Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

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Dr. Phillip Sturgeon A Pioneer of Automated Red Cell Typing, Reference Laboratories, and Rare Donor Registries

In this edition of *Immunohematology,* Dr. Phillip Sturgeon describes the beginning of automated blood typing. It is not often that one can read personal reminiscences of the origins of technical advances that revolutionized our field.

When I arrived at the Los Angeles Red Cross in 1978, a modification of Dr. Sturgeon's machine was hard at work ABO and Rh typing more than 1000 donors per day. This machine was replaced around 1980 with a French machine (the Groupamatic) that utilized microplates. Eventually, this machine was replaced by a Japanese machine made by Olympus. More recent modifications of this machine are used for typing all donors at American Red Cross National Testing Laboratories.

From 1948 to 1960, Dr. Sturgeon was the Chief of Hematology Research at Childrens Hospital, Los Angeles. In 1960, he was made National Associate Director of the Red Cross Blood Program and Director of the western branch (in Los Angeles) of the National Research Laboratory. Soon after his arrival at the Red Cross, Dr. Sturgeon started a reference laboratory (Dorothy McQuiston was the chief technologist) and a rare donor file. By 1963 he had a file of 10,000 donors with unusual blood types, and he was satisfying requests from blood centers in other parts of the United States. His master plan was to have at least ten other Red Cross centers doing the same type of work. He left the Red Cross in 1966 to become Professor of Pediatrics and Head of the Division of Hematology at the University of California in Los Angeles (UCLA), a position he held until 1973. He then carried out research at Cedars Sinai Hospital until 1980. During that time, I was honored to be a coauthor of one of his papers about an IgA Rh autoantibody (*Transfusion* 1979;19:324).

In addition to his seminal work on automation, Dr. Sturgeon published many important immunohematology studies. He contributed to our understanding of the serology, hematology, and biochemistry of "permanent in vivo mixed field agglutination" (polyagglutination). He was the first to describe the Lewis blood group substance, Le^x, and to describe the hematological abnormalities (e.g., stomatocytosis) associated with Rh_{null} phenotype.

Dr. Phillip Sturgeon is an extraordinary person and scientist. He is still contributing (by writing his scientific memoirs) while in his 80s, in retirement in Switzerland. In addition to his more than 122 scientific publications, Dr. Sturgeon has published articles on his experiences (e.g., skiing and trekking) in the Swiss Alps and the Himalayas. Selected titles of articles are: Mani Stones and Mantras; A Tibetan-English Beginner's Dictionary (published in 1999); Shopping in Kathmandu (published in 2000); and a letter to the editor (published in 1999) with the fascinating title Abdominal Recollections (The Loo at Nepalgunj)! He also is working on a biography of his father, Rollin S. Sturgeon, Sr., who was one of Hollywood's three movie pioneers.

In addition to all the above accomplishments, Dr. Sturgeon was awarded the Bronze Star and the Purple Heart in World War Two.

Young immunohematologists have to look no further than this man for an inspirational model.

George Garratty, PhD, FRCPath
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Automation: its introduction to the field of blood group serology

P. Sturgeon

Automation of blood typing was a thing of the future when I joined the American Red Cross (ARC) in Los Angeles, California, in 1960. Automation had found its way into a variety of medical laboratory procedures over the previous decade, but blood typing somehow had escaped. For each bottle of labeled blood released from a routine blood bank, the donors' cells and sera had to be tested using sera and cells of known types. Dozens of methodical, repetitious, time-consuming—not to mention boring—hand manipulations made automation's time long past due. Over the course of the next several years, not only was the need for automation met but a valuable tool was born, which opened up avenues of research applicable in both applied and basic medicine.

Development of the Single-Channel Prototype

Dr. Sam Gibson called me from his office at the ARC's National Headquarters in Washington, DC, to inform me of a 3-year research project that Dr. Creighton McNeil, a physician consultant to the Red Cross' Blood Bank in Salt Lake City, was engaged in with the Technicon Instrument Corporation (TIC), Chauncey, NY, the leading manufacturer of equipment for the automation of medical laboratory procedures. They had been attempting to adapt such equipment to the automation of blood typing, with their attention focused exclusively on the ABO system. The work had been financed by the ARC but the results of their efforts, though tantalizing, fell short of the mark. They had just notified Dr. Gibson that they had come to a final, joint decision to abandon the project, but to publish the results of their efforts in the hope that someone else would pick up the research.[1] Dr. Gibson explained to me that, because of the time, effort, and expense that had gone into the project, it was his hope, albeit a forlorn one, that I might find a way to perfect the machine. He asked me if I would be willing to make a trip to TIC and have a look. That was indeed a compliment to me and a challenge. Although I was willing to try, I had to acquaint Dr. Gibson with one major problem: I had heard of the AutoAnalyzer; however, I had never seen one and had not the faintest idea how it worked. This, Dr. Gibson must have realized, could well prove to be an advantage, and he noted that the additional investment of time, effort, and money, compared with all that had already gone into the project, would be negligible. He bid me make my way forthwith to TIC.

Mr. William (Bill) Smythe (Head of Research and Development, TIC) showed me to his laboratory and explained the prototype of the blood-typing AutoAnalyzer. "Living spaghetti bolognese" was the first thought to cross my mind. There it was, a jumble of fine plastic and glass tubes that were squirming about as rock roller pumps circulated all of the ingredients necessary to a blood-typing reaction through the maze. Due to my experiences as a boyhood backyard mechanic who had worked on Model T Fords and other antique cars, and to Mr. Smythe's lucid explanations of the principles involved in continuous-flow automation used by the AutoAnalyzer, I was able, within a brief time, to grasp how the ungainly machine typed blood. The impasse that Dr. McNeil and TIC had not been able to escape was that the A_2B blood type slipped through as type B.

The prototype machine had the sensitivity necessary to detect types A, B, and O, as well as the weaker A_2 variant; however, when A_2 is a component of the AB complex, A_2 becomes even weaker, and the prototype machine lacked the sensitivity to detect it. An A_2B donor's blood would come out as type B, and were it to be given to a type B recipient, it would result in a transfusion reaction—possibly even a fatal one. Despite their 3 years of trying, type A_2B continued to elude Mr. Smythe and Dr. McNeil.

Mr. Smythe gave me a demonstration of how, using the anti-B typing serum, the machine gave correct reactions, i.e., negative with type O and A cells, positive with type B and AB cells. With anti-A serum, there were

The author displays a Multichannel Colorimeter Recorder AutoAnalyzer—circa 1963.

the expected negative reactions with type O and B cells and all type A cells gave positive reactions. However, some type AB cells did not; those were A_2B cells. The plan, following that demonstration, was to pack up the machine and ship it out to Los Angeles, where research on increasing its sensitivity would be undertaken.

At that point, I asked Bill Smythe to let me have a closer look at all of the reactions that were taking place as the test ingredients flowed into, and through, the machine. I followed them closely as they were progressively mixed in small glass coils and pumped along plastic tubes, and as agglutinates that formed were allowed to slowly settle out in large glass reaction coils. It was the latter reaction—that of agglutination—that was not taking place with the A_2B cells. With the traditional manual method of testing, in which a drop of a dilute suspension of red cells was mixed on a glass slide with typing serum, the reaction took place in a few seconds. "What was the difference? Where along the way through the machine was the sensitivity being lost?"

The typing serum being used in the machine was the same as that used in the manual test. The volume of serum to the volume of cell suspension being tested was also the same, and the degree of mixing and the incubation time were all in favor of increased sensitivity in the machine. However, a closer look revealed one significant difference. In the traditional manual test, a very dilute cell suspension in saline solution is prepared from the whole-blood sample before mixing the cells with the typing serum. With the machine, the cells were being taken directly from the whole-blood sample, a very heavy suspension of cells, and were being mixed directly with the typing serum. Thus, the volumes were essentially the same as those used in the manual test, but the proportion of cells in relation to the antiserum was in great excess.

I drew Mr. Smythe's attention to this discrepancy, and he agreed that it would be a simple matter first to draw the whole-blood aliquot into a dilution circuit and then to resample from that circuit into the prototype machine. He felt he could get it set up in a few minutes. Based on my experience with the manual procedure, I suggested a dilution of the whole-blood sample (which character-istically is approximately a 50% cell suspension) to a 2 percent suspension, a 25-fold dilution. Thus the strength of the typing serum, in proportion to the number of cells, would become 25 times as potent. Before the morning was out we had the results. An example of an A_2B blood gave unequivocally clear-cut reactions. The breakthrough had come. The Auto-Analyzer was indeed practical for typing in the ABO blood group system, and, if it could be further refined to work for the more exacting Rh system, automation could be introduced into blood banking.

A prototype machine, with the above modification, plus some additional mechanical refinements, was sent to the Los Angeles ARC. There we made fine adjustments to the environment within the machine in which the agglutination reaction took place. Those included adjustments in the concentration of the salt solution, cells, and antiserum; length of incubation time; temperature; etc.; or, in laboratory parlance, in the necessary *serologic conditions,* all of which added further to the sensitivity of the machine. Twelve type A_2B bloods all gave strongly positive results; in fact, among them was a very rare and extremely weak AB variant, type A_3B, and it gave a positive reaction as well.[2] However, for the more stringent demands of Rh blood typing, those improvements were not enough.

In ABO automated typing, as well as in the traditional manual testing method, the most favorable serologic conditions for the agglutination reaction to take place are obtained with the red cells in a saline suspension. The typing serum is also diluted in saline; that method is known as the *saline agglutination test.* With the discovery of the Rh factor, it became evident that, with the saline agglutination test, Rh positive cells would not give a positive reaction with most Rh antisera—even those that came from patients who had had fatal Rh blood transfusion reactions. Similar contradictions were soon to emerge with most of the other blood group systems coming to light at that time.

Attempts to find the laboratory (serologic) conditions that would consistently detect these relatively obscure blood types followed four main courses. One was to carry out the test with a very heavy suspension of cells in their serum and with the antiserum diluted in a strong concentration of albumin, the *albumin agglutination test.* It improved the sensitivity but still fell short of that required to detect D^u. Another method was to allow the interaction of cells and antiserum to take place in saline solution and then, following several careful washings of the cells, to test them for adsorbed antibody with a rabbit antiserum that reacted in saline—the *antiglobulin,* or *Coombs, test.* This proved to be the most sensitive of all; however, it held little or no promise of being adaptable to automation, at least in a continuous-flow system. A third method was to reduce, in the medium in which the reaction is taking place, the electrostatic repulsive forces (forces that tend to keep the cells and antiserum apart). Changes in the salt concentration produce such an effect, as does the addition to the medium of large-molecular-weight substances such as gum acacia or polyvinylpyrrolidone (PVP); that is, *macromolecular substances.* The fourth method was to reduce the electrostatic repulsive charge on the cell surface by a preliminary treatment of the cell surface with protein-digesting enzymes, *the proteolytic enzyme test.* It was self-evident that the latter two would be readily adaptable to the continuous-flow system of the AutoAnalyzer, hence quite appropriate for testing for weak Rh variants and for other blood group systems.

Thus the first-stage ABO-cell-dilution circuit was modified to include an enzymatic treatment of the cells with *ficin,* an enzyme from figs. The ficinized cells were drawn off into the second stage and split into the ABO circuit and the Rh circuit, where Rh typing serum was introduced. It worked; Rh positive cells reacted well, including several types of the weaker variants. But the weakest of them all, D^u, did not. This was overcome by taking advantage of the third option listed above. Gum acacia was the macromolecular substance used, and D^u cells reacted well.[2] Thus, the A_2B hurdle had been cleared and now the Rh and D^u hurdles were also cleared; automated blood typing, as required for routine blood banking, was at last a reality! Moreover, beyond the needs for routine blood banking, it was implicit in the above developments that the way was now open for the application of AutoAnalyzer continuous-flow technology to typing of antigens in other blood group systems,[3] as well as to fields of laboratory medicine and medical research.

Transition from the Single-Channel to the Multichannel Prototype

Whatever may have been the minute and precise details of how automation of blood typing and its reporting evolved, its research had, by the end of 1963, reached the point where the *single-channel* prototype—one capable of performing but one test—had to be expanded into "*…a multi-channel machine which can integrate all of the results [from] the several tests being performed upon a single sample of blood.*"[2]

The task sounded like a straightforward problem of mechanics; it simply required eight duplicates of the original prototype to be coordinated into a simultaneous operation. But when that first multichannel prototype was put to work, a host of problems emerged. To keep the eight channels in phase with each other (in order that all of the tests from a single sample of blood came out on the recorder simultaneously) required modification in the flow circuitry and some further serologic refinements. To keep glass tubes and coils clean and to prevent plug formation along the way, various wash solutions (detergents) were tried.

The serologic modifications essentially fell into the domain of the ARC in Los Angeles, and the mechanical modification fell to Mr. Smythe at TIC. But, by strange coincidence, the solution to a mechanical problem, which was taking on insurmountable proportions, fell into my hands. The probe that aspirated the cells from the original blood sample could become plugged by tiny blood clots that would occasionally form in the original sample. Then, in normal operation, during the interval between samples, when the probe was in the wash position and aspirating saline solution, the plug

would not release. Thus, from all subsequent blood samples, no further results could be obtained; in effect the machine was irreversibly shut down. Attempts at preventing or dissolving the plug clots with modifications in the washing fluids failed.

