other cell types may mediate hemolysis with or without involvement of antibodies (Table 2),[1,16,17,2324–2627–3031–3435–37] whose effects are not as well studied as those of monocytes. The ADCC assay, which can be either lymphocyte- or monocyte-driven,[28–30] has been used to measure the 3rd pathway, but is not often applied in clinical diagnostics.[38]

If complement proteins attach to the RBC surface without inducing hemolysis directly or by cell recruitment, the complement degrades and the C3d,g component remains attached to the RBC for the remainder of its life span without much impairing of its survival. Up to 2 – 3 months after the event, complement-coated RBC continue to be detected by anti-C3d in the direct antiglobulin test (DAT),[39] a very sensitive, if unspecific, assay for a previous hemolysis that involved the activation of complement on the RBC surface.

Pathogenicity of antibodies

The clinical effect that an antibody can exert depends on many factors, including antibody class, antibody subclass, antibody specificity, thermal range, complement activating efficiency, and affinity. Only a few of these characteristics are routinely tested.[8] Even when such factors are quantified, correlation with clinical outcomes is often weak because of the complexity of the factors and their mutual interaction. Polymorphism of proteins, for instance, even when rare in a population for any single protein, may become relevant when a large number of proteins interact. Personalized medicine may allow further insight in the future.

The relationship between thermal range and complement activating efficiency is on example of this type of interaction. The thermal range of antibodies, such as cold agglutinins (often IgM, reacting at 4 °C up to 25 °C) or warm antibody (often IgG, best reactive at 37 °C but may react at 22 °C or below),[40] correlates with the efficiency of complement activation, known to require antibody binding at 25 °C or above, and can be tested when indicated. Most autoimmune hemolytic anemias (AIHA) are caused by warm-reactive autoantibodies of IgG type (warm AIHA)[41] and approximately 15% by cold-reactive antibodies of IgM type (CAD – cold agglutinin disease).[42] Cold-induced IgG intravascular hemolytic anemia (PCH – paroxysmal cold hemoglobinuria) is now rare and based on a special type of cold-reactive (Donath-Landsteiner) antibody, binding best to RBC at less than 20 °C but activating complement efficiently above 25 °C.[15] Approximately 0.1% of donors have warm-reactive autoantibodies, which are not clinically relevant when diluted in plasma pools, and approximately 1 % of donors have cold-reactive autoantibodies, most of which are clinically irrelevant. Specific tests for antibody class (IgG, IgM etc.), subclass (IgG_1 etc.) and affinity are not widely applied in transfusion medicine laboratories. More reliable and clinically relevant, however, is the antibody specificity, which is widely tested in the routine clinical setting.

Antibody specificity

The antibody specificity is most informative because it defines the (cognate) antigen that the antibody binds and, as much is known about the distribution of antigens in tissues or body fluids and their quantity on the RBC membrane and other cells, specificity can therefore predict pathogenicity. A few of the 35 blood group systems recognized today are restricted to RBC only, although many are expressed on other tissues as well (Fig. 2).[43–45] ABO antigens are widely expressed and often to a greater degree on tissue cells than on RBC. These antigens are also soluble and present in plasma and other bodily fluids in persons who “secrete” their ABO antigens, e.g. 80% of Europeans.

Immunoglobulin binding to any of the blood group antigens foreign to the carrier is called an alloantibody. Anti-A, anti-B and anti-A,B are more commonly called isoagglutinins.[46,47] They were formerly labeled as hemagglutinins[48,49] or isohemagglutinins[46] for their agglutination effect in vitro and isolysins[50] or hemolysins,[48–50] if they showed hemolytic activity in vitro as well.

Currently, 35 blood group systems and 342 antigens are recognized. The antigens of 7 blood group systems are constituted by carbohydrates; 4 other blood group systems involve the complement cascade, the CR1 complement receptor and 2 proteins (CD55 and CD59) that attenuate the complement attack on the RBC surface (Table 3).[51] The complement component C4 (Chido/Rodgers) is actually not synthesized by erythroid cells; rather the soluble protein is adsorbed onto the RBC surface from plasma of all persons expressing the protein.

RBC lacking Cromer (CD55)[52] or CD59[53,54] cannot attenuate a complement attack and are more prone to hemolysis.[55] Patients with inherited deficiencies are rare, but those harboring a paroxysmal nocturnal hemoglobinuria (PNH) clone with a subclinical or smoldering susceptibility to hemolysis are more common, lack the 2 aforementioned complement regulators and may manifest themselves when their pathologic RBC are exposed to an antibody challenge,[15] such as by IVIG infusion.

