

# Research Opportunities in Transfusion Medicine

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## Scope of the Problem

The field of transfusion medicine began 100 years ago, in 1900, with the discovery by Landsteiner<sup>1</sup> of the ABO blood group system. This discovery demonstrated that plasma proteins have defined specificities. These plasma proteins, later termed antibodies, recognize epitopes on red blood cells. These discoveries constituted a starting point for blood banking—collection and storage of blood—and for immunohematology, the serological investigation of blood group antigens (FIGURE 1). During the past 3 to 4 decades, significant advances have been achieved in improving the blood supply with respect to availability, safety, and fractionation into components, such as red blood cells, platelet concentrates, and plasma proteins.

Donors currently donate approximately 12 million units of blood annually in the United States.<sup>2</sup> Without these donations, many procedures and treatments, such as hematopoietic stem cell transplantation, complex cardiac and orthopedic surgery, and organ transplantation, would not be possible. A safe and adequate blood supply is a fundamental necessity to support state-of-the-art medical and surgical therapies. However, resources are now needed to translate advances in the biology of hematopoietic cells into newer cellular therapies and to investigate the unique immunological effects that result from transfusion of blood cells. This article discusses current status and progress in several aspects of transfusion medicine, including adequacy and safety of the blood supply, appropri-

In recent years, the translation of basic research in transfusion medicine has led to development of novel cellular therapies using well-characterized cell populations isolated from either bone marrow or blood (eg, hematopoietic stem and progenitor cells, T lymphocytes, dendritic cells). Refinements in cell therapies will make possible optimal stem cell engraftment, gene therapy, immunotherapy of cancer and infectious disease, and even solid organ regeneration. Moreover, the immune consequences of transfusion therapy are better appreciated and opportunities are at hand to prevent or blunt unwanted immune responses, such as platelet refractoriness and graft-vs-host disease. Transfusion medicine has become a broad, multidisciplinary field that has evolved beyond issues related to blood procurement and storage. The next series of advances in transfusion medicine will complement the current approaches of donor blood screening and viral/bacterial inactivation steps to ensure a safe and adequate blood supply.

*JAMA. 2000;285:577-580*

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ate use of transfusion therapy, development of novel cellular therapies, and manipulation and prevention of immune responses.

## Major Advances

Advances in transfusion medicine that have occurred during the past 25 years include reduction in risk of virally transmitted disease, pharmaceutical production of recombinant clotting factors, isolation and storage of stem and progenitor cell populations for transplantation, and genetic characterization of blood group antigens.

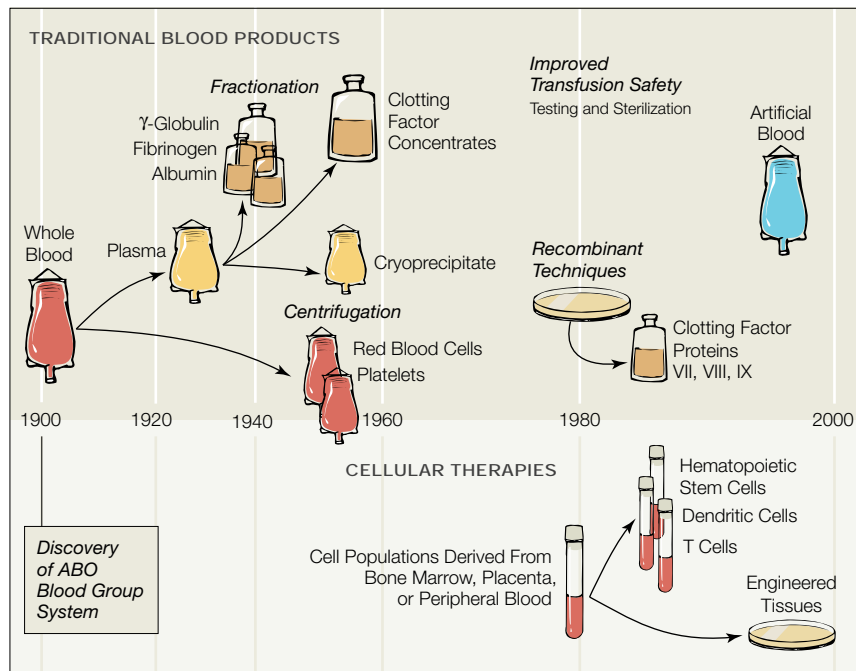
Reduction in transfusion-transmitted viral disease was achieved by a conversion from paid to volunteer donors, by improvement in donor screening, and by improvement of assays that detect viruses in donor blood. Since implementation of nucleic acid testing of donor blood, the estimated risks of hepatitis B (1 per 63 000 units), hepatitis C (1-3 per 1 million units), and human immunodeficiency virus (HIV)

(1-2 per 1 million units), are now minuscule (FIGURE 2).<sup>3</sup> At the same time, pasteurization and solvent-detergent treatment have virtually eliminated risk of HIV and hepatitis transmission through clotting factor concentrates and other plasma derivatives.