It occurred to me that the practical skills of a plumber had to be brought to bear on the problem. Why not try to keep the line clear by reversing the flow during the wash phase rather than introducing various cleaning solutions; i.e., to blow the plug back out of the probe. Knowing there were electronic valves that could automatically change flow directions, I brought this solution to the attention of Mr. Smythe. I suggested that he incorporate in the sampling line, immediately beyond the sample probe, a T-connection with a solenoid valve below. Then, between samples, in the washing phase, the valve could be programmed to direct a large quantity of saline solution into the system. That would come from a pump that would supply the amount needed farther along in the circuit and, in addition, a large excess, which in turn could only escape back through the probe. That was easier said than done, but Mr. Smythe took care of the details, and it worked.[4]

This was not just a glitch in AutoAnalyzer technology; the backwash principle now made it possible to present the device with whole-blood samples, as traditionally collected during routine blood donations. There would not have to be any extraordinary manipulations or precautions at the time of collection and, once the samples were in the laboratory, no preliminary manual loading into special sample cups would be required, nor would there have to be any washings or filtering. It held promise of permitting efficient testing of large-scale whole-blood sample collections from a normal blood-donor population. This would be the case not only for routine blood typing but also for research that required a large number of samples from normal populations. This little episode lingers in my memory as a quaint way in which knowledge that was not taught in medical school but was critical to medical research had to be called upon. Furthermore, it leaves me with some misgivings that, whatever may have been my contributions to the development of automated blood typing, the insertion of that T-fitting, in fact, may have been my most significant!

As the host of problems generated by the multichannel machine were overcome, a series of papers from my laboratory, identifying them and giving solutions, was published.[4-7] One of the remaining problem areas had its origin in the machine's two pieces of electronic equipment—the colorimeter and the recorder; there was one of each for each of the eight channels—16 in all. The colorimeter made an electronic measurement of the hemoglobin released from the red cells remaining after the agglutinates had been drawn off. The recorder traced out, as a series of separate peaks, the amount of the remaining hemoglobin detected by the colorimeter. A peak of full height meant a negative reaction, i.e., no agglutination had taken place. A flat valley meant a strongly positive reaction, i.e., all of the cells had been agglutinated and discarded. Peaks of intermediate height were a measure of agglutination reactions of intermediate strengths. With these two devices serving as the sensors of the blood typing reaction, the machine became known as the *colorimeter-recorder unit.* The interpretation of the tracings had to be done by visual inspection of the peaks and valleys; further processing of the data remained a clerical task as well.

At this juncture it became evident that at the rate at which the AutoAnalyzer could type blood, there would be a valuable saving in labor costs. A machine was put in service at the New York-New Jersey Red Cross Blood Center, where 10,000 bloods were tested in parallel with routine blood banking operations. It was observed that faults related to mechanical operations were the most frequently encountered, with incorrect phasing being the most common and time-consuming problem. The AutoAnalyzer colorimeter-recorder machine was determined to be a useful addition to the blood donor center's routine.[8] Nevertheless, it was also becoming worrisome that the complexity of the machine, with its demands for constant monitoring and adjustment of the phasing of the channels, was threatening to make the multichannel colorimeter-recorder unit too complex—and therefore impractical for routine blood bank use.

With those concerns in mind, it was Mr. Smythe alone who overcame the obstacle. In another application of Technicon Instruments, there was a very simple device that used a continuously moving broad belt of filter paper, and he recognized that it could be used to great advantage as the sensing system for blood typing. The agglutinates would be deposited on the filter paper for manual reading, and all of the associated complexities of manifolding required to get the remaining cells to the relatively ponderous colorimeters and recorders, including the latter two, could be done away with. Thus, a drastically revised system was devised. The serologic conditions did not have to be changed. The

agglutinates from each circuit were deposited as bright-red granular smudges in eight parallel rows on the moving filter paper. The nonagglutinated cells were drawn through the paper by vacuum and discarded. The absence of a smudge meant a negative reaction, i.e., no agglutination had taken place. A well-demarcated smudge meant a positive reaction, i.e., all of the cells had been agglutinated and were trapped on the filter paper. Further quantification of the smudges was not reliable; they essentially had to be read as either positive or negative.

A comparative study of the proficiency of the two machines was made by testing 3040 samples in the colorimeter-recorder machine and 4749 samples in the filter-paper machine. The latter proved to be faster, more efficient, and more reliable, and the consumption of reagents was one-third as great. Further, it was far less intricate, it had much less exposure to electrical and mechanical failures, it had less difficulty in keeping in phase, and its records were easily read and interpreted visually. It was estimated that two technicians operating one machine would be able, in one 8-hour day, to complete the ABO and Rh typing of all of the bloods collected by even the largest center in the world. Production models were soon under field trial in three American Red Cross Centers.[8,9]

Subsequent History of the AutoAnalyzer and New Technologies

It goes far beyond the scope of the present work to attempt a comprehensive history of automated blood typing subsequent to the events set out above. However, from the perspective of today's course of events, that is, one that sees the latest technological marvels almost outdated the day they reach the market, the history of automation did not follow an unusual pattern, albeit perhaps at a more relaxed pace. Sixteen years after those original publications on automation, it was estimated that 720 AutoAnalyzers had been sold.[10] Also of considerable note was that it was only 6 years later that the *Groupamatic* appeared. That was an entirely new device designed in France at the Centre National de Transfusion Sanguine, in Paris. This was more of a robotized version of manual test procedures; however, it worked well and had great speed, and data processing was readily incorporated in the system. To compete with the latter data processing capabilities, TIC introduced a new version of its machine, the *Auto-Grouper*, which similarly included a laser for reading sample numbers and integration of those with machine-interpreted results.[11] *"These two rival technologies for blood group automation and antibody quantitation were in fairly widespread use throughout the world in the mid-1970s."*[11] Suffice it to say, the problem of data processing proved to be a vastly more difficult one to solve than had been contemplated originally and, in retrospect, I am grateful indeed that it was my fate not to be drawn into it—hardly a field for one with a degree in medicine!

The late 1980s saw the introduction of yet another advance in technology, the *Olympus,* which used a unique micro-plate technology that does not require centrifugation.[12] Dr. George Garratty, Scientific Director of ARC Blood Services, Southern California Region, recently informed me that these machines are in current use in the ARC National Testing Laboratory and that the AutoAnalyzer was replaced about 1980.[13]

Thus, in the long view, it was in the range of 60 years after the discovery of blood types that automation was introduced into blood testing, and it took another 25 years for it to be perfected. Although contributions to the latter came from the efforts of scores of investigators working in laboratories of many nations, it was in the ARC and in the TIC laboratories that the pioneering discoveries were made. After a faltering start, it was from my colleagues' and my research that the technology entered a phase of rapid growth; this was essentially the opinion given by Peoples: *"The real breakthrough in practical automation for the blood donor center occurred when Sturgeon and co-workers*[5] *used polyvinylpyrrolidone…as a potentiator."*[11]

However, I believe that the time of the breakthrough antedated the introduction of PVP—so that it could be pinpointed more precisely, i.e., the time when, in 1960, Mr. Smythe and I were studying the faltering prototype machine in his laboratory. It was then that I suggested that the proportion of cells to antiserum needed major correction; and Mr. Smythe immediately took care of the problem. Because of that correction, we were privileged to observe for the first time a type A_2B blood give a positive reaction! Whatever may have been the short- and long-term consequences of that crucial event is probably difficult to say. However, it would not be too far-fetched to propose that, had the consequences not occurred, TIC would not have become involved in the automation of blood typing and the introduction of automation to blood banking could have been delayed for several years.

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Serologic aspects of treating immune thrombocytopenic purpura using intravenous Rh immune globulin

C.M. Savasman and S.G. Sandler

In patients with immune thrombocytopenic purpura (ITP), IgG autoantibody-coated platelets are phagocytized by mononuclear macrophages, primarily in the spleen. Intravenous Rh immune globulin (IV RhIG) has been used since 1983 to treat D+, nonsplenectomized patients with ITP. The beneficial therapeutic effect of IV RhIG is attributed to competitive inhibition of phagocytosis of IgG-coated platelets by IgG anti-D-coated D+ red blood cells (reticuloendothelial or Fc receptor blockade). Following infusions of IV RhIG in D+ ITP patients, the direct and indirect antiglobulin tests become transiently positive, reflecting passively transferred anti-D and other alloantibodies that were present in the infused IV RhIG. These consistent and predictable serologic findings contrast with the inconsistent and weak anti-D reactivity observed when D– women are treated with relatively small doses of intramuscular RhIG for Rh immunoprophylaxis. The pathophysiology of ITP and the effect of infusing IV RhIG in patients with ITP are illustrated in this review, using computer-generated figures. ***Immunohematology* 2001;17:106–110.**

Key Words: immune thrombocytopenic purpura, ITP, RhIG, Rh immune globulin, reticuloendothelial blockade, Fc receptors

When intravenous Rh immune globulin (IV RhIG; WinRho SDF™, NABI, Boca Raton, FL) is issued by a blood bank to treat a patient with immune thrombocytopenic purpura (ITP), it is the *only* time that a blood product is used with the specific intent of destroying the recipient's red blood cells (RBCs).[1] This unique circumstance has practical implications for interpreting postinfusion serologic test results and for selecting RBCs for transfusion in IV RhIG-treated patients.[2] The following review is intended to describe the rationale for treating ITP using IV RhIG; to illustrate the effect of IV RhIG, using computer-generated figures; and to provide suggestions for selecting RBCs if such patients require a transfusion.

Pathophysiology of ITP

ITP is an autoimmune disease in which immune dysregulation results in IgG autoantibodies with serologic specificity for epitopes on platelet glycoproteins.[3-6] Persons with thrombocytopenia may be classified as having primary or secondary thrombocytopenia. Persons with secondary thrombocytopenia may have immune destruction of platelets as a complication of another disease, such as systemic lupus erythematosus, Evans' syndrome, or an infectious disease (i.e., infectious mononucleosis); or they may have a disease due to decreased production of platelets, such as aplastic anemia. Some patients may have primary thrombocytopenia caused by immune destruction of circulating platelets for which no other illness has been diagnosed. In this situation the exclusion of other diseases is the basis for establishing the diagnosis of primary immune thrombocytopenia, i.e., ITP. Clinically, the most widely used classification categorizes persons with ITP as either children or adults. In this classification, the emphasis is on differences in the expression of ITP in children, who typically present with an acute and transient illness (i.e., less than 6 months' duration), versus adults, who typically present with an insidious, chronic course of thrombocytopenia (i.e., more than 6 months' duration). In children with ITP the proportion of affected males and females is approximately equal. In adults, as for all other autoimmune diseases, more females than males are affected.

In ITP, normal-appearing platelets are formed from morphologically normal megakaryocytes, but abnormal IgG autoantibodies attach immunologically to circulating platelets. IgG-coated platelets are detected by Fc

receptors on mononuclear macrophages, typically in the spleen.[7-9] The IgG-platelet complex causes the membrane of the splenic macrophage to invaginate, and IgG-coated platelets are phagocytized. This mechanism of mononuclear macrophage phagocytosis of IgG-coated platelets is illustrated in Figure 1. To the extent that bone marrow megakaryocytes do not increase production of platelets sufficiently to compensate for the shortened survival of circulating platelets, patients have a decreased platelet count and may develop clinical signs of thrombo-cytopenia. If the decreased platelet count is an isolated abnormality and there are no other factors contributing to a decrease in hemostasis, such as decreased plasma coagulation factor concentrations, the patient is likely to have minimal spontaneous bleeding and relatively few petechia ("dry purpura"). Other patients, whose thrombocytopenia is complicated by the coexistence of other hemostatic deficiencies, such as chronic liver disease and decreased coagulation factor concentrations, abnormal integrity of the capillary system, or drug-related platelet dysfunction, may experience more prominent signs and symptoms of thrombocytopenia, such as bleeding gums, metror-rhagia, and increased susceptibility to bruising ("wet purpura").

Treatment of ITP

The first successful treatment of ITP was reported in 1913, when Kaznelson reported the successful outcome of splenectomy in a patient with increased bleeding and susceptibility to bruising and a low platelet count.[10] After that report, splenectomy was the treatment of choice for ITP until 1950, when Wintrobe reported increased platelet counts in patients with purpura hemorrhagica after treatment with ACTH or cortisone.[11] For the next three decades, corticosteroids and/or splenectomy were the principal treatments for ITP. For refractory patients, other approaches to treatment were used, such as immunosuppressive drugs, plasmapheresis, and selective immune adsorption of autoantibodies, but these treatments were less likely to be successful and had a secondary role in the management of ITP. In 1981, Imbach et al. reported that intravenous immunoglobulin (IVIG) increased platelet counts in children with ITP.[12] Imbach's interest in the use of IVIG in ITP began when he noted that two children with low platelet counts who were being treated with IVIG (for other indications) had increased platelet counts after receiving IVIG. Clinical trials soon established the efficacy of IVIG for treating ITP. Splenectomy, corticosteroids, and IVIG became the primary treatments for ITP until 1983, when Salama et al. hypothesized that the beneficial effect of IVIG in ITP may be due to RBC antibodies present in IVIG that cause immune hemolysis of the recipient's RBCs by splenic sequestration of IgG-coated platelets.[13] Salama et al. postulated that anti-A, anti-B, and other RBC antibodies may have been responsible for hemolysis and decreased hematocrits they had observed in patients treated with IVIG. They stated that, while the main effect of IVIG in ITP may be macrophage Fc receptor blockade by individual IgG molecules, an even more efficient Fc receptor blockade may be induced by IgG-coated RBCs undergoing hemolysis in mononuclear macrophages. They conducted clinical trials using IV RhIG in D+ children and adults with ITP,[14,15] and the successful outcomes opened a new era for patients with ITP. Subsequent clinical trials using several different IV RhIG products in D+ patients established the efficacy of IV RhIG for the treatment of ITP (Table 1).[16-27]

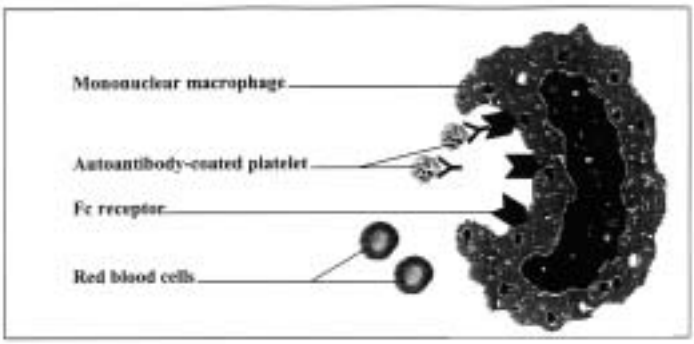

Fig 1. Pathophysiology of immune thrombocytopenic purpura. Normal platelets are phagocytized by mononuclear macrophages whose Fc receptors attach immunologically to the Fc portions of IgG platelet autoantibodies. The platelet count of peripheral blood decreases as IgG-coated platelets are destroyed, usually in the spleen.