Antibody quantity

While not the sole determinant of an antibody's pathogenicity, the clinical effect of any particular antibody will invariably be greater when it presents in larger quantity, typically gauged as a "high titer".[47] A "titer" is defined by the agglutination strength (reciprocal of serial dilution) of an antibody preparation.[14] Much data have been gathered recently for the titers of ABO antibodies in blood products[7,13,14,56–58] mainly in an effort to limit the adverse effects of platelet apheresis products.[10,59] Few donors have high titers (non-Europeans or previously pregnant), which may vary over time,[60] but those with excessive titers contribute inordinately to adverse effects[12,61] and eventually to the ultimate amount of ABO antibodies in IVIG products.

Only 1% of healthy donors may be expected to have antibodies against common blood group antigens other than ABO. These antibodies can be routinely tested for and products eliminated if titers are high or clinically relevant.[7,58,59,62,63] Other healthy donors may have antibodies to blood group antigens of low prevalence (1% or less in the population).[64] The titers can be high and clinically relevant.[65,66] Because antibodies to a low prevalence antigen are not systematically screened,[64] they are bound to occur in large donor pools. Such an IVIG batch administered to a large patient cohort can eventually result in an inadvertent match between the antibody and a patient with the low prevalence antigen. Antibody-mediated hemolysis by non-ABO antibodies can easily be missed without a proper evaluation of adverse effects.[67,68] Hitherto unknown side effects continue to be recognized.[69]

While antibodies cannot be eliminated by pooling, they do become diluted in large pools of donor plasma and titers are reduced in predictable ways. The limit of dilution is defined by the frequency of donors with antibodies,[65] particularly donors with high titers.[12,47] Enlarging the donor pool can only reduce the titers to some extent. Eventually one donor's plasma carrying a high titer is added to the pool and thwarts the effect of dilution. The process to reduce antibodies becomes more efficient if donors are tested for antibodies;[70] then, all donors with high titer or against low prevalence antigens can be excluded.

ABO antigens and A1 and A2 phenotypes

The ABO antigens A and B exist in several variations on the RBC surface.[46,71] The A or B type 1 antigens can be acquired from the plasma onto RBC in “secretor” persons. A and B type 2 antigens are the dominant ABO antigens on RBC and the primary target of clinically significant isoagglutinins. The A type 3 exists on all A cells (there is no B antigen equivalent) and A type 4 only on those of the A1 phenotype; antibodies directed against these low quantity RBC antigens are clinically insignificant.

The chemical basis of the A1 and A2 phenotypes has been resolved recently.[72] While the A1 phenotype is characterized by increased amounts of A antigen, there also exists a minor structural difference compared to the A2 phenotype: A1 individuals carry small quantities of A type 4 antigen and A2 individuals appear to lack this antigen (Fig. 3).[71,72]

The antibody commonly known as anti-A1 is directed against the A type 4 antigen and could be used to differentiate the A1 and A2 phenotypes. However, the routinely used laboratory reagents are usually diluted lectins that discriminate the A1 and A2 phenotypes purely on a quantitative basis, with A1 individuals having more A antigens and less H antigens than A2 individuals. A2 is a blood group phenotype, not an antigen. Reagents designed to detect the A2 phenotype are in fact anti-H reagents and a label “anti-A2 reagent” is formally incorrect. The amounts of the H and A types 2 and 3 and the presence of A type 4 determine the difference between the A1 (Fig. 3, upper panel) and A2 phenotypes (Fig. 3, lower panel).

Blood donors of blood group phenotypes O or B always have anti-A directed against all variations of the A antigen. Donors of A2 or A2B phenotype can have anti-A1 (anti-A type 4), sometimes of IgG type.[73] Most anti-A1 are not clinically relevant. Anti-A,B binds to the common regions shared between the A and B antigens. A plasma pool from a large number of donors would naturally contain a mixture of antibodies to all variations of the A and B antigens (Table 3). Hence, ABO antigen minutiae[74] may be considered if antibodies are to be removed completely from an IVIG product by absorption to ABO antigens. Fortunately, most of these antibodies show significant cross-reactivity and a single adsorbent may remove them, be they directed against A types 1, 2, 3 and 4 or B types 1 and 2.

