

## Historical Review

### KARL LANDSTEINER AND HIS MAJOR CONTRIBUTIONS TO HAEMATOLOGY

Karl Landsteiner was born in Vienna in 1868. He graduated from the Vienna Medical School in 1891, which dominated one of his most productive and formative periods in the following years. Although he devoted himself to research in bacteriology, haematology and immunology, he tried to maintain a close relationship with clinical medicine. Landsteiner's intellectual curiosity was centred on fundamental questions about the specificity of antisera directed against various antigens. His research with chemically modified proteins also led to the concept of the specificity of serological reactions, defined in his classical book on this subject. The attachment of small organic molecules to proteins, which in 1921 he called 'haptens', was the foundation for future immunological research into the nature of antibody-combining sites, antigenic determinants and antibody–antigen binding forces. In 1930, Landsteiner was awarded the Nobel Prize for his description of the human ABO blood group system, which he himself considered an accidental discovery.

Karl Landsteiner discovered human blood groups in 1900 and laid the foundation for the modern medical practice of blood transfusion. The ABO blood groups have a role in physiology beyond their importance for blood transfusion. In the past few years, red cell antigens (A and B carbohydrate structures) have been found on a variety of cells, tissues and proteins, indicating that these antigens might be involved in different physiological processes.

The blood group O has an edge in Darwin's concept of the survival of the fittest because it confers protection from vascular diseases. Many studies have established a relationship between the ABO blood groups and the risk of coronary heart disease, atherosclerosis and venous thrombosis (Souto *et al.*, 2000). Hypercoagulation is a risk factor for vascular diseases. It can be caused by excessive concentrations of proteins whose function is to maintain haemostasis. Such proteins include factor VIII (FVIII) and von Willebrand factor (VWF).

Lower concentrations of VWF and FVIII are found in the circulation of people who have blood group O than in those with blood group A, B or AB (Souto *et al.*, 2000). The mean VWF antigen concentration is 25% lower in blood group O than in other blood groups (Moeller *et al.*, 2001; O'Donnell & Laffan, 2001). This often leads to diagnostic difficulties in distinguishing mild forms of the inherited

bleeding disorder, type I von Willebrand's Disease (VWD), from a healthy blood group O (Nitu-Whalley *et al.*, 2000). Plasma phenotypes differ between OO and non-OO genotypes. Recently a link was found between the ABO gene locus and the VWF antigen concentration, and one was suggested between the ABO gene locus and FVIII coagulation activity (Souto *et al.*, 2000). Blood groups influence the VWF antigen level not only in normal subjects but also in heterozygous VWF-deficient subjects who have a null allele with certain missense mutations in the VWF gene. The heterogeneity of plasma phenotypes in heterozygous carriers for type 3 VWD might be due to blood groups (Castaman & Elkenboom, 2002).

Von Willebrand factor is a large multimeric glycoprotein that is synthesized in endothelial cells and megakaryocytes. It has a dual role in haemostasis. In primary haemostasis, VWF is essential for the adhesion of platelets to sites of vascular injury. In its second role, VWF is crucial to intrinsic coagulation because it binds to the amino-terminal part of FVIII and preserves it in the circulation (Schwarz *et al.*, 2002). Mature VWF is heavily glycosylated, containing 12 ASN-linked 10 Ser/Thr-linked oligosaccharide chains. The N-linked oligosaccharide chains contain the ABO blood group antigens (Matsui *et al.*, 1993; Sarode *et al.*, 2000). Studies on ABO-mismatched bone marrow transplantations indicate that platelet-derived VWF synthesized in megakaryocytes does not contain these blood group antigens in contrast to the endothelial-cell-synthesized VWF found in plasma (Matsui *et al.*, 1999). The only other known plasma proteins that contain ASN-linked ABO blood group antigens are alpha 2 macroglobulin and FVIII (Matsui *et al.*, 1993). The biological function of blood group antigens on VWF and FVIII is still not clear but blood group sugar chains might influence the functional properties of VWF such as ristocetin-induced platelet agglutination or the metabolic clearance of VWF and its susceptibility to proteolysis (Sarode *et al.*, 2000).