Mechanism of Action of IV RhIG in ITP

Figure 1 illustrates how splenic mononuclear macrophage Fc receptors become activated by circulating IgG-coated platelets in untreated ITP, which become phagocytized. Figure 2 illustrates how an infusion of IV RhIG in a D+, nonsplenectomized patient with ITP modifies this situation and increases the circulating platelet count. Shortly after the infusion of IV RhIG, phagocytosis of IgG-coated platelets is competitively inhibited by IgG anti-D-coated D+ circulating RBCs (reticuloendothelial or Fc receptor blockade). Assuming a typical or representative platelet count for

Table 1. Selected reports of clinical trials using IV RhIG (anti-D)

Year	First author[ref #]	Principal findings
1984	Salama, A.[14]	Of ten patients treated with IV RhIG (Biotest, Frankfurt, FRG), eight had an increase in platelet count.
1986	Salama, A.[15]	Of 15 chronic and two acute patients with ITP treated with RhIG (Zentralinstitut fur das Bluttransfusionswesen, Hamburg, FRG; HypRh$_o$-D, Cutter, Emeryville, CA) who were Rh(D)-positive, two cases responded to anti-Rh$_o$(D) insignificantly (increment < 20,000/μL) while all others were considered good or excellent.
1986	Becker, T.[16]	Of 15 D+ children with ITP treated with IV RhIG (Zentralinstitut fur das Bluttransfusionswesen, Hamburg, FRG) all were found to have a rise in platelets.
1988	Oksenhendler, E.[17]	IV RhIG (Biotranfusion, France) was effective in at least nine out of 14 Rh(D)+ patients with HIV-related ITP.
1991	Bussel, J.B.[18]	Of 43 nonsplenectomized patients, the mean initial platelet count was 22,000/μL and the mean increase in platelet count was 95,000/μL after treatment with IV RhIG (WinRho, Winnipeg Rh Institute of the University of Manitoba, Winnipeg, Canada).
1992	Gringeri, A.[19]	Of 51 HIV ITP patients, including HIV-related, treated with IV RhIG (Rhesuman, Berna, Italy; Partogamma, Immuno, Italy), 67 percent showed increases in platelet counts.
1992	Andrew, M.[20]	Of 25 patients with ITP, 90 percent responded to IV RhIG (WinRho; Rh Pharmaceuticals, Winnipeg, Manitoba, Canada).
1993	Caglayan, S.[21]	Of 19 children with ITP, 15 responded to IV RhIG (Rleso-Gulap, Seru, Austria) with an increase in average platelet number to 76,000/μL after being administered 100 μg for 5 consecutive days.
1994	Borgna-Pignatti, C.[22]	Of seven patients with ITP, five had an increase in platelet count (> 100,000 cells/μL) after treatment with IM RhIG (Partobulin, Immuno, or Partogamma, Biagini, Pisa, Italy).
1994	Blanchette, V.[23]	Although IV RhIG (Sandoglobulin, Swiss Red Cross, Basel, Switzerland; WinRho, Winnipeg Rh Institute, Winnipeg, Canada) was easier to administer, the rate of platelet response was significantly slower than that observed in children randomized to receive IV IgG.
1996	Godeau, B.[24]	Of seven patients with chronic ITP, only one patient showed transient response while all others showed no response to a monoclonal anti-D (MONO-D, Laboratoire Francais du fractionnement et des Biotechnologies, Les Ulis, France).
1997	Scaradavou, A.[25]	Of 261 nonsplenectomized ITP patients, 72 percent of patients responded to IV RhIG (WinRho or WinRho SD, Cangene, Winnipeg, Manitoba, Canada) with an increased platelet count > 20,000/μL.
2001	Newman, G.C.[26]	Children with acute ITP receiving IV RhIG (WinRho, Cangene Corporation, Winnipeg, Manitoba, Canada) 75 μg/kg/d had overnight platelet increases in seven out of nine cases.
2001	Bussel, J.B.[27]	There was no overall relationship between response to IV RhIG (WinRho) or IV IG and response to subsequent splenectomy. However, both a good platelet response in adults to the last IV RhIG and a hemoglobin decrease of $\geq$ 2.0 gm/dL appeared to predict response to subsequent splenectomy.

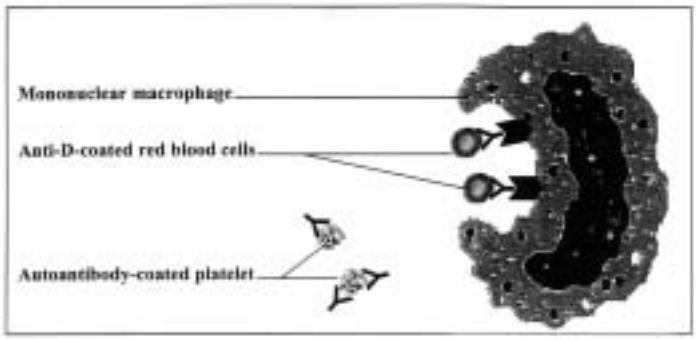

Fig 2. Postulated mechanism of action of IV RhIG (anti-D) for inducing a transient macrophage Fc receptor blockade to treat ITP in D+ patients. Infused anti-D attaches immunologically to D antigen sites on the patient's D+ circulating RBCs. Assuming a normal RBC count of approximately 5.0 million/μL and a decreased platelet count of 10,000/μL, IgG-coated RBCs compete for phagocytosis by mononuclear macrophages with a numerical advantage of 500:1. The result is a decrease in circulating RBCs and an increase in circulating platelets. The effect has been described as a "medical splenectomy" and, although the platelets remain coated by IgG, they typically function adequately after surgical splenectomy.

a patient with ITP of approximately 10,000/μL and a normal RBC count of 5,000,000/μL in that patient, the numerical competition of approximately 500 (IgG-coated RBCs) to 1 (IgG-coated platelet) favors phagocytosis of IgG-coated RBCs. Presumably, the physical bulk of phagocytized RBCs leverages the comparatively small dose of IV RhIG (50μg/kg) to result in a comparable degree of Fc receptor blockade as would a much larger dose of IVIG (1gm/kg). This reticuloendothelial or macrophage Fc receptor block, which has been referred to as a "medical splenectomy," was described earlier by Shulman et al. in their studies of immune destruction of platelets in patients with hereditary spherocytosis.[28] IV RhIG is not indicated for persons who are D– or have had a splenectomy, since clinical trials have shown IV RhIG to be ineffective in these patients.[29]

Serologic Aspects

Most blood bank technologists are acquainted with the weak and transient reactivity of anti-D in the plasma of D– women after an intramuscular injection of conventional RhIG (IM RhIG) for Rh immunoprophylaxis at 28 weeks of pregnancy or after delivery of a D+ infant. Because IM RhIG is injected in muscle and consists of only approximately one-tenth the dose of IV RhIG that is used to treat ITP, circulating anti-D is detectable in the recipient's plasma for only a few days to a few weeks, if at all. Anti-D titers after injections of IM RhIG are low and do not interfere with Rh(D) type-specific transfusions of serologically compatible D– RBCs to D– women, because there is no serologic incompatibility. In the case of treating D+ patients with ITP with IV RhIG, the circumstance is quite different because (1) a much higher dose of anti-D is given, (2) it is given as an IV bolus, and (3) it is given to a person whose D+ RBCs are serologically incompatible. Thus, an ITP patient treated with IV RhIG not only will develop a positive antibody detection test (indirect antiglobulin test), but also will develop a positive direct antiglobulin test (DAT). Although the composition of alloantibodies in both IM and IV RhIG is similar, and it reflects the expected serologic specificities of alloantibodies in D– alloimmunized women, the considerably larger dose of IV RhIG for ITP causes more alloantibodies to be detectable in recipients' plasma, such as anti-C, -E, and -G.[2] These additional alloantibodies may be detected both in the eluate from the DAT-positive samples and by antibody screening by the indirect antiglobulin test.[2,30] There is relatively little data on the transfusion of D+ RBCs to patients who have been treated for ITP with IV RhIG. However, in our experience[1] and in that of others,[31] it seems prudent to transfuse D– RBCs, unless there is an urgent need to continue the treatment of ITP to increase the platelet count.[1] In that latter situation, it may be argued that by giving D+ RBCs one brings the full dose of IV RhIG to effect competitive inhibition of macrophage Fc receptor function, supporting the primary therapeutic goal of raising the platelet count.

Note: Figures 1 and 2 were produced using Microsoft Word 2000 word processing software. Images were created using Adobe Illustrator software, modified using MGI Photosuite, and inserted in Microsoft Word where needed. Symbols were created using WordArt in Microsoft Word.

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Easy method for determining the frequency of O^1 and O^2 alleles in Brazilian blood donors by PCR-RFLP analysis

A.C. Batissoco, M.C. Zago-Novaretti, V.J. Bueno, P.E. Dorlhiac-Llacer, and D.A.F. Chamone

Serologic ABO blood typing is routinely performed using anti-A and anti-B sera to distinguish four phenotypes (A, B, AB, and O). Restriction fragment length polymorphisms (RFLPs) and DNA sequence studies offer the possibility of direct ABO genotyping. We used polymerase chain reaction-RFLP analysis to determine the frequency of O^1 and O^2 alleles in 82 unrelated blood donors in São Paulo, Brazil, known to be group O. Genomic DNA was extracted from blood leukocytes by a modified salting-out method. Different genotypes (O^1O^1, O^1O^2, O^2O^2) were identified after digestion with restriction enzymes *Kpn*I, *Hpa*II, and *Alu*I, followed by agarose gel electrophoresis. Of 82 samples analyzed, 74 were O^1O^1, 7 were O^1O^2, and 1 was O^2O^2. These results showed the frequency of O^1O^1, O^1O^2, and O^2O^2 genotypes to be 90.24 percent, 8.53 percent, and 1.22 percent, respectively, in blood donors in São Paulo, Brazil. ***Immunohematology* 2001;17:111–116.**

Key Words: ABO blood group, *ABO* genotypes, O^1 allele, O^2 allele, blood donors, PCR, RFLP, blood group frequencies.

The ABO blood group system is the most clinically significant system in transfusion medicine. Antigens of the ABO system consist of an *A* or *B* carbohydrate structure carried on the substrate H antigen. The *A* and *B* glycosyltransferase encoded at the *ABO* locus on chromosome 9 define which specific carbohydrate is added to the end of the H substance oligosaccharide chains, GalNAc α1-3 for A and Gal α1-3 for B, respectively.[1] The ABO blood group is determined serologically by using anti-A and anti-B sera, which are able to distinguish four phenotypes (A, B, AB, and O).

In 1990 the nucleotide sequences of the alleles at the *ABO* locus were defined.[2,3] Since then, polymerase chain reaction (PCR) combined with diagnostic restriction enzyme digestion or with the use of allele-specific primers, based on several single-base substitutions between *ABO* alleles, have been used for direct genotyping of the ABO blood group system.[1,2,4-6]

The use of restriction fragment length polymorphisms (RFLPs) and DNA sequence data has produced new and important information about genetic diversity in the human population. Molecular genetic studies of the *ABO* genes have shown that the structural sequences of the *O* allele are identical to that of the primordial *A* gene (A^1) with the exception of a single nucleotide (nt) deletion at position 261 (261 del G) in the *O* gene, which causes a shift in the reading frame that prematurely codes for a stop codon. This results in translation of a truncated protein without the enzymatic activity of an A transferase.[1-6] This common *O* allele is designated O^1.

Another *O* allele, designated O^2, differs from the A^1 allele in the substitutions 297, 526, 802, and 1096, resulting in two amino acid substitutions and lack of glycosyltransferase activity.[1,4,6-13] This variant is a hybrid *A/B* nucleotide sequence and it can be defined by typing as *A/B* at nt 261, as *B* at nt 526, and as A^1 at nt 703.[6,13]

The O^1 allele is very common, whereas O^2 is less common.[6-10,13-18] The O^2 frequency in the Danish population is 3.7 percent,[13] whereas among blacks and Caucasians the frequency is 4.7 percent.[7] The O^2 allele is absent among Amazon Indians.[7,9]

The aim of our study was to detail a method to evaluate *O* gene polymorphisms wherever DNA sequencing is not available and present *O* allele frequencies in blood donors at Fundação Pró-Sangue/Hemocentro São Paulo, Brazil.

Materials and Methods

DNA samples

DNA was extracted from EDTA-blood samples obtained from 82 group O blood donors at Fundação Pró-Sangue/Hemocentro de São Paulo, São Paulo State,

Brazil. The ABO blood group was determined serologically, using Olympus PK7200 automated equipment, and confirmed by tube test technique as described elsewhere.[19]

DNA preparation

Genomic DNA was extracted from the blood samples by a modified salting-out method described by Miller and colleagues.[20] Briefly, 1mL of homogenized whole blood was centrifuged at 1200 g for 5 minutes and the supernatant was removed. Blood cells were lysed with 1 mL of Tris Buffer 1 (10 mmol/L Tris-HCl, pH 8.0; 10 mmol/L KCl; 10 mmol/L $MgCl_2$; 2 mmol/L EDTA, pH 8.0) that contained 25 g/L Triton X-10. After centrifugation, the pellet was washed × 2 with Tris Buffer 1, lysed with 220 μL of Tris Buffer 2 (10 mmol/L Tris-HCl, pH 8.0; 10 mmol/L KCl; 10 mmol/L $MgCl_2$; 2 mmol/L EDTA, pH 8.0; 0.4 mol/L NaCl; 10 g/L SDS), and incubated for 15 minutes at 56°C. Cellular proteins were removed by precipitation after addition of 100 μL of 5 mol/L NaCl. DNA was isolated by ethanol precipitation and solubilized in TE buffer (100 mmol/L Tris-HCl, pH 8.0; 1 mmol/L EDTA).