Complement and immune cells

Apart from antibodies, the 2 remaining critical players in hemolysis are complement and immune cells (Table 2). Activation of complement proteins and their binding to the RBC surface is the major cause of intravascular hemolysis,[39,75] and can also lead to permanent extravascular sequestration by the reticuloendothial system (RES) with subsequent phagocytosis by macrophages. A temporary sequestration in the RES may shorten RBC survival. If the complement activation is aborted and only C3d,g residues remain on the RBC surface (Table 1), the RBC survival is considered normal, although C3d has been reported to facilitate phagocytosis by monocytes.[76,77] Complement fragments induce a multitude of other effects including cell activation, which may led to life-threatening systemic inflammatory response syndrome (SIRS).[78]

Cell-mediated hemolysis is modulated by the activity of the RES and its macrophages. The macrophages can be hyperactive in clinical situations such as infection, inflammation,[79] autoimmune hemolysis, sickle cell disease, thalassemia, or cytokine release.[8,78,79] They can become hypoactive, when their Fc-receptors are blocked by immune complexes (systemic lupus erythematosus), maternal anti-HLA or by certain drugs (corticosteroids).

Monocyte Monolayer Assay (MMA)

Originally described in the early 1980s, the MMA was applied to determine the pathogenicity of alloantibodies[18,19] and autoantibodies.[20,21] Optimization and validation allowed the assay to distinguish between clinically significant and insignificant alloantibodies of IgG type.[80] IgM cannot itself provide a second signal for phagocytosis (Table 1), and IgM-mediated RBC-bound complement is an inefficient stimulus for phagocytosis.[4]

Dr. Garratty summarized his 20 years' experience with the MMA in 2004:[81] An MMA was considered negative when ≤ 5% reactivity was observed, that is monocytes with adhering RBC or phagocytized RBC or both. A negative MMA indicated that incompatible blood could be given without risk of an overt hemolytic transfusion reaction, although it does not guarantee a normal long-term survival of the transfused RBC. Only specialized laboratories offer MMA, and even health care systems in large Western countries may have no routine access to the assay. Assays for hemolysis induced by other cells (Table 2) are not readily available for clinical use and, even if antibody-mediated, hemolysis effected by cells other than monocytes and macrophages remains difficult to gauge. Adverse reactions to IVIG that cannot be explained solely due to ABO incompatibility may yield insight into the other cellular mechanisms, if systematically evaluated by specialized laboratories.[7,8,79] Such research might improve our understanding of hemolysis and contribute to blood product quality generally, even beyond IVIG products.

Transfusion reactions caused by non-ABO antibodies

The appearance of a non-ABO antibody within one month of a transfusion may represent either its passive infusion from the donor plasma, or an anamnestic immune response in the recipient. The latter phenomenon is also known as a delayed hemolytic transfusion reaction (DHTR) or, if there is no apparent hemolysis, a delayed serologic transfusion reaction (DSTR). The proportion of anamnestic immune responses to transfusions that cause hemolysis is not always easy to determine, but has been estimated in several studies to be approximately one third.[82,83] However, the presence of hemolysis can be obscured by other disorders with laboratory signs mimicking hemolysis, for example liver disease, or by blood loss.[39] When hemolysis does occur, it is often considered to be of minimal clinical consequence, but clinicians may fail to recognize the subtle effects such hemolysis may have on the patient's underlying condition. Only if the antibody-mediated hemolysis is the single or clearly attributable factor for an adverse effect or death, does the transfusion reaction become reportable to the Food and Drug Administration (FDA). This may explain a possible under-reporting of fatalities secondary to non-ABO antibodies, as is widely perceived by practitioners of transfusion medicine.[9,84]

Despite a possible under-reporting, non-ABO antibodies still represent the second most commonly reported cause of fatal transfusion reactions in the hemovigilance systems of many large Western countries,[9,85–87] including the US.[88] If under-reporting was considered,[9] non-ABO antibodies might represent the most common cause of all fatal transfusion reactions today, exceeding by far the fatalities due to transfusion transmitted infections.

Parenthetically, in the US the most common cause of fatal transfusion reactions is transfusion-related acute lung injury (TRALI), a clinical syndrome reportable without any evidence of antibodies in the blood product.[89] Because no causative agent needs to be discerned in the product, there may be a fatality properly reported as TRALI and attributed to blood products which did not actually cause any transfusion reaction. Such false attributions cannot occur with antibody-mediated hemolysis in the current reporting practice, and it may therefore be conjectured that TRALI is in fact not a more common cause of transfusion-related mortality than immune-mediated hemolysis.