Advances have also been made in hematopoietic stem and progenitor cell transplantation for both hematologic and nonhematologic malignancies. The realization that stem and progenitor cells circulate in peripheral blood and that these cells can be mobilized from bone marrow using cytokines has led to outpatient cytopheresis procedures.<sup>4</sup> Transfusion medicine specialists are now involved in collection, in vitro manipu-

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**Figure 1.** Evolution of Transfusion Medicine Cellular Therapies

Whole blood transfusions were the mainstay of transfusion therapy until the 1950s. Fractionation of plasma into derivatives such as albumin and clotting factor concentrates was introduced in the 1940s. Using centrifugation, whole blood was separated on a wide scale in the 1960s into various cellular and acellular components. Beginning in the 1970s, platelet and granulocyte products were also prepared using apheresis. During the 1980s, various steps were undertaken to reduce the risk of infectious disease transmission, including the development of recombinant clotting factor proteins. During the past 2 decades, it became possible to isolate different cell populations from bone marrow and peripheral and placental blood with defined functional properties. This led to development of cellular therapies for hematopoietic stem cell transplantation and immunotherapy for infectious diseases and cancer. The discovery that hematopoietic stem cells can differentiate into cells of other lineages such as neuronal and hepatic cells suggest the potential for tissue engineering.

lation, and storage of hematopoietic stem and progenitor cells for both allo-transplantation and autologous transplantation.

Historically, blood group antigens were defined only by serological means, and their expression was thought to be limited to red blood cells. During the past 2 decades, establishment of the molecular basis for the majority of these blood group antigens has led to the realization that many of these antigens are expressed on other tissue cells. In addition, the biological functions of some of these antigens have been defined (eg, as receptors for pathogens or as ion transporters in the cell membrane<sup>5</sup>). It is now possible to develop blood group antigen testing by genetic approaches.<sup>6</sup> Genotyping may lend itself to screening larger numbers of units in the blood supply and may be more amenable to laboratory automation.<sup>7</sup> This

development could therefore enhance compatibility testing by selecting donor red blood cell units, which genetically match the red blood cells of the recipient. As a result, alloimmunization would be less likely.

### Current Scientific Foundation

The indications for transfusion of blood products continue to evolve. The hemoglobin concentration alone is an inadequate indication for red blood cell transfusion but improved clinical guidelines have not yet been defined. The importance of this issue is underscored by evidence that suggests that more liberal use of red blood cell transfusion may possibly harm younger, less severely ill patients in the intensive care unit.<sup>8</sup> Similar questions pertain to the indications for platelet concentrates, plasma, and specialized blood products (eg, leukoreduced cellular products, cytomegalo-

virus seronegative blood, washed red blood cells, fresh blood).

Alloimmunization is a major clinical problem in transfusion medicine, particularly in the setting of patients with multiple transfusions who become refractory to both red blood cells and platelet concentrates. For example, patients with hemoglobinopathies, such as sickle cell disease, rely heavily on transfusions for prevention and treatment of stroke, treatment for acute pulmonary disease, and preparation for surgery.<sup>9</sup> Unfortunately, a large percentage of sickle cell disease patients (25%-30%) develop multiple alloantibodies and autoantibody syndromes and become refractory to transfusion. Similarly, patients undergoing stem cell transplantation and aggressive chemotherapy often require frequent and sustained platelet transfusion support. A significant proportion of patients (30%) develop anti-HLA antibodies, resulting in platelet refractoriness.<sup>10</sup> A different aspect of alloimmunization pertains to graft-vs-host disease, in which transfused allogeneic T cells react with antigens on host tissues. These allogeneic T cells are transfused via traditional blood components (eg, packed red blood cells and platelet concentrates) or via stem cell products for hematopoietic stem cell transplantation.

There is also a deliberate use of alloimmunity in transfusion medicine, which involves the transfusion of allogeneic T cells to induce a graft vs leukemia/tumor effect for treatment of residual or recurrent tumor.<sup>11</sup> In addition, transfusion of viable donor leukocytes may induce ill-defined immunosuppression, referred to as the immunomodulatory effect, leading to tumor recurrence and postoperative infections.<sup>12</sup> It has been argued that more research is needed to understand the biology and clinical implications of the immunomodulatory effect before expensive strategies, such as universal leukoreduction of blood products, are instituted.<sup>13</sup>

### Current Cutting-Edge Research

Current research is aimed at reducing viral transmission of blood products.