PCR amplification

DNA analysis was performed initially by the PCR amplification of genomic DNA, followed by digestion with the restriction enzyme *Kpn*I to identify the deletion at position 261 of the exon 6 of the glycosyltransferase gene, which is the common O^1 allele.[6-8,11-14] The primer pairs, mo46-5′-CGGAATTCACTCGCCACTGCCTGGGTCT C-3′/mo57-5′-CGGGATCCATGTGGGTGGCACCCTGCCA-3′, amplified a fragment of 252 bp that contains nt 261.[6,8]

For the samples not cleaved by enzyme *Kpn*I we used the restriction enzymes *Hpa*II and *Alu*I.[8,10,16,21,22] For this amplification we used the primer pairs mo 71-5′-GGGCCTAGGCTTCAGTTACTC-3′/mo101-5′-CGGGATCCCCGTCCGCCTGCCTTGCAG-3′ that amplified a fragment of 843 base pair (bp) of the exon 7 of the glycosyltransferase gene, the O^2 allele.[8]

PCR amplification of 0.1 μg of genomic DNA was performed in a final volume of 50 μL, containing 1mM $MgCl_2$, 400 μM dNTP, 3 units (U) Taq polymerase (Gibco Life Technologies, Gaithersburg, MD) and 30 pmol of each primer.

Cycling was 95°C/5 minutes, 95°C/1 minute, 64°C/1 minute, 72°C/1 minute for 35 cycles and 72°C/10 minutes on Peltier MJ Research-PC 200 Thermal Cycler (Labtrade do Brasil, São Paulo, Brazil). Postamplification gels to detect the ABO-DNA fragments were run to check whether the amplification was successful.

To ensure that no contamination by external DNA or PCR products had occurred during the preparation of the samples and the PCR reagents, a water blank was included as a negative control in parallel with the test sample.

Enzyme digestion

The digestion mix (20μL), containing 0.5 U of restriction endonucleases *Kpn*I, *Hpa*II, and *Alu*I (Gibco Life Technologies) in a 10 × concentrated buffer, was added separately to the tube containing the PCR product. Digests were incubated at 37°C for 3 hours in a water bath (Fanem Ltda. Mod.146, Brazil).

Electrophoresis separation

Cleavage products were separated electrophoretically for 1 hour at 100V (Gibco BRL-Horizontal Gel Electrophoresis Apparatus) and visualized under an ultraviolet light board (EAGLE EYE™ II—Stratagene System/Dept. Tumoral Biology, São Paulo, Brazil) using 2.5% agarose gel containing 0.5 μg/mL ethidium bromide from Sigma (St. Louis, MO, USA). Results were documented with a CCD monochromatic camera.

Results

We initially used DNA analysis by PCR for 82 group O individuals to detect the single nucleotide deletion at position 261 that produces the most common type of O allele, O^1, and we subsequently expanded the analysis to include detection of the O^2 allele.[8,16]

The primer pair mo-46/mo-57 yielded a PCR product of 252 bp, which in the presence of the 261 mutation (261 delG) is cleaved by the restriction enzyme *Kpn*I to yield the O^1 allele.[6,8] The primer pair mo71/mo101 amplified an 843-bp DNA fragment, which when cleaved by the restriction enzymes *Hpa*II and *Alu*I yields the O^2 allele.[8,16]

After cleavage with enzyme *Kpn*I, O^1O^1 samples yield two DNA fragments, one with a size of 164 bp and the other with a size of 87 bp. The O^1O^2 samples, in addition to the 164-bp and 87-bp fragments, yield the undigested 252-bp fragment. In the O^2O^2 sample only the intact 252-bp PCR product is found (Table 1).

Figure 1 shows a schematic representation of the *ABO* gene regions amplified by PCR and digested by restriction enzyme *Kpn*I. After digestion, the fragments were separated and identified through electrophoresis in 2.5% agarose gel (Fig. 2).

Table 1. Fragments obtained after digestion with *Kpn*I and *Hpa*II and the percent of the alleles found in the samples analyzed

Samples	Origin	Genotypes	Fragment size/*Kpn*I	Fragment size/*Hpa*II
74 (94.24 %)	whites, blacks, mulattos, and others	O^1O^1	87/164	——
7 (8.53%)	whites	O^1O^2	87/164/252	309/204/137/119/96
1 (1.22%)	whites	O^2O^2	252	309/204/137/119

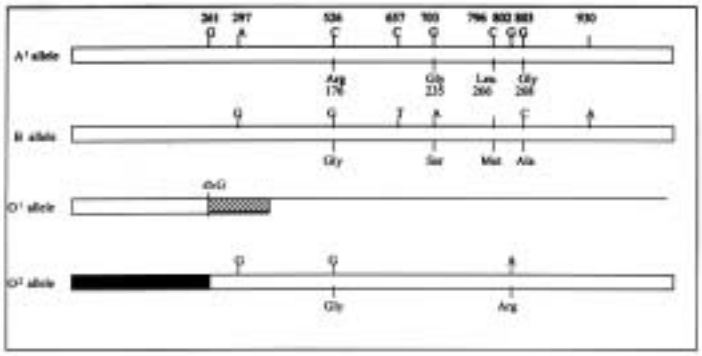

Fig. 1. Schematic comparison of the nucleotide and deduced amino acid sequences of A^1, B, O^1, and O^2 alleles.[3]

Sequences not determined

Entirely different deduced amino acid sequence in the ordinary O allele due to a frame shift causing a single-based deletion

For the samples that did not yield the restriction site with the enzyme *Kpn*I, the restriction enzymes *Hpa*II and *Alu*I were used to confirm the presence of the O^2 allele in the O^1O^2 and O^2O^2 genotypes.

After cleavage with the restriction enzyme *Hpa*II, the samples yield the fragments 309 bp, 204 bp, 137 bp, 119 bp, and 96 bp for the O^1O^2 genotypes and 309 bp, 204 bp, 137 bp, and 119 bp for the O^2O^2 genotype (Table 1 and Fig. 3).

With basis in apparent fragment sizes, and the specificity of the enzyme *Hpa*II (CCGG) and the nt sequence of the *ABO* gene, Olsson and Chester[8] correlated the mutation C526G, specific for B and O^2, with the CCGG sequence at nt 1094-1097.[8] Digestion with the enzyme *Hpa*II of PCR-amplified sections of exon 7 from individuals with the B and O^2 alleles gave a fragmentation pattern after electrophoresis that differed from that of similarly treated samples of other blood groups.[8]

The enzyme *Alu*I digests the DNA fragments of individuals with the G703A mutation only present in the B allele and absent in the O^2 allele. [13,16,21,22]

Table 1 summarizes the results for blood samples from 82 donors analyzed by PCR-RFLP using *Kpn*I and

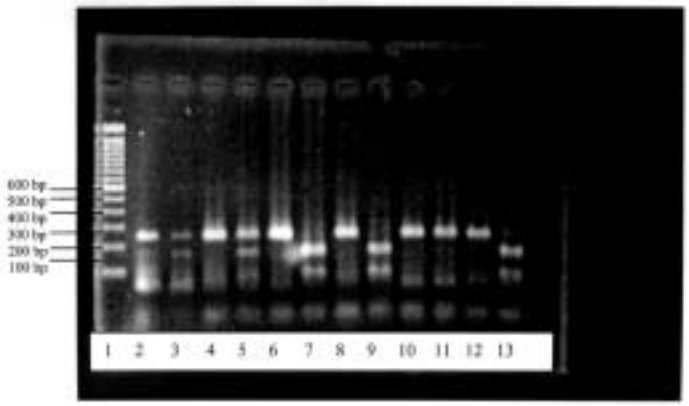

Fig. 2. 2.5% agarose gel after digestion with restriction enzyme *Kpn*I of PCR-amplified *ABO* gene. Lane 1: molecular size marker (100 bp by DNA ladder); lanes 2,4,6,8,10, and 12: undigested 252-bp (exon 6) PCR fragments (samples O^1O^2, O^1O^2, O^1O^1, O^1O^1, O^2O^2, and O^1O^1, respectively); lanes 3, 5, 7, 9, 11, and 13: DNA amplified with the primer pairs mo46/mo57 and digested with the enzyme *Kpn*I; lanes 3 and 5: O^1O^2; lanes 7, 9, and 13: O^1O^1, and lane 11: O^2O^2.

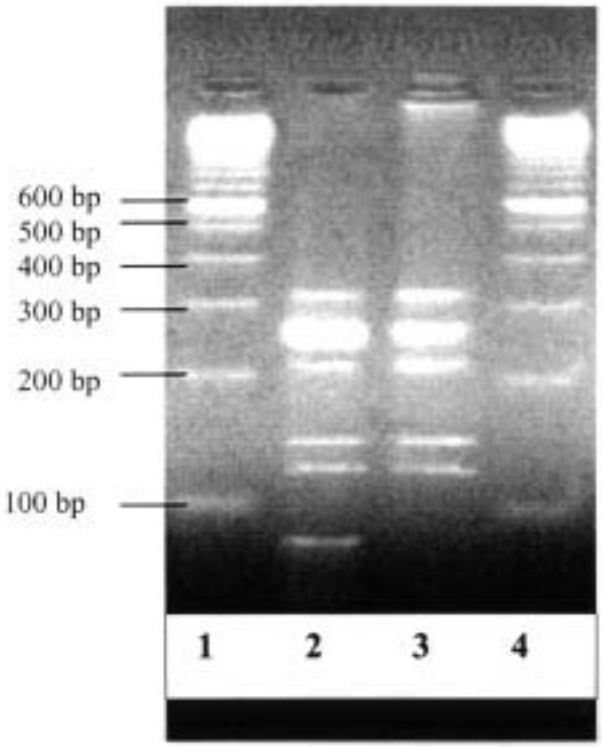

Fig. 3. 2.5% agarose gel after amplification with the primer pairs mo46/mo57 and mo71/mo101 and digestion with restriction enzyme *Hpa*II. Lanes 1 and 4: molecular size marker (100 bp by DNA ladder); lane 2: O^1O^2 (309 bp, 204 bp, 137 bp, 119 bp, and 96 bp); lane 3: O^2O^2 (309 bp, 204 bp, 137 bp, and 119 bp). The fragment 252 bp is the result of the amplification with the primer pairs mo46/mo57 undigested with the enzyme *Hpa*II, whereas the other fragments are the result of the digestion of the fragment 843 bp amplified by primers mo71/mo101 with the enzyme *Hpa*II.

*Hpa*II. The majority of the donors (74) appeared to be O^1O^1, seven appeared to be O^1O^2, and only one O^2O^2 sample was found. All contamination controls were negative by electrophoresis and genotyping.

From 82 group O blood donors, 49 (59.8%) were white, 15 (18.3%) were black, 9 (11%) were mulattos, 6 (7.3%) were Amerindian and white, 1 (1.2%) was Amerindian and black, 1 (1.2%) was Amerindian and Asiatic, and 1(1.2%) was of Asiatic origin. The O^2 allele was found only in samples from white donors.

Discussion

The major molecular polymorphism of the ABO blood group system was elucidated in 1990.[2,3] Subsequently, new alleles with different mutations were described and new methods to investigate them were developed. Comparison of the nt sequences of the coding regions of various *ABO* alleles has revealed differences in which total loss of enzymatic activity, as indicated by the resulting O phenotype, is serologically characterized by the complete absence of A and B antigen expression on erythrocytes.[2-5]

A variant of this *O* allele with the same structural defect in the form of the O_{261} deletion, but with additional nt substitutions, has been identified as an O^1 allele.[2,4]

The original *O* allele, O^1, is structurally identical to the original A^1 primordial gene, with the exception of the characteristic single nt deletion in the 5′ end of the coding sequence (O_{261}) that introduces a shift in the reading frame. The resulting protein, if any, is a truncated peptide of 114 amino acids without resemblance to the *A* and *B* transferases.[7] It is not surprising that identifying such a change at the level of genes leads to a better understanding of the ABO blood group system.

Johnson and Hopkinson,[23] using denaturing gradient gel electrophoresis on the PCR product from the exon coding the O_{261} deletion, showed that multiple *O* alleles might exist.[23] Furthermore, in a recent study of a B^3 subgroup individual, an allele different from the previously described *O* allele characterized by the O_{261} deletion was predicted to represent a new *O* allele.[4]

The second *O* allele detected, O^2, is a mutant in which the nt 261 deletion is absent, and it differs from the A^1 allele in four nt substitutions, 297, 526, 802, and 1096, resulting in two amino acid substitutions.[4,8,10] These two amino acid substitutions were found to abolish the activity of the transferase expressed in vitro.

In our donor study, we found a frequency of 1.22 percent for the O^2O^2 genotype and 8.53 percent for the O^1O^2 genotype only in the samples from white donors (Table 1).

This study demonstrates that, using molecular genetic techniques for *ABO* genotyping, it is possible to distinguish different *O* alleles. *ABO* genotyping for blood banking, forensic, and population studies is an important advance and allows us to distinguish some seemingly serologically identical phenotypes. DNA sequencing of the *A* and *B* genes showed that they are highly similar, differing by only seven nts, resulting in four amino acid substitutions (residues 176, 235, 266, and 268).[2,3,23]

Traditional blood group serology, which is based on immunoreactivity among *A-*, *B-*, and *H*-active carbohydrates with specific reagents, does not provide information on *ABO* genotypes. There are several cases in which extensive serologic investigation failed to determine the ABO blood group.[24] We used DNA analysis to determine the relative frequencies of the two types of *O* alleles, O^1 and O^2, among individuals of different racial groups.