Hemolysis caused by ABO antibodies

Transfusion of ABO incompatible RBC

The third most common cause of fatal transfusion reactions are hemolytic transfusion reactions caused by ABO antibodies following ABO incompatible RBC transfusion.[88] These could theoretically be eliminated completely, as all ABO incompatible RBC transfusions occur by error.[90] Such transfusions are lethal for the patient in approximately 10% of cases.[91] Fortunately, approximately half of the involved patients have no adverse effects[91] and remain asymptomatic.[87,92] The clinical outcome depends very much on the volume of ABO incompatible RBC transfused. When recognized early by a severe reaction, and stopped after a few milliliters, survival is excellent. RBC volumes of greater than 50 mL are associated with a lethality of 20%.[91] If transfusion continues because reactions are masked, such as by anesthesia, severe and lethal transfusion reactions become very likely. Even the many patients who survive may well suffer permanent organ damage, which is important to consider. Regarding any ABO incompatible exposure, the objective is not only that the patient survives or has no obvious reportable side effects; we aim to avoid all permanent side effects from incompatible ABO antigens and antibodies.

Transfusion of ABO incompatible plasma or IVIG

Exposure to the antibodies of mixed Ig types (IgM, IgG and IgA)[93] in ABO incompatible plasma is rarely fatal.[12,61,62] The risk from IVIG products may differ as the predominant antibodies are IgG type. When incompatible antibodies are transfused by plasma, platelet products or IVIG, the soluble A and B antigens compete with the cellular A and B antigens in “secretor” persons, less so in “non-secretor” persons,[8,11,94,95] and may inhibit the hemolytic effect of IgM antibodies, less so of IgG antibodies,[96] in some patients.[79] Incompatible ABO antibodies are also captured by various tissue cells (Fig. 2), which further dampens a hemolytic effect. However, many clinical effects remain unknown;[8,46] whether they may reduce glycosylated plasma protein concentrations, generate immune complexes,[97] or damage endothelia is yet to be demonstrated.[98]

The theoretical risk of hemolysis following the infusion of ABO incompatible antibodies may be stratified based on the recipient's ABO phenotype and the attenuation by soluble A and B antigens in secretors. This predicted risk does not differ much among ethnicities, despite the wide variability of ABO and Secretor phenotypes (Table 4).[43,459599,100101] Up to 40% of recipients may have a high risk to develop hemolysis according to this model, while more than 40% have no risk at all. The risk can be evaluated individually by *ABO*, *FUT2* (*SE*)[99,100] and *FUT3* (*LE*) genotyping[99] using molecular immunohematology methods, which are particularly useful after recent transfusions.[102]

Do we understand immune RBC destruction?

Dr. Garratty summarized his experience once by stating, "Nevertheless, after more than 30 years researching this area, I am sometimes embarrassed to realize how much I cannot explain" [2] and listed questions that are pending answers by ongoing research.

Why do some auto- and alloantibodies in blood products with low titers cause severe hemolysis, whereas some antibodies of the same specificity in blood products with high titers do not? Do differences in the clinical severity and treatment response relate to the relative efficiency of macrophage-induced phagocytosis versus cytotoxicity? Despite many proposed mechanisms, why is there no conclusive test predicting a hyperhemolytic transfusion reaction in patients with sickle cell disease?

In addition, how do we define "clinical significance"? Is it safe to always consider delayed serologic transfusion reactions as clinically *in* significant? What defines the threshold to consider a hemolytic transfusion reaction as clinical significant? After all, both types of transfusion reactions damage tissue to some degree and it depends on the patients and their clinical conditions as to how well the inflicted damage is tolerated acutely and long term.

Concluding remarks

Most reported hemolytic transfusion reactions associated with IVIG are due to ABO alloantibodies, and although reducing the titer of the anti-A and anti-B in IVIG will help lower the number of cases with hemolytic anemia, there will remain a few cases associated with low titer in the blood product. The only way to stop ABO mediated hemolytic transfusion reactions is to have no antibodies against any of the 4 ABO antigens A, B, A,B and A1 in the blood products. Perhaps cases of IVIG-associated hemolysis will continue to occur even if the products contain no anti-A and anti-B? It may turn out that there are non-ABO antibodies or other factors in the products causing hemolysis, and this possibility should eventually be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The author dedicates this publication to the memory of Professor George Garratty PhD FRCPath, who died on March 17, 2014 at the age of 78. Being an iconic figure in the areas of serology and hemolysis, George had a

lifetime of contributions to these fields until his last year of life. He shared much of his insight as editor, thus contributing to the improvement of very many publications. George will be remembered most of all by his sincerity.