The first description of the congenital dysfunctional state of VWF in causing bleeding was published by Erik von Willebrand (von Willebrand, 1931), 1 year after Karl Landsteiner was awarded the Nobel Prize in Physiology or Medicine for the discovery of human blood groups.

FIRST CLASSIFICATION OF BLOOD GROUPS:  
WIENER KLINISCHE WOCHENSCHRIFT,  
14 NOVEMBER 1901

In his 17th publication, a 1.5 page communication in the *Wiener Klinische Wochenschrift* (the organ of the Royal and Imperial Society of Physicians in Vienna), which appeared

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**Tabelle III, betreffend das Blut von fünf Puerperae und sechs Placenten (Nabelschnurblut).**

Sera						
Lust. . . . .	+	+	-	-	-	+
Tomsch. . . . .	-	-	+	-	-	-
Mittelb. . . . .	-	-	+	-	-	-
Seil. . . . .	-	-	+	-	-	-
Linsm. . . . .	+	+	+	-	-	+
<b>Blutkörperchen von:</b>	<b>Traum.</b>	<b>Linsm.</b>	<b>Seil.</b>	<b>Freib.</b>	<b>Graupn.</b>	<b>Mittelb.</b>

Fig 1. The results of Karl Landsteiner's original 1901 blood group experiments. The table shows the results obtained when mixing the sera and red blood cell suspensions from five of his colleagues and himself. (*Wiener klinische Wochenschrift*, 1901, 14: 1132). Reprinted with permission from the Institut für Geschichte der Medizin, Vienna.

on 14 November 1901, Karl Landsteiner classified blood into three groups according to its agglutination properties (Landsteiner, 1901). Landsteiner was working at the Department of Pathological Anatomy at the University of Vienna at this time.

Landsteiner cross-tested sera and red cells from six healthy scientists: five co-workers and himself. He found that none of the sera reacted with their own red cells but Dr Pletsching's serum reacted with Dr Sturly's cells and Sturly's serum reacted with Pletsching's cells. This suggested at least two classes of antibodies were involved, which Landsteiner named anti-A and anti-B. Accordingly, Dr Sturly carried the A antigen on his red cells but had anti-B antibodies in his plasma and Dr Pletsching had the B antigen on his red cells and anti-A antibodies in his serum. Landsteiner's own cells contained neither A antigen nor B antigen but both antibodies, indicating what is now called blood group O (Fig 1). The fourth and rarest group, AB, in which both antigens are present on red cells, and the serum contains neither anti-A nor anti-B antibodies, was described 1 year later by Sturli (Landsteiner's pupil) and von Decastelo (von Decastelo & Sturli, 1902). Landsteiner noted in his paper that his laboratory colleagues' sera did not react with their own red cells (Landsteiner, 1901), an observation that could be considered as the first description of self-tolerance. Landsteiner also mentioned that he had obtained similar results from a patient with haemophilia and 10 additional healthy volunteers, but he did not include these results in a table in the paper.

Landsteiner found that the agglutination reaction could be carried out with dried blood samples. He reproduced his results after 14 d of drying and furthermore realized the importance of this observation for forensic purposes. It struck the scientist as strange that he found so few different agglutinins in his subjects. He puzzled over the reasons for the agglutinins and referred to Philipp Eisenberg of the Second Department of Internal Medicine in Vienna, who thought that they were caused by the resorption of red cell particles. Landsteiner, however, rejected this idea because in earlier experiments with animals he had not been able to produce autoagglutinins after injecting the animals with their own suspended red cells. Landsteiner seemed to favour

the theory of agglutinins forming as a result of a physiological decay of organ tissue but ultimately he was not able to resolve whether they were a phenomenon of auto-immunization induced by the resorption of red cell particles or were linked to diseases from which people had recovered. Now, 100 years later, the exact triggers responsible for the production of anti-A and anti-B in humans have yet to be identified.

The paper concluded with a sentence in which Landsteiner stated that his observations might explain the variable clinical consequences of human blood transfusion. The style of this paper is defensive in some parts. He states, referring to a footnote published earlier, that the experiments he presents show 'my (previous) results do not require any correction'. In a footnote to this article, he complained about Eisenberg alternately attacking and confirming his work. He seems to have been angered by Eisenberg's failure to cite his work in the text, although it was included in the reference list.