Differences in the DNA of these transferase genes can be determined by PCR amplification with primers that amplify exon 6 and exon 7 of the *ABO* gene, followed by RFLP analysis of the PCR product. We used RFLP analysis to study the DNA from 82 group O individuals to detect the O^1 and O^2 alleles.[6,8] The primers used (mo46/mo57) amplify a 252-bp DNA fragment that contains the nt 261 of the glycosyltransferase gene. The single nt deletion found in the common *O* allele (O^1) creates a *Kpn*I site, which produces after digestion the 164-bp and 87-bp fragments in the samples that have the O^1 allele.[6,8] The digestion with the enzyme *Hpa*II, used in the samples that were not cleaved with the enzyme *Kpn*I, gave a fragmentation pattern that differentiated the *B* and O^2 alleles from the other alleles treated similarly.[8] The primer pair mo71/mo101 amplifies an 843-bp DNA fragment which, when cleaved by enzyme *Hpa*II, produces the fragments 309 bp, 204 bp, 137 bp, 119 bp, and 96 bp for the O^1O^2 genotype and fragments 309 bp, 204 bp, 137 bp, and 119 bp for the O^2O^2 genotype[8] (Fig. 3). Fragments smaller than 96 bp are detectable, but are not necessary for an unambiguous interpretation.

Our data show that 100 percent of *O* genotyping results were in concordance with the serologic results.

The aim of this study was to evaluate a rapid and simple genotyping method for O^1 and O^2 alleles that

can be easily performed in an immunohematology laboratory.

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Case report: four donors with granulocyte-specific or HLA class I antibodies implicated in a case of transfusion-related acute lung injury (TRALI)

A. Davoren, O.P. Smith, C.A. Barnes, E. Lawlor, R.G. Evans, and G.F. Lucas

A 54-year-old female patient with a history of chronic liver disease and portal hypertension was admitted for an elective cholecystectomy. Preoperative evaluation revealed a prolonged prothrombin time of 17.4 seconds (control 12 to 15.5 seconds). Six units of fresh frozen plasma (FFP) were prescribed after failure of correction of the coagulopathy with intravenous vitamin K (10 mgs). During infusion of the fifth unit of FFP, the patient became acutely dyspneic. Arterial blood gas analysis revealed marked hypoxemia (PO_2 6.58 kPa) and the chest X-ray showed new diffuse bilateral alveolar infiltrates. The patient remained hypoxemic with unstable oxygen saturations over the following 7 days, during which time she required 60 to 100 percent oxygen administered by face mask. Intravenous methylprednisolone (200 mgs) was given for 5 days. Mechanical ventilation was not required. The lung infiltrates gradually cleared over 3 to 4 days and the patient showed clinical improvement after 1 week. Four of the donors of the implicated units of plasma were female and all had a history of pregnancy. Two donors had HLA class I antibodies and two had granulocyte-specific antibodies detectable in their serum. In crossmatch studies, granulocyte-reactive antibodies from two donors bound to granulocytes from the patient, which suggested that these antibodies were clinically relevant. These clinical and serologic findings support a diagnosis of transfusion-related acute lung injury (TRALI). ***Immunohematology* 2001;17:117–121.**

Key Words: TRALI, transfusion reactions, lymphocyte, leukocyte- and granulocyte-specific HLA class I antibodies

Transfusion-related acute lung injury (TRALI) is a serious, potentially life-threatening complication of blood transfusion therapy.[1,2] It presents clinically as acute dyspnea associated with hypoxemia and bilateral lung infiltrates on chest X-ray in the absence of other apparent causes, usually within 6 hours (but may be up to 24 hours) of blood transfusion.[1,2] TRALI is generally associated with the transfusion of blood products containing plasma in which HLA (human leukocyte antigen) class I and II or granulocyte-specific antibodies, which react with recipient leukocyte antigens, are present.[2,3,4] More recently, biologically active lipids in stored blood components have been implicated in the pathogenesis of TRALI.[5,6] Popovsky and Moore reported an incidence of TRALI of 0.02 percent per unit transfused,[2] while an incidence of approximately 1 in 2000 was found in a study of patients who received general anesthesia and one or more blood products.[7] TRALI is generally considered to be underreported.[2,8,9] The donors of implicated blood products are usually multiparous females (≥ 3 pregnancies).[2]

The precise mechanism of TRALI is unknown. Current evidence suggests that interaction between donor antibodies and recipient leukocyte antigens appears to cause complement activation and neutrophil sequestration in the pulmonary microvasculature. Activated neutrophils release proteases and other substances that damage the pulmonary capillary endothelium, causing increased vascular permeability and alveolar and interstitial edema, impaired gas exchange, hypoxia, and the clinical manifestations of respiratory distress.[1,2]

The clinical manifestations of TRALI usually improve within 48 to 96 hours of onset; pulmonary infiltrates recede in 1 to 4 days, but may last for up to 7 days.[1,2] The condition can occur at any age and occurs with a similar frequency in both males and females.[9] In the absence of invasive hemodynamic monitoring of the patient, TRALI is difficult to distinguish clinically from cardiogenic pulmonary edema due to fluid overload or other cause. Laboratory investigation is often

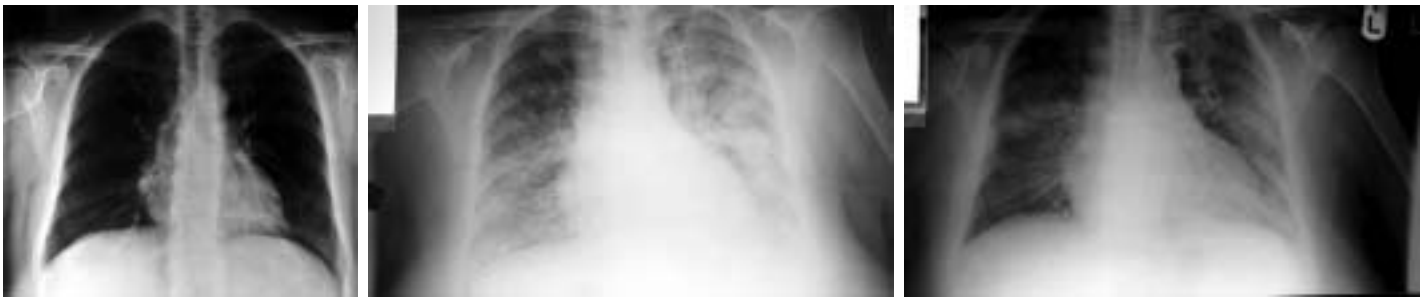

Fig. 1. Chest X-rays of patient with coagulopathy who developed transfusion-related acute lung injury (TRALI); (a) pretransfusion, (b) immediately post-transfusion, (c) 48-hours later.

complicated by the detection of antibodies in more than one donor and the inability to determine which antibodies are clinically significant.

Case Report

A 54-year-old female was admitted for an elective cholecystectomy. Apart from a long-standing history of cholelithiasis, the patient had a background history of chronic liver disease of unknown etiology, portal hypertension with hypersplenism, and gastrointestinal bleeds. She was a non-smoker and did not consume alcohol. Her medication on admission consisted of spironolactone, ranitidine, and folic acid. She had no known drug allergies and had no underlying cardiac or pulmonary problems.

Preoperative evaluation revealed a prolonged prothrombin time of 17.4 seconds (control 12 to 15.5 seconds) and the activated partial thromboplastin time was within the normal range (32.4 seconds). Moderate thrombocytopenia was present (platelet count: 64×10^9/L). The chest X-ray at the time of admission showed that the lung fields were clear and that the heart was not enlarged (Fig. 1a). The patient's coagulopathy was not corrected by intravenous vitamin K (10 mgs) administration on the day prior to surgery and six units of fresh frozen plasma (FFP) were prescribed immediately preoperatively. Shortly after commencement of the fifth unit of FFP, the patient became acutely unwell and dyspneic and began coughing. She had no chest pain, rash, or pruritis, and no significant drop in blood pressure. The patient's temperature fell from 36°C to 35.5°C at the time of the reaction, increasing to 38°C 1 hour later. The transfusion was discontinued and oxygen was administered by face mask. Arterial blood gas analysis showed marked hypoxemia, with PO_2 6.58 kPa, PCO_2 4.8 kPa, and O_2 saturation of 87.6 percent while breathing 40 percent oxygen. Chest X-ray showed new extensive bilateral alveolar infiltrates (Fig. 1b). Widespread crepitations were audible on auscultation of the chest.

A diuretic was administered (frusemide 40 mgs) intravenously for the first 2 days after the onset of symptoms. The patient remained oxygen dependent (60 to 100 percent concentration), with oxygen saturations varying between 89 and 95 percent for 7 days. Mechanical ventilation was not required. Intravenous methylprednisolone (200 mgs/day) was prescribed on day 2 and continued for 5 days. Eight days post-transfusion, blood gas analysis showed considerable improvement (with PO_2 8.89 kPa, PCO_2 4.54 kPa, and O_2 saturation of 95%) while breathing room air. The infiltrates on the chest X-ray showed gradual clearance over 3 to 4 days (Fig. 1c). The subsequent clinical course was complicated by a staphylococcal septicemia that responded to intravenous antibiotic therapy.

Despite the significant morbidity associated with this transfusion reaction, the patient made a good recovery and proceeded to cholecystectomy 1 month later. Her coagulation profile was satisfactory perioperatively and corrective therapy was not necessary. The postoperative course was uneventful.

Materials and Methods

Four of five donors of the plasma units transfused were female. The fifth donor was a male donor with no history of blood transfusion and was eliminated from further investigation. Serum samples from the patient and the four female donors of the transfused FFP units were tested for the presence of granulocyte-specific and lymphocyte-reactive antibodies, using the chemiluminescence test,[10] granulocyte immunofluorescence test (GIFT),[11] lymphocyte immunofluorescence test (LIFT),[12] and a panel of human neutrophil antigen (HNA)-typed granulocytes. The monoclonal antibody immobilization of platelet antigen (MAIPA) assay[13] was used to detect HLA class I antibodies.

Granulocytes and lymphocytes from the patient were isolated by using the whole blood lysis technique,[14] and crossmatch studies were performed by incubating these cells with sera from the four implicated donors. The patient's granulocytes were also incubated with her own serum in an indirect "auto" test. Immunofluorescence tests were evaluated using a flow cytometer.

Results

The serologic results are summarized in Table 1. HLA class I antibodies were detected in donors 2 and 3, granulocyte-specific antibodies with specificity for HNA-1a were detected in donor 4, and granulocyte-specific IgG and IgM antibodies of unknown specificity were detected in donor 1. HLA class I antibodies were also detected in the patient's serum. The patient's granulocytes typed as HNA-1a–, HNA-1b+.

Table 1. Summary of serologic results

Donor number	Antibodies Detected
1	Granulocyte antibodies reactive in GCLT and GIFT (IgG and IgM)
2	HLA class I (IgG) antibodies by MAIPA assay, reactive in GCLT, GIFT, and LIFT
3	HLA class I (IgG) antibodies by MAIPA assay, reactive in GCLT, GIFT, and LIFT
4	Granulocyte antibodies reactive in GIFT (IgM) with HNA-1a specificity
Patient	HLA-class I (IgG) antibodies by MAIPA assay, reactive in LIFT (IgG)

GCLT: granulocyte chemiluminescence test
GIFT: granulocyte immunofluorescence test
LIFT: lymphocyte immunofluorescence test
MAIPA: monoclonal antibody immobilization of platelet antigen assay for HLA class I antibodies

In crossmatch studies, the sera from donor 1 (who donated the FFP being administered immediately prior to the reaction) and donor 3 bound granulocyte-specific IgG and IgM antibodies to the patient's granulocytes (Table 2), suggesting that these antibodies may have caused the TRALI. Platelets from the patient were incubated with serum from the implicated donors, but HLA class I antibodies reactive with the patient's platelets were not detected in the MAIPA assay.

Table 2. Crossmatch studies

Donor number	GIFT (IgG)	GIFT (IgM)	LIFT (IgG)	LIFT (IgM)
1	Weak positive	Positive	-*	-
2	-	-	-	-
3	-	Positive	-	-
4	-	-	-	-
Patient	-	-	-	-

Granulocytes/lymphocytes from the patient were incubated with serum samples from the implicated donors and the patient's own serum. Antibody binding was evaluated by immunofluorescence tests. The serum from patient 1 bound IgG and IgM and the serum from patient 3 bound IgG to the patient's granulocytes.
GIFT: granulocyte immunofluorescence test
LIFT: lymphocyte immunofluorescence test

*: Negative

Discussion

More common causes of acute respiratory insufficiency in association with blood component transfusion, which should be excluded before a diagnosis of TRALI is made, include fluid overload, anaphylaxis, and transfusion of a bacterially contaminated unit. In contrast to patients with cardiogenic pulmonary edema, patients with TRALI (noncardiogenic pulmonary edema) will have normal central venous and normal or low pulmonary artery wedge pressure. Therefore, without invasive hemodynamic monitoring, cardiogenic and non-cardiogenic pulmonary edema can be extremely difficult to differentiate with certainty. However, in the absence of other apparent causes, the sudden onset of chills and fever, cough, and increasing dyspnea closely related to the transfusion of blood products is consistent with a clinical diagnosis of TRALI. The hospital blood bank and transfusion service should be notified of any potential case of TRALI, so that appropriate investigation of the involved donors and patient can be initiated.

TRALI has been associated with every type of blood component containing plasma, even if the quantity of plasma is very small.[2] Recently, TRALI has been documented after infusion of intravenous immunoglobulin.[15] In the majority of cases, HLA or granulocyte antibodies are identified in donor plasma.[2,9] Recent evidence supports a role for biologically active lipids, which accumulate during blood storage and act as neutrophil priming agents, in the pathogenesis of TRALI.[5,6]

The majority of TRALI patients make a complete recovery,[2] but there is an associated mortality of 5 to 10 percent, and the condition is associated with considerable morbidity.[2,3,9] In the United States, TRALI is the third most common cause of death related to transfusion.[16] The difficulties involved in the diagnosis of TRALI are clear from the most recent report of the serious hazards of transfusion (SHOT) initiative in the United Kingdom (1999-2000).[17] In the SHOT study, careful analysis of 18 cases for which a questionnaire had been completed suggested that six were probable cases of TRALI, nine were possible cases, and three were unlikely. In many cases serologic investigation was

incomplete, and in only a minority of cases was a crossmatch between donor sera and patients' cells performed.