The author expresses his appreciation to the FDA for the honor of having been chosen to deliver Dr Garratty's presentation, who had to decline participation on the weekend before the Workshop, and thanks Stephen M Henry and Franz F Wagner for advice in preparing Figure 3; and also Donald R Branch, Gregory A Denomme, Harvey G Klein, Urs E Nydegger, Kshitij Srivastava and Ingeborg von Zabern for review and helpful comments; Allan B Hoofring and David A Stiles for help with Figure 3; and Elizabeth J Furlong for English edits.

This work was supported by the Intramural Research Program of the NIH Clinical Center.

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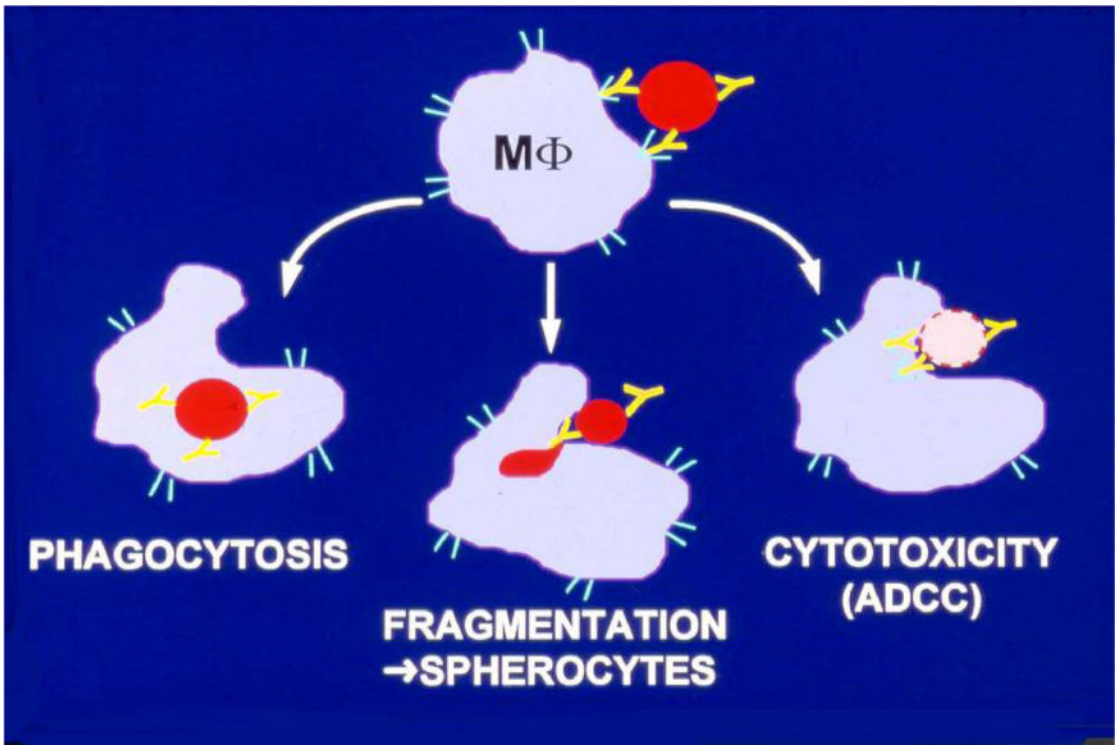

Figure 1.

Antibody-mediated destruction or alteration of a red blood cell (RBC). An effector cell recognizes an RBC by antibodies that are bound to the RBC's cell membrane. Three mechanisms can lead to the RBC's destruction or alteration: (i) An RBC is engulfed by a macrophage (MΦ) and lysed intracellularly (phagocytosis). (ii) An RBC is partially phagocytized (fragmentation), but the altered RBC (spherocytes) escapes the immune attack by the macrophage (MΦ) and remains circulating. (iii) An RBC is attacked by a macrophage (MΦ) and lysed extracellularly (ADCC, antibody-dependent cell-mediated cytotoxicity). Modified from Garratty.[2]