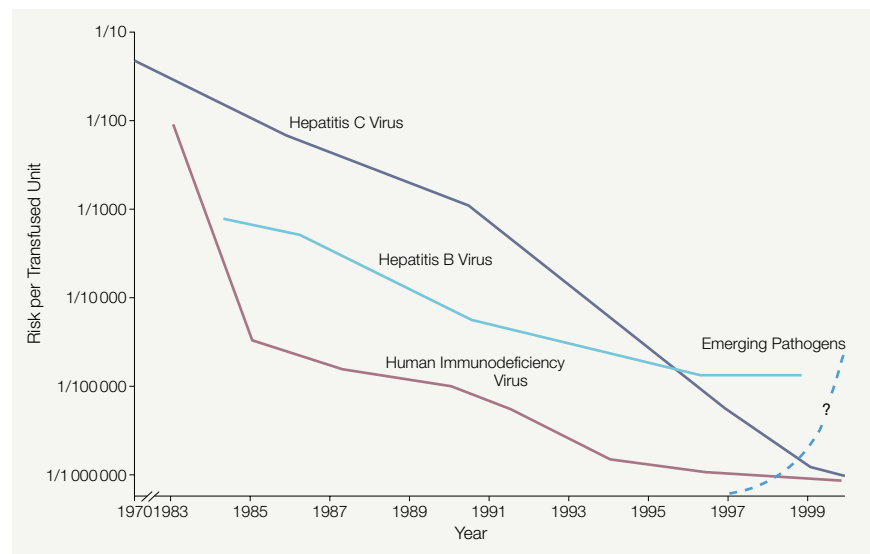
Several viral/bacterial inactivation methods are being investigated for cellular products that are not amenable to solvent-detergent treatment or pasteurization.<sup>14</sup> Also, artificial blood substitutes (now in phase 3 trials) ultimately may have clinical utility, but they are unlikely to replace the volunteer donor blood supply.<sup>15</sup>

With respect to cell therapies, attempts are under way to define hematopoietic stem cells and to understand stem and progenitor cell replication and differentiation.<sup>16</sup> These studies may lead to better approaches for ex vivo stem and progenitor cell expansion. Also, stem cell homing (eg, chemokines) and engraftment (eg, facilitator cells) are critical for hematopoietic stem cell transplantation.<sup>17</sup> Other cell therapies in development include T-cell therapies, for their graft vs tumor effect (ie, residual/recurrent chronic myelogenous leukemia), and the ex vivo generation of dendritic cells, for use in tumor vaccines.<sup>18,19</sup> Progress also is being made in differentiating the cells (and surface molecules) of the immune system that generate immune responses to foreign and host antigens.<sup>20,21</sup> As a result, experimental research and clinical trials are ongoing to find ways to either prevent or blunt autoimmune or alloimmune responses by costimulatory molecules (eg, using anti-CD40, CTLA4-Ig).<sup>22-24</sup>

### Critical Needs

Basic principles of quality and risk management support establishment of evidence-based guidelines. A network of clinical research centers in transfusion medicine is essential to improve the effectiveness of the research that this discipline requires. Basic research analyzing the control of hematopoiesis is critical to expansion of hematopoietic cells and important to development of novel cellular therapies. Several issues must be resolved to advance hematopoietic stem cell transplantation. For example, which cells or factors are necessary for long-term engraftment? Which cells exist in the bone marrow environment and how do they influence hematopoietic growth and differentiation? Which factors in-

**Figure 2.** Estimated Risks of Transfusion-Transmitted Diseases



The dramatic decrease in risk of transfusion-transmitted viral disease was accomplished by conversion from paid to volunteer whole blood donors in the 1970s, increased direct questioning of donors regarding risks for the viruses, and increased testing of collected donor blood for antibodies, antigens, and nucleic acid of these viruses starting in the 1970s.<sup>3</sup> Risk of hepatitis B may decrease further with universal childhood vaccination. Not shown in the graph are the rare risks of other transmissible diseases. The dashed line indicates the unknown risks, if any, of new viruses and other potentially emerging pathogens, such as prions that cause new variant Creutzfeld-Jakob disease.

fluence the retention and trafficking of hematopoietic progenitor cells to and from the bone marrow environment?