The recommendation Landsteiner made 1 year later to use blood grouping for paternity cases suggests that he believed that the A-B classification was genetic but he did not elaborate on this (Owen, 2000). The four blood groups were well established by 1902 and completely described in Landsteiner's monograph on haemagglutination and haemolysis (Landsteiner, 1907). In 1910, von Dungern and Hirszfild showed through family studies that agglutinogens A and B have a Mendelian pattern of inheritance, suggesting two genes, A and B, each with a recessive allele (von Dungern & Hirszfild, 1911).

Today, 100 years after Landsteiner's description of the two blood groups (antigens A and B), 270 blood group determinants are known (Daniels, 2001). Of these, 227 belong to one of 26 blood group systems. Almost all the genes of the 26 systems have been cloned and sequenced, and the location at the respective chromosomes identified. The molecular bases for most of the blood group polymorphisms are also known. In contrast, only little is known about the function of blood group antigens and the physiological role of polymorphisms, which have been shown to influence the immune response to allogeneic transfusion. Most red cell antigens are located on integral

membrane polypeptides and glycoproteins. Blood group antigen bearing proteins have a diversity of biological functions, ranging from adhesive properties, playing a role in the context of sickle cell disease, to complement-related functions and enzymatic or transport functions (Rao *et al.*, 1991; Parsons *et al.*, 1999). The genes responsible for ABO blood group polymorphisms do not encode the antigen directly but produce glycosyl transferases that regulate the addition of monosaccharides to an oligosaccharide substrate (Schenkel-Brunner, 2001). Antibodies to the A and B antigens described by Landsteiner are found in all adults who do not carry the corresponding antigens on their red cells. Most antibodies to other blood groups are induced by immunization with antigen-positive red cells after exposure through transfusion or pregnancy.

#### EARLY BLOOD TRANSFUSIONS

The first scientific communication on blood transfusion was made to the Royal Society in London on the 16 September 1666. A report on dog–dog blood transfusion from the physiologist R. Lower was read to the Society which was sufficiently impressed to request the experiments, in which blood was infused from one dog's cervical artery directly into another's vena jugularis, be repeated at the next monthly meeting. Lower also pointed out that transfusion did not alter the character or behaviour of the dog. Following the demonstration, most experiments were successful. Death of a recipient animal was rare but transfusion between different species was often associated with death (Benedum, 1996).

The first successful blood transfusion to a human was in 1667 when the French physicians Denis and Emmerez transferred 9 ounces of blood from the carotid artery of a lamb into the vein of a young man. This was followed by other similar procedures until a fourth patient died. This unlucky patient had already had one successful transfusion but after the second, 'his arm became hot, the pulse rose, sweat burst out over his forehead, he complained of pain in the kidneys, the next day the urine was dark, in fact black'. These symptoms are consistent with a haemolytic reaction caused by immunization of the patient to the sheep's blood given in the first transfusion. Finally, a third transfusion resulted in the patient's death. The patient's wife accused Denis of having poisoned her husband and sought redress in what was probably the first lawsuit for death from blood transfusion. Denis was exonerated but the court nevertheless made a ruling prohibiting such transfusions in the future unless prior approval was obtained from the Académie de Médecine. The French parliament, the Royal Society and the Catholic Church subsequently issued a general prohibition against transfusions. For 150 years, transfusion was banned from orthodox medical practice (Blakemore & Jennett, 2001).

The detrimental effects of transfusing animal blood into humans were not clarified until the 19th century. In 1875, Landois and Ponfick found that human serum was able to haemolyse or clump red cells from other mammals. The ability of animal serum to react with red cells of another species was attributed to the presence of natural antibodies,

heterohaemolysins or heteroagglutinins (Landois, 1875). In 1898, Bordet showed that both antibodies increased in titre after immunization of rodents, which explained the harmful results of repeated transfusions of sheep blood to the same patient by Denis (Bordet, 1903).

Landsteiner conducted similar experiments on the production of immune heteroagglutinins by injecting blood from one animal into another but withdrew the manuscript when Bordet's publication appeared (Wiener, 1969).