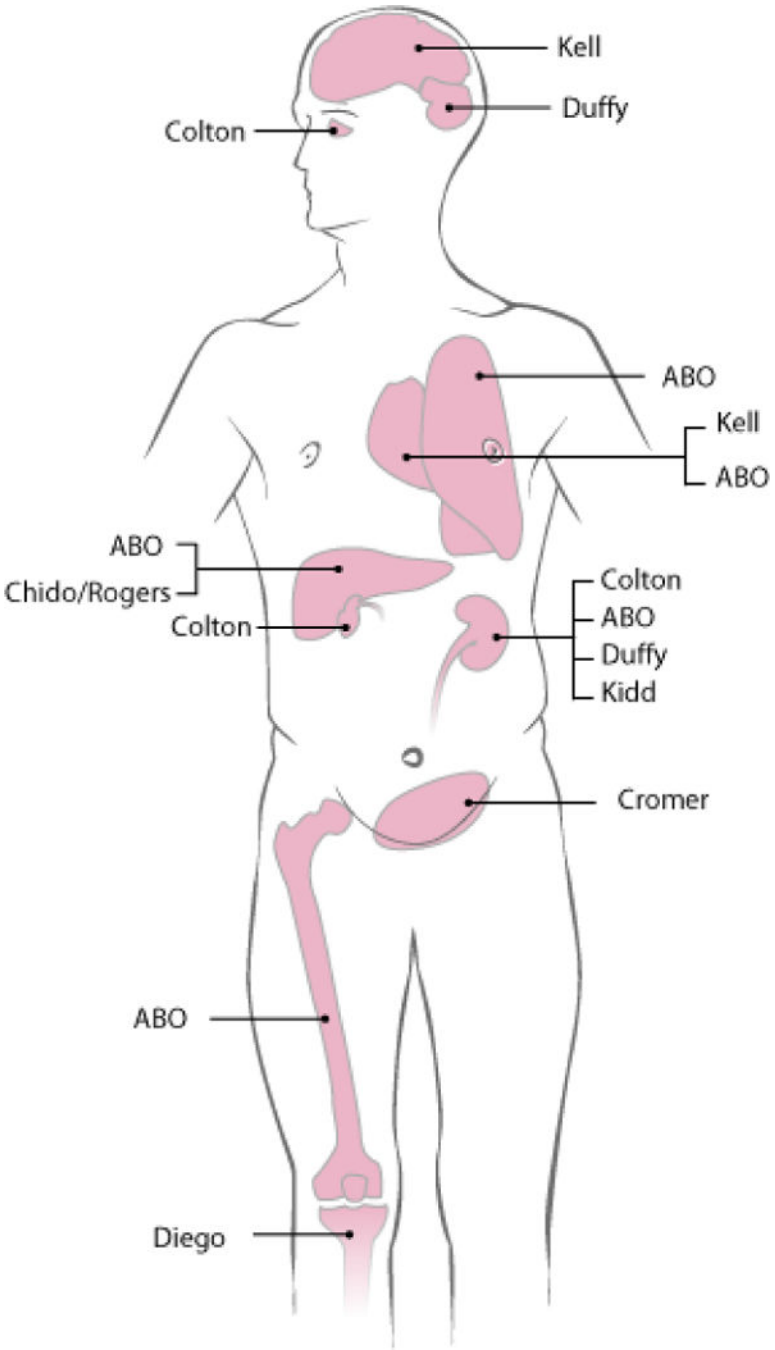

Figure 2.

Organ distribution of blood group antigens. A schematic representation for the antigen distribution of some clinically relevant blood group systems is shown. ABO antigens are formed by carbohydrates expressed on the surfaces of many cells in the human body. When an individual carries active ABO genes, the ABO antigens are expressed on red blood cells (RBC) and even more strongly on some tissues other than RBC, such as kidney, lung, liver and intestine. The ABO antigens are also soluble in the plasma of persons and then attach

passively to cells that do not express the ABO antigens by themselves.[43] Modified from Nydegger et al.[44] and reprinted with permission.