In the present case, the close temporal association between the plasma infusions and the onset of dypsnea and hypoxemia was strongly suggestive of TRALI. The typical chest X-ray changes, along with the positive donor serology and positive crossmatch between the patient's granulocytes and the sera from two of the donors, further support the diagnosis. All four of the implicated female donors in this case had granulocyte-specific or HLA class I antibodies. Laboratory results do not indicate which of these antibodies (or combination of antibodies) initiated the TRALI, although crossmatch studies provide an indication of the clinical relevance of antibodies. That the infused blood products were FFP tends to reduce the likelihood that the patient's own HLA antibodies reacting with donor leukocyte antigens could have caused the TRALI or that immune complexes formed between donor antibodies and leukocyte antigens may be implicated.[18] Similarly, a role for the HNA-1a antibodies in donor 4 is unlikely, as the patient's granulocytes typed as HNA-1a–, HNA-1b+. Laboratory investigation eliminated ABO blood group incompatibility between the donors and the patient. Blood cultures taken from the patient after the event were sterile, as were samples from the implicated plasma bags, and an atypical pneumonia screen was negative. Serial cardiac enzymes and D-dimers were normal, which suggests that neither a myocardial event nor a pulmonary embolus was a factor in this case. The four female donors implicated in this reaction all had a history of pregnancy (average two pregnancies), but none had been transfused. In the crossmatch study, only antibodies from two of the donors (nos. 1 and 3) reacted with granulocytes from the patient, which suggests that these antibodies might be the most clinically relevant. However, the serum from donors 2 and 4 contained HLA class I and HNA-1a antibodies, respectively, and because both these specificities have previously been implicated in TRALI, all four implicated female donors have been counselled and excluded from the donor panel.

A recent randomized controlled study in intensive care patients found that plasma from multiparous donors was associated with impaired lung function in recipients when compared with control plasma (no history of pregnancy or transfusion).[8] It has been previously suggested that the use of blood components from multiparous donors should be restricted.[1,2] One study found HLA antibodies in 26 percent of female plateletpheresis donors with a history of three or more pregnancies.[19] However, despite 9000 donations by the 324 women in that study, none were implicated in a clinical episode of TRALI. It is generally agreed that the exclusion of such donors would lead to the loss of many safe donors and lead to a substantial reduction in the donor pool.

The development and implementation of standardized protocols for investigation of suspected TRALI cases would give us a better insight into the true incidence of the condition. Efforts to increase awareness among clinicians about this potentially fatal complication of transfusion therapy may also lead to increased reporting, improved diagnosis, and better understanding of this condition, thus facilitating steps to prevent TRALI.

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One thousand seventy antibodies detected only by a 2-stage papain test: wanted and unwanted positive reactions

D. Castellá, J. Cid, M. Panadés, and C. Martín-Vega

Despite the wide use of the antibody detection test for unexpected antibodies, controversy still remains regarding the use of enzyme-treated red blood cells. Over a 6-year period, 72,573 samples from 49,863 patients submitted for pretransfusion compatibility testing were examined for unexpected antibodies. The antibody detection tests included a low-ionic-strength solution (LISS) indirect antiglobulin test and a two-stage papain (2SP) test. One thousand and seventy of the 2267 (47%) antibodies tested by 2SP were reactive only by the 2SP test. Overall, the 2SP test detected only 0.6% of antibodies considered to be clinically significant (10 examples of anti-c and 2 examples of anti-e). The slight additional safety provided by detection of clinically-significant antibodies is overshadowed by the high number of clinically-insignificant antibodies detected by the 2SP test. ***Immunohematology* 2001;17:122–124.**

Key Words: antibody detection, enzyme-only antibodies

The main argument for the routine use of enzymes in compatibility testing would be their ability to detect clinically-significant alloantibodies that may be undetected by other methods.[1] In the past, published studies on this topic were often based on the analysis of selected cases.[2-4] However, recent studies show the lack of clinical significance of red blood cell (RBC) alloantibodies detected only by enzyme methods,[5,6] and a survey on pretransfusion testing showed that only 0.1 percent of 1051 facilities still use proteolytic enzymes for routine screening.[5]

The objective of the present study was to analyze retrospectively the specificity of 2666 antibodies detected in samples from 49,863 patients (5.3%) and the serologic methods that demonstrated reactivity.

Materials and Methods

Over a 6-year period, 72,573 samples from 49,863 patients submitted for pretransfusion compatibility testing in Barcelona, Spain, were examined for unexpected antibodies. The antibody detection tests included a low-ionic-strength solution (LISS) indirect antiglobulin test (IAT) and a two-stage papain (2SP) test.

The microtube column cards with and without an anti-human globulin (IgG/C3D) reagent in the gel were supplied by DiaMed-ID®, Iberica. The reagent RBCs used for antibody detection and identification are selected monthly from donors of known phenotype and prepared in our center. The papain was obtained from Merck® and prepared as follows:

- Solution 1: 1 gram of papain in 100 mL 0.9% sodium chloride
- Solution 2: 3.6 grams of $Na_2HPO_4 \cdot 2H_2O$ in 100 mL distilled water
- Solution 3: 0.446 grams of L-cysteine hydrochloride in 200 mL 0.9% sodium chloride

The three solutions were mixed and 1 mL was added to 1 mL of RBCs washed × 3. The RBCs were incubated for 7 minutes at 37°C, washed × 3 with 0.9% sodium chloride, and made up to an 0.8% suspension.

For both the 2SP and LISS-IAT tests, 25 μL of serum or plasma were mixed with 50 μL of the 0.8% papain-treated or LISS-suspended RBCs. The tests were incubated at 37°C for 15 minutes in microtube columns in buffered gel or AHG cards, respectively, then centrifuged in a DiaMed-ID centrifuge for 10 minutes, and read.

Results

Over a 6-year period, 72,573 samples from 49,863 patients yielded 7735 positive reactions (10.6%) in screening for unexpected antibodies. From the 7735 positive samples, 2666 individual samples were available for this study (Table 1).

Further investigation of these 2666 samples disclosed the presence of a single antibody in 1165 samples and two or more antibodies in 300 samples. Positive direct

Table 1. Total number of antibodies detected in samples from 2666 patients and the number of the total detected only by two-stage papain (2SP)

Specificity	Total Number	2SP only
D	239	9
C	35	17
E	450	258
c	33	10
e	3	2
C^w	14	5
D+C	83	1
D+E	12	1
E+C	4	2
E+c	21	0
D+C+E	12	0
K	95	0
K+E	18	0
Jk^a	23	0
Jk^b	2	0
$Fy^{a‡}$	26	0
$Fy^{b‡}$	2	0
Le^a	128	95
Le^b	50	40
M‡	32	0
P_1	16	14
S‡	7	0
$E+Fy^a$	6	0
Jk^a+E	8	0
$E+Le^a$	8	6
E+S	2	0
I,H,HI	18	11
$E+Kp^a$	2	1
s‡	1	0
Multiple alloantibodies	115	6
Nonspecific	370	301
Autoantibodies	432	291
Positive DAT*	258	Not done
Passive antibodies†	141	Not done
Number	2666	1070 positive out of 2267 tested (47%)

* Direct antiglobulin test
† Anti-D, -A, -B
‡ These antigens may be denatured by enzymes rendering them nonreactive in 2SP

antiglobulin tests (DATs) and passively-transfused antibodies (21 anti-A and 1 anti-B due to placental transfer of maternal antibody, 119 from Rh (D) immune globulin, and platelet transfusions) were seen in 258 and 141 cases, respectively. The remaining 802 reactive samples demonstrated either a nonspecific agglutination pattern (370 samples) or autoantibodies (432 cases) (Table 1). We further analyzed these 802 reactive samples according to the serologic methods in which they were reactive. It was found that 592 (73.8%) of the 802 samples reacted only by 2SP.

We also analyzed single Rh antibody specificities detected according to the methods in which they were reactive. Nine of 239 anti-D antibodies (3.8%) were detected only by 2SP. In addition, seventeen of 35 anti-C (48.5%), 10 of 33 anti-c (30.3%), 258 of 450 anti-E (57.3%), and 2 of 3 anti-e (66.6%) were detected only by 2SP.

Other specificities, such as antibodies against Kell, Kidd, Duffy, and MNSs antigens, were always detected with LISS-IAT, whereas antibodies against H, Lewis, and P system antigens were mainly identified by 2SP (Table 1).

Discussion

The reason for performing a pretransfusion screening for antibodies is to detect any unexpected alloantibody in the recipient that might result in an increased destruction of transfused RBCs.[1] Further studies are usually needed to distinguish between clinically significant and insignificant antibodies and to identify the antibody's specificity in order to select antigen-negative RBCs for transfusion. Positive antibody detection tests due to antibodies that are not expected to be clinically significant are troublesome, since they lead to costly and time-consuming studies that eventually will prove to have been unnecessary.

The most frequently encountered alloantibodies reacting only by 2SP were those to antigens of the Lewis, H, and P systems. Antibodies against antigens of these systems are usually considered to have no clinical significance.[7] More controversial would be the possible relevance of enzyme-only Rh system antibodies, since all Rh antibodies are traditionally regarded to be clinically significant.[8] In the present study, we detected 258 out of 450 anti-E antibodies by only 2SP, but other studies have demonstrated that enzyme-only anti-E antibodies do not decrease the posttransfusion survival of incompatible RBCs and are not implicated in delayed hemolytic transfusion reactions (DHTRs).[9-10] We detected 17 out of 35 anti-C antibodies by only 2SP, but anti-C alone is rare[4] and it is frequently detected with anti-D.[8] Only 12 out of the 275 remaining Rh antibodies reacted only in 2SP. In one study,[7] only one of 19 patients with an enzyme-active antibody who received antigen-positive blood had a DHTR.

Transfusion services that do not use enzymes routinely as an antibody detection test have not reported an increase in the incidence of DHTRs due to E, Lewis, or P_1 antibodies that would be expected if the high frequency of these papain-only antibodies in the present study and in other studies is taken into account.[7]

In summary, in this study, the use of 2SP resulted in detection of enzyme-only, clinically significant antibodies in only 12 (0.6%) of 1864 patients: ten examples of anti-c and two examples of anti-e (Table 1). In our opinion, the slight additional safety provided by the detection of these antibodies does not compensate for

the high number of unwanted positive reactions produced by the 2SP, since they lead to additional costly studies and result in unnecessary delays in transfusing patients.

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Switching donor cells as a major source of error in compatibility testing

B.J. Padget and J.L. Hannon

The most likely cause of fatality in blood transfusion is transfusion of the wrong unit of blood to a patient. This type of error is usually attributed to improper patient identification at the time of sample collection or transfusion. A retrospective analysis of the results of an external proficiency testing program identified a common source of error occurring during laboratory testing that has not been previously reported. Results were analyzed when major errors were assigned to laboratories for obviously switching donor units in compatibility testing and/or subsequent investigation. In 24 surveys sent to extended testing (Level A) laboratories and 18 sent to basic testing (Level B) laboratories, the antigenic composition of the two donor cells made it possible to determine whether the cells had been switched. Seven errors were assigned to Level A participants for switching donor units during testing, constituting 38.9 percent of the 18 major errors assessed. Level B participants were assigned eight errors for switching donor units, 26.7 percent of the 30 major errors assessed. Approximately one-third (31.3 percent) of major errors committed on 42 proficiency testing surveys were caused by switching of donor cells during compatibility testing. This type of error may result in transfusion of an incompatible donor unit. ***Immunohematology* 2001;17: 125–129.**

Key Words: compatibility testing, crossmatch errors, transfusion reactions, proficiency testing

Published data indicate that the most common cause of transfusion-related fatality is transfusion of a unit of blood to the wrong patient. Linden et al.[1] reported that one in 12,000 allogeneic red cell units is transfused to the wrong patient, with one in 600,000 units causing death related to ABO incompatibility. This is supported by data collected by Newman.[2] The majority of such errors occur as a result of improper patient identification at the time of pretransfusion sample collection or the transfusion of blood intended for one patient into another.

Sazama reviewed 355 transfusion-related deaths reported to the Food and Drug Administration (FDA) between 1976 and 1985. One hundred and fifty-eight of 256 (62%) classifiable cases were attributed to acute hemolysis. ABO incompatibility was confirmed as the cause of hemolysis in 131 cases, with an additional 12 cases also likely due to an ABO mismatch. Other antibody-antigen reactions were responsible for hemolysis in 9 cases. Forty-five of 158 (29%) incidents were directly related to laboratory errors, including 20 serologic errors and 16 cases relating to incorrect samples or records. Sazama concluded that "wherever errors could be made, errors occurred."[3]

Linden et al.[1] analyzed errors that occurred during a 22-month period resulting in the administration of an incorrect unit of blood or of blood to an unintended recipient. The data were consistent with Sazama's findings, with 25 of 104 (25%) incidents the result of errors occurring in the laboratory. Another notable finding was that 20 percent of the events involved a sequence of errors.

Data from the Serious Hazards of Transfusion (SHOT) study in the United Kingdom, reported at the International Society of Hematology (2000), reviewed transfusion complications over a 3-year period (1996–1999).[4] Of 575 fully analyzable cases, there were 28 deaths related to transfusion, an additional nine deaths possibly related to transfusion, and 111 incidents of major morbidity. Three hundred and thirty-five of 618, or 54.2 percent, of the cases reported were related to transfusion of the wrong unit of blood to a patient. Major sources of error included collection of the wrong unit of blood from the blood bank refrigerator and failure of the bedside identification process. In many cases, errors could be attributed to a sequence of procedural failures in preparing a unit for transfusion. One hundred and thirteen of 575 (20%) incidents were attributed to laboratory errors; these included failure to consult and/or heed historical records as well as incorrect grouping, crossmatching, and labeling.[4]

Retrospective analysis of the results of an external laboratory proficiency program has identified an additional common type of error that occurs when donor red blood cells (RBCs) are switched during

laboratory testing. The American Association of Blood Banks (AABB) Standard 5.13.1 requires that donor RBCs for crossmatch be obtained from an originally attached segment.[5] The RBCs are then washed with saline solution and suspended in a manner appropriate to the planned method. Accurate labeling when RBCs are transferred from the segment to the test tube is critical. As well, the addition of RBCs to any test must include an identification check. A third possible type of error occurs at the time of recording of results. Identification errors at any of these steps may result in assignment of test results to the incorrect unit. When the critical antigen types of the units differ, this may have adverse consequences.