FIRST REFERENCE TO ISOAGGLUTINATION:  
CENTRALBLATT FÜR BACTERIOLOGIE, VOL. 27,  
357–362, 1900

The short article in the *Wiener Klinische Wochenschrift* describing human blood groups was a follow-up to a footnote on another paper published by Landsteiner 1 year earlier in a bacteriological journal (Landsteiner, 1900). The footnote, which seems out of context with the text of the article, mentioned the phenomenon of isoagglutination of human blood for the first time and stated that serum from healthy humans not only agglutinates red cells from animals but also frequently agglutinates red cells from other humans. Landsteiner left open whether this observation was due to original individual differences or was caused by bacterial damage but did mention that the agglutination reaction was more pronounced in blood from people who were seriously ill than in blood from healthy subjects.

The paper, with its footnote, was submitted on 10 February 1900 when Landsteiner was working at the University Department of Pathological Anatomy under Weichselbaum. It is divided into four parts and is a rather confusing paper describing, amongst other things, experiments on exsanguinating dogs to obtain fluids from various organs after perfusion. In part one, he described the inhibitory effects of rabbit serum towards rat trypsin. Landsteiner found that the sera from guinea pigs, rabbits and rats differ in their capacity to inhibit trypsin. Nowadays it is obvious that Landsteiner's observations were due to the effect of serine protease inhibitors, especially alpha-1 antitrypsin, rather than specific antibodies against trypsin. In Landsteiner's experiments, freshly homogenized organs such as liver and spleen as well as fresh blood inhibited trypsin activity, but after heating the organs and blood this inhibitory effect was abolished. This section of the paper was entitled, 'The presence of antifermentative (perhaps equivalent to "anti-enzymatic" today) substances in the organisms'. Landsteiner had wanted to investigate how animal tissue is protected from endogenous proteases. He came to the conclusion that the underlying mechanism was associated with humoral or cellular immunity of tissues towards enzymes.

The first reference to Landsteiner's famous footnote, on the agglutination of human red cells by serum of other humans (even before Landsteiner's original article on the agglutination of normal human blood), can be found in a publication by Josef Halban, who later held the chair of the Department of Gynaecology at Vienna University. In his paper, Halban described agglutination experiments with

serum from women who had delivered normal healthy infants (Halban, 1900). He built his studies on Landsteiner's observation that human serum usually agglutinates red cells not only from other species but also from other humans. Halban collected blood from the mother and fetus through the vagina immediately after delivery and studied the effect of the serum from one on the red cells of the other. He believed that the agglutination he observed was due to mutual immunization between mother and child through naturally occurring substances.

#### THE DISCOVERY OF THE RHESUS (Rh) FACTOR

The introduction into clinical practice of the ABO blood grouping test for selecting donors made safe transfusion possible. As early as 1921, Unger reported intragroup transfusion reactions and recommended that, after the appropriate ABO donor had been identified, additional tests should be carried out to exclude the possibility of a recipient serum agglutinating the donor's red cells (Unger, 1921). After blood banks were established in the 1940s, thousands of transfusions were given daily and the incidence of intragroup haemolytic reactions increased (Wiener, 1969). Often, patients who had received previous blood transfusions without reactions became immunized against agglutinogens present in the donor's red cells but absent from their own. In 1939, Levine, with whom Landsteiner discovered the MN types, reported an unusual case of intragroup agglutination (Levine & Stetson, 1939). This woman, who had been admitted to the Bellevue hospital in New York, had a stillbirth. Her fetus was macerated and she needed a transfusion. She had not previously received any blood transfusions and was given whole blood from her husband who was also group O. Within 10 min, she developed severe symptoms and more bleeding. A cross-match revealed that her serum agglutinated her husband's cells. Of a total of 104 group O blood samples tested against her serum only 21 were compatible. Levine suggested that there had been an isosensitization in this woman caused by 'products' from the fetus.

The discovery of the Rh factor by Landsteiner and Alexander Wiener in 1940 provided the pathophysiological basis for erythroblastosis and possibly explained the Levine case as having been caused by isosensitization to Rh antigen (Wiener, 1969).