Alloimmunity is a common consequence of blood cell transfusion. Further studies are needed to elucidate the mechanisms involved in generation of these immune responses. Such studies may focus on definition of target antigens (eg, tumor antigens, antigens in graft-vs-host disease, immune cell types, and the membrane molecules involved), cell origin (eg, host vs donor), the role of cytokines in establishing and perpetuating an alloimmune response, and the role of chemokines and chemokine receptors in homing immune cells. To date, few animal models exist that can be used to study alloimmune responses to blood cell elements. A special need exists to develop murine models to take advantage of existing reagents; the ability to inactivate murine background genes and knock-in human genes may be useful to study the induction, prevention, and modulation of immune-hematological responses. Such models may help define the role of specific immune cells (eg, T cells, dendritic cells), the role of do-

nor vs host antigen-presenting cells, and the role of certain receptors (eg, Fcγ).

### Forecast of Major Advances

Current problems with blood shortages can be solved by finding ways to increase blood donations, by establishing donor criteria that maintain a safe blood supply without turning away safe donors, and by more accurately defining the indications for transfusions. Blood group genotyping will improve compatibility testing and selection of appropriate donor blood. In addition, genotyping will more precisely define fetal risk for neonatal hemolytic disease. Transfusion safety will be further enhanced by improved genetic testing of donated blood for known pathogens and by use of viral/bacterial inactivation steps suitable for cellular products. While reduction in the transmission risk of hepatitis B, hepatitis C, and HIV infection during the past 15 years is a major advantage, it is important to continue surveillance for newly emerging viruses and other pathogens (eg, prions, associated with variant mad cow disease) (Figure 2).<sup>25</sup>

Research Opportunities and Forecast: Transfusion Medicine	
Key Research Opportunities	Forecast
Enhancement of Blood Transfusion Practices Develop Measures to Better Assess Parameters for Transfusion (eg, Oxygenation, Hemostasis) and the Quality of Blood Components Prior to Transfusion and to Improve Blood Administration Practices	Optimal Blood Utilization Prevention of Overtransfusion and Undertransfusion; Improvement of Efficacy; Prevention of Adverse Effects
Pathogen Inactivation Develop In Vitro Approaches of Pathogen and Leukocyte Inactivation  Surveillance for Emerging Pathogens  In Vitro Manipulation of Blood Products	Safety of the Blood Supply Prevention of Pathogen Transmission and Adverse Effects of Leukocytes (eg, Transfusion-Associated Graft-vs-Host Disease)  Artificial Blood Substitute
Investigation of Plasticity and Commitment of Multipotential Stem Cells	Development of Tissue Engineering (eg, Liver Cell/Organs, Neuronal Cells, Cardiac Valves, Muscle)
Identification of Cytokines and Chemokines in Hematopoietic Development	Advances in Collection, Storage, Differentiation, and Ex Vivo Expansion of Stem Cells for Transfusion, Transplantation, and Gene Therapies
Mechanisms of Alloimmunization to Blood Cells and Plasma Proteins Approaches to Tolerance Induction to Allogeneic Cells Used for Transplantation/Transfusion; Masking of Blood Group Antigens	Treatment/Prevention of Ineffective/Refractory Transfusions; Prevention or Blunting of Graft-vs-Host Disease; Prevention of Hemolytic Disease of the Newborn and Hemolytic Transfusion Reactions
Immunobiology of Bone Marrow and Circulating Lymphocytes	Development of Novel Cellular Therapies: Defined T Cells Specific for Immunotherapy for Tumor or Pathogens, Progenitor Cell Populations With Enhanced Engraftment and Minimal Graft-vs-Host Disease

Advances in cell and molecular biology of immune and hematopoietic cells will make it possible to isolate and grow cells in vitro for specific cell therapies.<sup>26</sup> Such therapies will include hematopoietic stem and progenitor cell populations with optimal engraftment and minimal graft-vs-host disease,<sup>27</sup> dendritic cells for tumor vaccines, T-cell populations defined for their antitumor/antiviral effects,<sup>28</sup> and stem and progenitor cells that are rendered resistant to infectious disease or genetically modified to correct genetic disorders. Since hematopoietic stem cells are capable of differentiating into cells of different lineage (eg, liver cells), it may possible to generate human tissue and blood cells in vitro for therapeutic applications.<sup>29</sup>

Further understanding of alloimmune responses after transfusion may

lead to ways to mitigate or prevent unwanted consequences of transfusion, such as graft-vs-host disease and refractoriness to platelet transfusion. Collectively, this interdisciplinary research effort will translate into safer and more effective transfusion therapy with a wide range of applications.

**Funding/Support:** The Albert and Mary Lasker Foundation provided honoraria to Drs Silberstein and Toy for preparation of this article.

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