Study Design

An external proficiency testing program distributes survey challenges to 116 hospital laboratories in two Canadian provinces. Participation in the program is mandated by the respective provincial laboratory accrediting bodies as one requirement for licensure. The testing level of the participants ranges from laboratories performing basic testing only (ABO and Rh(D) typing, antibody screen, and crossmatch) to immunohematology reference laboratories. Facilities are categorized as Level A laboratories if they perform extended testing; antibody identification; and, in some cases, complex serologic investigation, in addition to basic testing. Laboratories categorized as Level B perform basic testing only. Level B laboratories refer any problem samples to Level A facilities for investigation.

Two challenges are sent bimonthly. Each challenge consists of a simulated sample of a patient's RBCs and plasma, and two donor RBCs. The red cells are shipped in 7 mL Vacutainer® tubes (Becton Dickinson and Company, Franklin Lakes, NJ) as unwashed RBCs suspended in Physiosol Solution (Dominion Biologicals Limited, Dartmouth, NS, Canada). Participants prepare aliquots of the donor samples for testing, a process similar to that of preparing a sample for testing from a segment attached to a donor unit.

Survey challenges monitor preanalytic sample evaluation, testing methods, test results, and postanalytic follow-up. Participants are expected to process the survey samples as they would a patient's sample. The results submitted by participants are evaluated on the basis of test results, interpretation of results, use of appropriate procedures, and clerical accuracy.

Results from five referee laboratories are assessed prior to evaluation of participant responses to ensure that sample quality has not been compromised during the shipping process.

A major error is assessed when such an error would result in a physician receiving an erroneous or misleading report that could result in injury to the patient. When proficiency is found to be deficient, a letter requesting remedial action is sent to the participant. The participant is then required to reply, outlining corrective action. If the performance of the laboratory remains deficient despite attempts to resolve the problem, the issue is then referred to the Advisory Committee on Laboratory Accreditation for review and possible action.

Trend analysis of participant results for the year 2000 indicated that a number of major errors had been assigned when laboratories had switched the donor RBCs in compatibility testing and/or subsequent investigation. These preliminary data led us to retrospectively analyze the results for the two program levels over a 48-month period (March 1997 to February 2001) with the aim of determining the actual rate of this type of error.

The antigenic composition of the two donor RBCs provided with the survey made it possible to determine whether the cells had been switched during testing in 24 surveys sent to 42 Level A laboratories and 18 surveys sent to 74 Level B laboratories (Table 1). On the remaining 24 Level A and 30 Level B challenges distributed during the study period, the necessary testing on the donor RBCs did not allow for differentiation of the cells; therefore, these surveys were not included in the analysis.

Table 1. Major errors assessed

	Level A		Level B		Total	
	No.	%	No.	%	No.	%
Surveys	24	—	18	—	42	—
Participants	42	—	74	—	116	—
Opportunities for error	1008	—	1332	—	2340	—
Errors due to switching cells	7	0.7	8	0.6	15	0.6
Other major errors	11	1.1	22	1.7	33	1.4
Total major errors	18	1.8	30	2.3	48	2.1

Results

A total of 48 major errors were assessed on the 42 surveys included in the data analysis. Eighteen errors were assigned to Level A participants and 30 to Level B

participants (Table 1). The overall rate of major errors was 1.8 percent for Level A laboratories and 2.3 percent for Level B laboratories (Table 1). The classifications of the major errors assessed on these survey challenges are outlined in Table 2.

Table 2. Classification of major errors

Error category	Switching cells	Other errors	Total
ABO typing	3 (38%)	5	8
Rh(D) typing	4 (25%)	12	16
Crossmatch	5 (50%)	5	10
Crossmatch/antigen typing	2 (100%)	0	2
Antigen typing	1 (33%)	2	3
Other	0 (0)%)	9	9
Total	15 (31%)	33	48

Seventy-nine of 116 (68%) participants did not receive a major error on the 42 surveys and 29 of 116 (25%) participants were assessed only one major error. Table 3 outlines the number of major errors per participant.

Table 3. Errors per participant

No. of errors	Level A	Level B	Total
0	28	51	79
1	11	18	29
2	2	4	6
3	1	0	1
4	0	1	1
Total	42	74	116

Seven errors were assigned to Level A participants for switching donor cells during testing, constituting 38.9 percent of the 18 major errors assessed and an error rate of 0.7 percent. Eight errors were assigned to Level B participants for the same errors, 26.7 percent of the 30 major errors assessed and an error rate of 0.6% (Table 1). As results are assessed and corrective action taken within 10 to 14 days of the initial testing, it was not possible to retrospectively identify errors resulting from incorrect serologic testing as compared to clerical errors. One Level A participant was assigned two major errors for switching cells. This occurred as the result of switching cells between two surveys which were distributed at the same time. No other participant was assigned more than one error in this category during the study period.

Three of the errors resulted in determination of incorrect ABO typing on the donor units. These errors accounted for 38 percent of all major errors in ABO typing (Table 2). In two cases type A and AB units were switched; the third case involved type A and O units.

Four errors involved incorrect Rh types on donor cells, 25 percent of all major Rh typing errors (Table 2). One participant received two errors for switching the Rh-positive donor units of one survey challenge with the Rh-negative units of a second challenge. Two other cases involved switching of the RBCs when both a D+ and a D– unit were distributed for crossmatch with a D– patient.

In eight cases, the RBCs were switched during compatibility testing and/or subsequent investigation. These eight errors constituted 53 percent of the total major errors assessed in this area of testing (Table 2).

Five Level B laboratories reported an antigen-positive donor unit as crossmatch compatible in the presence of the corresponding antibody, as a result of switching the two donor RBCs. Three of the surveys involved anti-E and two, anti-K. It is unclear whether the cell suspensions were incorrectly labeled or the results were switched at the time of recording. Two Level A participants also reported an antigen-positive donor unit as suitable for transfusion in the presence of an antibody. The antibodies present in the patient samples were again anti-E and anti-K. The laboratories performed both the crossmatch and antigen typing on cell suspensions that were incorrectly identified.

The final situation involved a patient sample with anti-Fy^b and anti-A_1. The donor units were both A_1+ and crossmatch incompatible. However, the participant performed Fy^b typings on donor RBC suspensions that were switched and reported incorrect antigen typing results.

Discussion

The possible clinical consequences of the errors described in this paper are disturbing. However, in a clinical transfusion setting, incorrect ABO or Rh (D) typing of a donor segment should be detected due to a discrepancy with the unit labeling. Although an incorrect ABO or Rh typing is an error of major significance in any situation, transfusion of an ABO or Rh incompatible unit should not occur if laboratory procedures for reconciling test results with unit labeling are adhered to. As previously noted, a significant finding of the SHOT study was the occurrence of multiple procedural failures contributing to a transfusion incident.

Although many institutions no longer routinely perform serologic crossmatching, AABB Standard 5.13.1 requires this testing when clinically significant antibodies are detected in a patient's sample.[5] A study by Kuriyan and Fox reported that an antiglobulin crossmatch was required for 10.6 percent of units

selected for patients.[6] Incorrect identification of cell suspensions in these situations would result in apparently compatible donor units having the potential to cause a serious transfusion reaction.

Although Level B facilities routinely refer antibody samples to a reference laboratory for investigation and provision of compatible units for transfusion, some are located in remote areas where emergency transfusion occasionally is required prior to completion of the antibody investigation. The emergency protocol in such hospitals may allow the issue of crossmatch-compatible units prior to receipt of fully crossmatched units from the larger facility. Errors such as those noted in the proficiency surveys would result in release of incompatible units, with the possibility of serious injury to the patient.

Participants in the program are encouraged to treat the proficiency testing samples in exactly the same manner as patient samples. However, one assumes that proficiency samples are generally tested with more care than routine samples. This would indicate that the error rate may represent an underestimate of the actual rate.

Participants who are assigned errors are required to implement corrective action. Most participants document retraining of personnel as their corrective action. As no participant was assigned a second error for switching donor cells subsequent to the first error, the corrective action procedure appears to have been effective in these laboratories. However, laboratories should also analyze their processes internally to ensure that these types of errors do not occur. If such an error does occur despite process controls, measures must be in place to ensure that the nonconformance is detected prior to release of blood for transfusion. One option to consider is a verification step to confirm that the cells tested are from a segment removed from the correct donor unit. Use of the segment number as well as the donor identification number might aid in this check, although the use of two identifiers is labor-intensive and may introduce an additional opportunity for error. To ensure that the donor RBCs are added to the appropriate test tube, participants might consider labeling the test tube with the donor number. As well, when donor units are deemed compatible in an antibody situation, repeat testing of RBCs obtained from a second segment attached to the compatible units may be appropriate.

The use of processes that minimize congestion of test tubes in the test rack would also help prevent such errors. Chapman et al.[7] report that the use of a computer crossmatch for routine testing reduces stress on staff and creates laboratory benches that are less cluttered, therefore allowing staff to concentrate on proficient testing.

It should be noted that a higher rate of errors due to switching cells occurred in the Level A facilities. These are the laboratories that are most often staffed by technologists dedicated to transfusion medicine testing. Technologists in Level B laboratories are usually generalists, and often they work in several laboratory areas concurrently. Therefore, the assumption that this type of error is related to a lack of technical expertise and/or experience in the procedure may not be accurate.

Conclusion

Fatality due to blood transfusion is most often caused by transfusion of the wrong unit of blood to a patient. While this is usually attributed to improper patient identification at the time of pretransfusion sample collection or transfusion, laboratory errors also may contribute to these events. One common source of error identified during external proficiency analysis is the switching of donor RBCs during testing.

Approximately one-third (31.3%) of the major errors committed on 42 proficiency testing surveys were caused by the switching of donor RBCs during compatibility investigation. The errors resulted in the reporting of incorrect ABO and Rh typing and compatibility results. These errors could result in the transfusion of an incompatible donor unit.

To ensure that compatibility testing results are accurate and, therefore, increase the safety of transfusion, laboratory practitioners must include steps in laboratory procedures to confirm that testing results have been assigned to the correct donor unit.

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COMMUNICATIONS

Letters From the Editors

Review: 2001

This year has been both exciting and sad. We said our last farewells to several friends and colleagues, and we were greatly saddened by the deaths of John Case and Bill Sherwood, who were editors and supporters of *Immunohematology,* and Ruth Sanger and Fred Stratton, who were two of the greats of the blood grouping field. Jane Haber was a technologist who began her career about the same time as the antiglobulin test. We are privileged to publish some of her career remembrances in this issue under "Those Were the Days." They all will be missed.

We thank the many authors who contributed the outstanding articles to the four issues published in 2001. We are proud that many of the authors are from outside the United States and want to share their experiences from around the world. Please continue to consider *Immunohematology* when you have interesting, original, and review information to be published.

We thank our editorial board, whose names are published in each issue. A special bow to them for their important suggestions for improving the journal and their constant support. Board members also serve as peer reviewers.

Last, but never least, we thank the following individuals who assisted us in reviewing and selecting papers for publication:

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The success of the journal is directly related to the quality of papers. We hope the authors, editors, and reviewers will accept our sincere gratitude and admiration for your contributions.

The readers, authors, and editors sincerely thank Ortho-Clinical Diagnostics for their 11th year of support. Not only do they contribute financially, they also distribute each issue to the members of the Ortho Bankers Club. We all owe them a great debt of gratitude.

The "new" *Immunohematology* Web site is now 2 years old and one of the finest of any publication (we may be prejudiced). Six years of the journal, cover to cover, are available to anyone who logs on to redcross.org/immunhematology or redcross.org/pubs/immuno and uses the password (2000) to access the site. The user can read, subscribe, search by word or phrase, send a letter to the editor, obtain instructions to authors, and so much more.

Finally, we ask those of you who have new data needing publication, or a review article in the word processor, to consider *Immunohematology*. Without your red-cell, white-cell, and platelet serology and transfusion, education, computer, and other appropriate manuscripts, there would not be a journal.

Delores Mallory
Editor-in-Chief

Mary McGinniss
Managing Editor

Congratulations and Thanks

It is with great pleasure that we congratulate Christine Lomas-Francis, MSc, who this year was awarded the Ivor Dunsford Memorial Award by the American Association of Blood Banks. Christine Lomas-Francis received the much-deserved award for her exceptional contribution to blood group serology. Ms. Lomas-Francis is the technical editor for *Immunohematology.*

We also would like to thank Sonia Connor, Copy Editor for *Immunohematology* for the past 5 years. She has an amazing ability to sort through all the scientific writing and put it in the correct form and order, and we shall miss her enthusiastic and caring help and guidance. We welcome our new Copy Editor, Lucy Oppenheim, and look forward to more expertise.

Delores Mallory
Editor-in-Chief

Mary McGinniss
Managing Editor

"Those Were the Days"

Note: Exerpts from a talk prepared by Jane Haber late '70s or early '80s

I feel as if I should arrive in a wheelchair. When you are suddenly asked to recall your personal experiences in a field, you realize that you have evidently had the same job for a long time. How did I get to this point? I grew up in blood banking. When I first went to work in the central laboratories of The New York Hospital, blood banks didn't exist. I was earning $100 a month. The blood bank at the New York Hospital started in 1939 in the Department of Obstetrics. It moved to the main hospital in 1940. The first technician who was in charge of the blood bank went to medical school and the second one went to war. This was three wars ago—the Second World War. It was what I thought I would like to do, but since I was the only one in the then-central laboratories that knew even a small amount of blood banking, I was persuaded to take on the job temporarily. Temporarily has become a very long time. I have only worked for two bosses. My first boss, Dr. Ralph Stillman, retired, and the second, Dr. Aaron Kellner, is my present boss. This I think is unusual in itself because today blood bankers tend to jump around from job to job. I think that Dr. Kellner's influence and my close association with Dr. P. Levine were the most important factors in making me a true blood banker.