Alexander Wiener did not work at the Rockefeller Institute, where Landsteiner had his laboratory at the time, but in the serological laboratory at the chief medical examiner's office in New York City. Wiener was interested in studying the evolution of agglutinogens M and N in anthropoid apes and monkeys. He obtained a series of anti-M and anti-N sera from rabbits, allowing him to study the inheritance of MN types (Wiener, 1938). Although all the anti-M sera reacted with human red cells, some strongly agglutinated rhesus monkey red cells and some did not. Absorption of the antisera with human M cells completely removed the antibody specificity for monkey red cells. Rabbits immunized with red cells of rhesus monkeys gave strong anti-M reactions after absorbing the sera with human

N cells. When the anti-M agglutinin from the anti-rhesus rabbit sera was absorbed, red cells from 85% of Caucasians were agglutinated. The new blood factor identified by this reagent was different from all factors discovered previously and was named Rh factor. Although this finding was made in 1937, publication was delayed until 1940 to allow the methods for production of anti-rhesus sera to be improved (Landsteiner & Wiener, 1940).

Initially, it was believed that animal and human antibodies identified a common antigen, Rh, on the surface of both rhesus and human red blood cells, but this was not the case. The original terms 'Rh factor' and 'anti-Rh', introduced by Landsteiner and Wiener, have persisted. The heteroantibody was re-named 'anti-LW' (after Landsteiner and Wiener) and the human alloantibody was re-named 'anti-D' (Avent & Reid, 2000).

The Rh blood group system is the most polymorphic of all human blood groups and is composed of at least 45 antigens. The D antigen is highly immunogenic and induces an immune response in 80% of D-negative people when transfused with 200 ml of D-positive blood. Therefore, in most countries, D-typing is routine for blood donors and recipients. Alloantibodies directed against Rh antigens are usually immunoglobulin (Ig)G and cause destruction of transfused red cells or fetal red blood cells in haemolytic disease of the newborn (HDN). This disease is caused by maternal IgG antibody passing through the placenta, binding the fetal antigen-positive red blood cells and destroying them, leading to anaemia (Avent & Reid, 2000).

Before the prophylactic use of Rh immunoglobulins (anti-D globulin) was introduced, maternal anti-D antibodies frequently caused fetal brain damage, as a result of the increased levels of bilirubin (Kern icterus), and death. The mechanism underlying the prevention of maternal anti-D production after receipt of prophylactic Rh immunoglobulin could be due to antigen blocking or a central inhibition of the immune response. Prophylactic Rh immunoglobulins are usually given by intramuscular injection. Rh immunoglobulins are also used for treating idiopathic thrombocytopenia, when they are given intravenously. The primary mechanism of action for this indication is believed to be an immunological blockade of Fc receptors within the reticuloendothelial system, preventing entrapment of antibody-coated platelets with a subsequent rise in the circulating platelet count (Ware & Zimmerman, 1998). Today's methods for obtaining Rh immunoglobulin for a therapeutic hyperimmunoglobulin preparation follow Wiener's original 1943 procedures for obtaining anti-Rh antibodies for diagnostic purposes. In his search, Wiener found the most convenient source of anti-Rh sera were people already sensitized by pregnancy or transfusion. During World War II, Wiener prepared anti-Rh serum for the armed forces by injecting small Rh-positive red cells into people who were already sensitized and could induce a very strong anamnestic response. The best source of anti-Rh serum came from male Rh-negative volunteers immunized with a small dose of Rh-positive red cells. At least two injections, 4 months apart, for the production of specific high-titre anti-Rh antibodies were required (Wiener, 1969).

## POLIOMYELITIS

At the meeting of the Royal and Imperial Society of Physicians in Vienna on 18 December 1908, Landsteiner reported the successful experimental transmission of poliomyelitis to monkeys through i.p. injection of homogenized spinal cord from a 9-year-old boy who died after 4 d of polio at the Children's Clinic in the Wilheminen Hospital. This fundamental experiment received no attention but laid the foundation for the prevention of this disease (Schwick, 1991). Although the cause of polio was unknown and believed to arise from 'various infections', the presentation was not followed by any discussion. It took 45 years from this oral communication for the first polio vaccine, developed by Salk, to become available in 1955 and be followed by Sabin's oral polio vaccine in 1960.