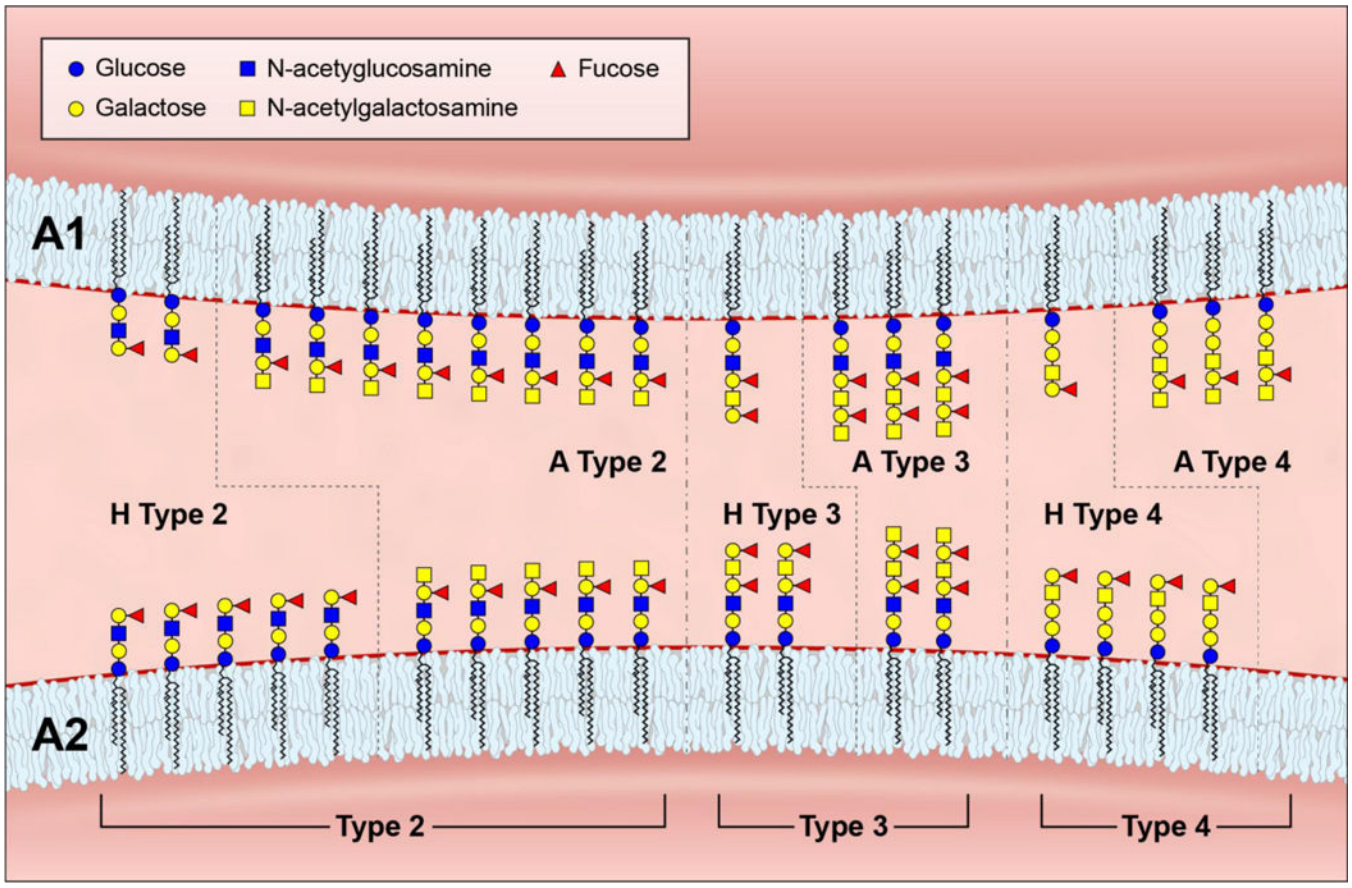

Figure 3.

Schematic simplified model of the glycosylation patterns in the A1 and A2 blood group phenotypes. The elongated, branched and repetitive carbohydrate structures on the RBC surface end in various types of H and A antigens: In an A1 phenotype (upper panel), most of the H antigens (types 2 to 4) are transformed to their A antigen analogues (types 2 to 4). The A type 4 antigen represents the A1 antigen.[72] In an A2 phenotype (lower panel), many H type 2 antigens remain unchanged and are the targets of anti-H lectins; also, most H type 4 antigens remain unchanged. Hence, the number of A type 2 and 3 antigens is reduced and the A type 4 antigen is lacking in an A2 phenotype. The Secretor/Lewis antigens (H and A type 1 antigens, not shown) are adsorbed from plasma onto the RBC surface in Le(a-b-) secretors and occur in ratios like the H and A type 4 antigens. The antigens are shown as glycolipids in their simplest form; the same antigens also exist on glycoproteins and commonly as large extended glycans with greater than 60 residues (not shown). The ratios among the antigens are not absolute but for demonstration purposes only. Modified from Flegel and Wagner[71] according to Svensson et al.[72]

Table 1

Examples for molecules involved in the bonding between RBC and monocytes/macrophages

Receptor on monocytes/macrophages*	Proteins that can be bound to the RBC surface
Fc	IgG_1, IgG_3, IgA, possibly IgG_2
CR1	C3b, iC3b†
CR3	iC3b
CR4	iC3b

*Monocytes, floating in the blood stream (intravascular), and macrophages, residing in tissues (extravascular), are the same cell type.