When my blood bank first started, the only tests that were done were ABO, Rh, and saline crossmatches. If we had an Rh-negative patient, the sample was sent to Dr. Phillip Levine for confirmation. At that time, the differences between R1 and R2 were poorly defined or understood, and the sera we had were miserable and unreliable. ABO was done on slides and Dr. Chown's capillary method was used for Rh. We now do all ABO and Rh tests in tubes (and test all Rh-negatives for D^u), but we still use the Chown capillary method for Rh. Transfusions were given with care and as they were successful, the volume grew from approximately 3500 transfusions in 1940 to our present volume of 16,000 blood transfusions per year.

The first major step that was a little out of the ordinary did not occur until about 1952. By then, it was realized that there were many antigens capable of stimulating antibody formation other than Rh. Antibody screening procedures had to be developed. We started using enzymes for antibody screening—first trypsin, and now ficin, which is still in use.

In 1953, one of our house staff was operated on for a gastric ulcer. Following surgery, he displayed signs of a hemolytic transfusion reaction. In the course of working up the transfusion reaction, it was found indeed that he had had a hemolytic transfusion reaction. Both pretransfusion and posttransfusion specimens were compatible with the blood that he received, by saline and enzyme crossmatch. When the indirect Coombs crossmatch was done, the pretransfusion specimen was compatible but the posttransfusion specimen was incompatible and the antibody was identified as Fy^a. He had been previously transfused in South America. From that time on, every single crossmatch that has been done at The New York Hospital has had both a saline and an indirect Coombs compatibility done. Every patient and every donor is screened for antibodies using an enzyme technique and an anti-human globulin. Soon after this experience, we prepared a paper about it, and I presented it to several groups in many parts of the United States. At that time the constant queries following my presentation were invariably "Do you have the time for a Coombs test?" and "How do you sell the administration on the added cost of Coombs serum?"

What happened in the next 20 years? In 1954, we found 13 examples of anti-Kell in a routine transfusion population, exclusive of obstetrical cases. Antibodies really did exist in people who needed transfusions.

In 1960 Dr. Richard Silver from the Department of Hematology went to South America and had the opportunity to go into the Brazilian jungle. In the valley of the Motto Grosso live what is left of five tribes of primitive Brazilian Indians. With great difficulty he bled about 100 of them, and shipped the specimens to me. ABO, Rh, and extensive testing for all the antigens for which we had typing serum was done. We found what we thought was a new allele in the Kidd system. Just as we were arriving at these conclusions the latest copy of *Transfusion* arrived. We were scooped. In the journal was a report of one patient from Hawaii who appeared to be Jk(a–b–). In our Indian series, there were five such individuals. We had some strange S, s, U results, too. Despite the efforts of several geneticists, Dr. L.C. Dunn at Columbia, and Dr. E. Hackel from Michigan State, no conclusions were forthcoming. In light of present knowledge, our results could have been important.

In 1966–67, we were involved along with many other large teaching hospitals in the preclinical trials of what is now RhoGAM™. Rh-negative women who were not immunized and had Rh-positive, Coombs-negative babies were put into two groups—one group received RhoGAM™ and the other a placebo. The blood bank did antibody titers on these women for 6 months. We did this double blind. We did not know which were test patients and which were controls. In fact, as the result of these clinical trials, RhoGAM™ was released for all women in 1968. With the adoption of the present abortion law in New York State, we now give it not only to women post-delivery, but to all Rh-negative, non-immunized abortion patients. Although a small number of failures have been reported, you will live to see the virtual elimination of hemolytic disease of the newborn due to anti-Rh_o. It is an unusual occurrence that the cause of a disease and its prevention can be accomplished in 30 years.

Until 1966, the International Hematology and Transfusion Societies held a joint meeting every 2 years. In 1954, this meeting was in Paris, and I was given the opportunity to go if I visited blood banks along the way. My first stop was London, and I arrived with a letter to Robert Race, whom I had met in the USA. I tried to find the Lister Institute. I was going to Mecca—Race and Sanger were my gods and *Blood Groups in Man* my bible. The Lister Institute was such an old, run-down building that I passed it several times before I realized it was what I was looking for. They now have a brand-new, modern wing, which I saw last year. I met Ruth Sanger and we had lunch. Everyone was extremely cordial and later Race and Sanger insisted on taking me out to dinner. We went to a lovely place on the Thames frequented by the English princesses, one of whom is now Queen Elizabeth, where we had an excellent dinner.

In London, then as now, there were two large donor centers—one at each end of London. I visited one of them. There were huge rooms filled with women washing glassware and tubing. Labor was cheap, and disposable equipment was very expensive. Plastic bags were not even on the horizon. I ended my visit to England by going to Cambridge to visit Dr. Robin Coombs. Cambridge is a beautiful old university town, and Coombs' laboratory was on the top floor of an old building. To reach it you climbed a spiral staircase. At that time his most cherished possession was a centrifuge he had made from an old aluminum pot. The building, the staircase, and the lab itself made me feel I was at King Arthur's court, visiting Merlin.

I left London with a letter to Dr. Van Loghem in Amsterdam. On my first morning there, I found there wasn't a language problem but there was a telephone one. After 45 minutes I finally made contact with Mia Vanderhart, who was Van Loghem's assistant. I went downstairs in the hotel with the address clutched in my hand and asked the concierge to get me a taxi to go to this address. He smiled and said it was across the street and proceeded to escort me there. They did all ante-natal screening and helped solve problem cross-matches.

Paris was next—the meeting was at the Sorbonne. I visited some of their laboratories and saw my first electron microscope in Bessis' laboratory. He is the one responsible for the first beautiful pictures of red cells that look like doughnuts. I flew home from Paris, arriving with only enough time to change my luggage and go to Washington to present the previously mentioned report on the Kell antibodies.

In 1956, the meeting was in Boston, which was a letdown, but things got better. In 1958, it was in Rome and in 1960, it was in Japan. Blood bankers are an international fraternity, and they do get around. Even then the size of the meetings was large, and there were many simultaneous sessions as well as sessions in languages other than English, where you wore a headset and could hear the paper in English, even though the speaker was talking in another language. It is very hard to follow a paper in this fashion.

Japan was whole new world. It is a strange sensation to see signs that you can in no way interpret. When we left the hotel, we were given a card with the name of the hotel in Japanese. This could be shown to the cab

driver so we could get back. As expected, the meeting had many Japanese speakers whose idea of spoken English is radically different from ours.

1962, it was in Mexico City, where everyone who was accustomed to living at sea level had trouble adjusting to the altitude. Following this meeting, we went to South America. A minor revolution in Argentina caused some change in our plans. In Chile, the American Embassy said it was safe to go in, while the British Embassy said if you were a British subject, it was not advisable to go. We delayed the trip one day and had no trouble. When we got home, no one here seemed aware that there had been any trouble in Buenos Aires. While in Buenos Aires we visited a large research center devoted to hematology research.

1964, the meeting was in Sweden, where the high spot was seeing where the Nobel Prize is awarded. There is a long flight of stairs you have to descend. It is just as well that I will never win the Nobel Prize, because I could never come down those stairs gracefully.

In 1966, it was in Australia, which would need a paper of its own. Though it was August, and supposed to be spring, it was cold and damp. Men gave papers in overcoats and mufflers.

In 1969, it was in Moscow. Red Square is just as you have seen it pictured. The meeting was at the university, in one of the largest halls I have ever seen. The high spot was a visit to the hospital where they collect cadaver blood. It was a "live" demonstration. I have faked enough demonstrations for photographers to recognize a real cadaver. In 1972, it was back in the USA, in Washington.

The last 5 years has seen the extensive use of component therapy. Whole blood is no longer the best therapeutic product. Blood banks are now rated in terms of the number of packed cells they use. If 30 percent of transfusions are not packed cells, investigation of transfusion practices should be started. We use about 50–55 percent packed cells and should do better. A single unit of whole blood can now supply the needs of five patients—packed cells for anemic patients; platelets for leukemic patients; cryoprecipitate for hemophiliacs; fresh-frozen plasma for patients with liver disease; and white cells, the use of which is still quite limited and experimental. Tight inventory control is needed to utilize more effectively the available blood supply, especially during seasonal shortages. Our discard rate is less than 2 percent, which is well below the national average of 16 percent. Education of house staff and the basic philosophy of no one patient owning a unit of blood can accomplish this.

Today my blood bank is a large operation, i.e., 16,000 blood transfusions per year. Open heart surgery started in 1958, when we did 47 cases. In the first 6 months of this year, we did 206. We do most of the kidney transplants done in the New York metropolitan area, using both related and cadaver kidneys. We have done two bone marrow transplants. We are just starting the use of matched platelets in certain situations.

What is in the future for the blood bank? I think the field is still wide open. The number of clinically important antibodies seems to increase each year. From five Rh antigens, we are now able to identify more than 100 clinically important ones. Automation is well on the way. Present autoanalyzers are 10 times more sensitive than hand methods for antibody detection. I would like a quick, easy method to screen for white-cell antibodies such as we have for red-cell antibodies. This could help eliminate pyrogenic reactions. The use of frozen blood should become cheaper, and the reconstituted cells must have a longer shelf life than the present 24 hours. This could help abolish chronic blood shortages. Much is still to be done on platelet preservation and the need for matched platelets. In spite of HLA testing and good matches, why are some kidney transplants rejected? Is it due to white cells or other immunological mechanisms?

Why should anyone want to be a blood bank technician? It's a 24-hour-a-day job, 365 days a year. The blood bank is one of the most critical laboratories in the hospital. There is no room for human error. You have to have a thirst for knowledge, a desire to read and learn, and an ability to recognize results that seem unusual, even if you don't know why, and a willingness to go beyond the ordinary needs of the job. This probably can be defined as total dedication. Would you be willing to be transfused with the blood you have just crossmatched?

Blood banking has been good to me. I have made a great many friends and have had many warm and exciting professional associations. Several years ago, at the AABB meeting in San Francisco the invocation was given by a priest who drew a comparison between a transfusion of blood and the wine used in the communion service. Playing God is not easy, and you do this every day. The satisfaction comes when you finally do go home—you know that someone is alive because of your efforts.

Jane Haber

IN MEMORIUM

Jane Haber was blood bank supervisor at the New York Hospital, Cornell Medical Center, for 44 years, 1943–1987. She retired in 1987 to Boca Raton, Florida, and died peacefully on December 20, 2000.

Ms. Haber received her bachelor's and master's degrees from the University of Michigan. She was very active in state and national organizations, both as a member of the board and president of the Blood Bank Association of New York State and as a member of the board of the American Association of Blood Banks (AABB). She also served on many AABB committees, including the Editorial Board of *Transfusion*.

Jane Haber
1916 – 2000

In 1979 she received the Ivor Dunsford Memorial Award from the AABB, which stated "In recognition of her pioneering work spanning the first forty years of modern blood banking during which she created the model of the specialist blood bank supervisor as a cornerstone of hospital transfusion service and as a 24-hour-a-day consultant to the practicing physician in the care of patients; for her appreciation and promotion of innovation in immunohematology and its application in the hospital blood bank, particularly the Coombs cross-match technique; and for her unswerving dedication to the training of technicians, house staff, and blood bank physicians."

In 1983, she received an award from the New York Blood Center for her pioneering role in American blood banking and support of the New York Blood Center.

Those who knew Jane knew her to be fiercely independent, loyal, and honest. She was truly a leader and pioneer in the profession.

IN MEMORIUM

Ruth Sanger
1918 – 2001

Ruth Sanger was born June 6, 1918, in Queensland, Australia. She was educated at Abbotsleigh School in Sydney and Sydney University. She joined the Red Cross Blood Transfusion Service in Sydney in 1940 and worked in the blood plasma drying plant and then in the blood grouping laboratory. She went to England after the war and worked for a year with the famous blood group researcher Dr. R.R. Race at the Medical Research Council (MRC) Blood Group Unit in London. Dr. Sanger completed her PhD at London University in 1948 and returned to Sydney. In 1950, she returned to Lister Institute to work with Dr. Race, whom she married and with whom she collaborated until his retirement in 1973. Dr. Sanger continued as director until her retirement in 1983. Her contributions to human blood group research are beyond enumeration. One of her contributions was coauthorship of one of the most important texts ever published about blood groups, *Blood Groups in Man,* by Drs. Race and Sanger. Six editions were published and it was the bible of blood groups for more than 30 years. Ruth Sanger was brilliant and erudite, and she had a marvelous dry humor that could be discovered in her writings and her interaction with her many friends and coworkers. Because of the exciting work at the MRC Blood Group Unit, people from around the world came to study and work with Dr. Sanger and her group. The unit played a central role in defining most of the hundreds of blood group antigens identified over 40 years.

Dr. Sanger received many awards for her work, and she became an honorary member of many societies around the world. She was elected a Fellow of the Royal Society in 1972, and she and Dr. Race received the Karl Landsteiner Memorial Award, the highest honor awarded by the American Association of Blood Banks.

Dr. Sanger was warm, kind, intelligent, and charming. She was particularly kind and encouraging to new serologists and investigators and she will be remembered with great respect and affection by all who were privileged to know her.

ANNOUNCEMENTS

Monoclonal antibodies available. The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive direct antiglobulin test. Anti-Rh:17 is a directly agglutinating monoclonal antibody. Anti-Fy^a, anti-K, anti-Js^b, and anti-Kp^a are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. **Contact:** Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org.

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3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 6 under Preparation
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