†iC3b is an inactive derivative of C3b and degrades to C3d,g, which is the last step in vivo and can remain attached to the RBC surface for the rest of the RBC's life span. In vitro, C3d,g can be further cleaved, using trypsin, to C3d.[4]

Fc – Fc-receptors. They exist in variations.[15]

CR1 – complement receptor 1, CD35, blood group system Knops

CR3 – complement receptor 3, CD11b + CD18, integrin alpha-M

CR4 – complement receptor 4, DC11c + CD18, intergrin alpha-X

Table 2

Immune cells known to cause hemolysis

	Hemolysis		
Cell type	**Phagocytosis & Fragmentation**	**ADCC**	**Cell type carries Fc-receptors**
Monocytes/macrophages*	Yes[1,16,17,23]	Yes[23]	Yes
Granulocytes†	Yes[24]	Yes[25]	Yes
Dendritic cells	Yes[26]	No[26]‡	Yes
Natural Killer (NK) cells	No[27]§	Yes[28–30]	Yes
Cytotoxic T cells//	No[31]	Yes[32–34]	Yes

*Macrophage names vary depending on the tissue: Kupffer cell (liver), Langerhans cell (dermis), microglia (brain), and osteoclast (bone). They also occur as subcapsular sinusoidal or medullary macrophages (lymph node) or splenic, bone marrow, intestinal, alveolar and intraocular macrophages.[35] Monocytes can differentiate into dendritic cells, which is a different cell type.[36]

†Neutrophils, eosinophils and basophils are called mast cells when found in tissues.

‡There is no ADCC by dendritic cells (APC – antigen presenting cells), forcing them to engulf cells, such as RBC, necessary for their antigen processing function.

§NK cells do not phagocytize RBC, but can function as phagocytes for other cell types.[37]

//also known as ‘Killer’ cytotoxic T cells

Table 3

Known blood group systems constituted by carbohydrates or complement-related proteins

Blood group		Carbohydrate or involved protein	Defined antigens	
ISBT number	System name		Number	Examples
1	ABO	Sugar	4	A, B, A,B and A1
3	P1PK	Sugar	4	P1PK1, P1PK3, NOR
7	LE	Sugar	6	Le^a, Le^b
17	Chido/Rodgers	C4a/C4b	9	Ch1, Rg1
18	H	Sugar	1	H
21	Cromer	CD55*	18	Cr^a
22	Knops	CD35†	9	Kn^a, McC^a, Sl1, Yk^a
27	I	Sugar	1	I
28	GLOB	Sugar	1	P
31	FORS	Sugar	1	FORS1
35	CD59	CD59‡	1	CD59.1

*DAF – Decay Accelerating Factor

†CR1 – complement receptor 1

‡previously described as HRF20 – homologous restriction factor, MIRL – membrane inhibitor of reactive hemolysis, and MACIF – membrane attack complex inhibitory factor

Table 4

Risk model for hemolysis caused by anti-A and anti-B in blood products, such as IVIG, based on ABO and Secretor phenotypes in the recipient

		Population*					
		East Asian		European		Sub-Saharan African	
Risk	**Recipient ABO and Secretor phenotype**	**Frequency**	**Total**	**Frequency**	**Total**	**Frequency**	**Total**
High	A1 or A1B, non-secretor†	3%	35%	7%	40%	7%	31%
	A2, B or A2B, non-secretor	3%		4%		9%	
	A1 or A1B, secretor	29%		29%		15%	
Low	A2, B or A2B, secretor	22%	22%	16%	16%	20%	20%
None	O, secretor or non-secretor	43%	43%	44%	44%	49%	49%
Total			100%		100%		100%

*Calculations based on published population data for ABO and Secretor genes: Secretor phenotype in sub-Saharan Africans was set at 90% (Europeans 80%, East Asians 90%[99]).[95,100]

†The highest risk in the general population may occur in non-secretor (genotype *FUT2*se* homozygous) recipients of A1 Le(a-b-) phenotype (genotype *ABO*A1* homo- or heterozygous and *FUT3*le* homoyzgous).[43,45,101] An even higher risk is found in patients carrying ABO antigens on their RBC but not on their tissue cells nor soluble in their plasma,[11] which is a rare acquired condition such as after hematopoietic progenitor cell transplantation.[8]