Although the i.p. injection of spinal cord material to rabbits, guinea pigs and mice caused neither disease nor histological changes in the respective neural tissue, the two apes which were inoculated by Landsteiner developed classic symptoms of polio and one of them died. Landsteiner continued his transmission experiments with spinal cord from another child who died during a polio epidemic in 1908 (Landsteiner & Popper, 1909). In collaboration with the Pasteur Institute in Paris, Landsteiner did filtration and inactivation experiments with material containing polio, and through immunization studies laid the foundation for diagnostic procedures. Landsteiner, together with Constantin Levadit and Ilitsch Mentschnikoff from the Pasteur institute, was the first to provide evidence for the viral cause of polio (Schwick, 1991).

## NOBEL PRIZE LECTURE: 11 DECEMBER 1930

Landsteiner was awarded the Nobel Prize in Physiology or Medicine 30 years after his description of human blood groups in the *Wiener Klinische Wochenschrift*. By this time, he had left Europe and was working at the Rockefeller Institute in America. Landsteiner did not consider his 'accidental' discovery of blood groups as his most significant contribution to medical science. Pleased as he was to receive the award, he would have preferred to be honoured for his work on the specificity of serological reactions.

In his Nobel lecture entitled, 'Individual differences in human blood' given on 11 December 1930, Landsteiner expressed his surprise at the large number of transfusions that were being conducted and said the use of the technique might well have been taken too far (Landsteiner, 1990). According to statistics made available to him, some 10 000 transfusions were given in New York during 1919 and alone in one hospital, the Bellevue, there were 1467 transfusions between 1926 and 1929. These included two with fatal results due to blood group incompatibility.

In Landsteiner's mind, the most obvious indication for a blood transfusion was acute or chronic anaemia, resulting from wounds or from large haemorrhages occurring in childbirth or caused by gastric or duodenal ulcers. He pointed out that the beneficial effect of blood transfusions, which in haemorrhaging often saves the patient's life, was

of course primarily as a result of blood replacement. An important factor here was that the transferred erythrocytes retained their functional capacity in the circulation for several weeks. Other important effects were haemostasis due to increased coagulability and he presumed also stimulation of blood regeneration in the bone marrow.

Landsteiner referred to shock following severe injury as another area of application and said it was assumed that in these patients the introduction of blood was more beneficial than the injection of the isotonic solutions recommended during the First World War. Likewise, he said, transfusions had often been given with great success after major operations not only to replace blood but also to serve as a stimulant. He added that, furthermore, American surgeons recommended such treatment before major operations when the patient was in a weakened condition.

Landsteiner went on to report that good results had also been obtained with haemophilia, thrombocytopenic purpura and, to some extent, with agranulocytosis, carbon monoxide poisoning and burns. In several other conditions in which transfusion therapy had been attempted, e.g. septicaemia, the results had, however, been doubtful.

The practical procedure of determining the patient's blood group before transfusion had taken quite a while to materialize because it was perceived as less important than the major challenge of overcoming blood clotting. It was not until 1915 that the use of an anticoagulant (citrate) solved the clotting problem. This was in time to ease the suffering in the First World War, when transfusions were used extensively and the value of cross-matching was clearly established.

Landsteiner closed his Nobel Prize lecture by voicing his concern about the existence of differences between individual proteins that could cause antibodies to form, a problem he thought had not been investigated sufficiently. Immunogenicity in general and antibody formation towards therapeutic proteins after replacement therapy continue to be a hot topic today. Landsteiner saw reason to hope that studies of patients with adverse events would help to confirm suspected underlying mechanisms and perhaps reveal the unknown causes of transfusion reactions, and thus finally to virtually eliminate the 'slight risks which transfusion still involves'.

The political environment in Vienna in the thirties is reflected in a newspaper clipping which appeared in November 1930 on the occasion of the award of Landsteiner's Nobel Prize: 'Let us hope that those who for years have introduced all kinds of political, ultra-national and religious criteria into scientific work will realize how detrimental their attitude is for a university city' (Speiser, 1961). History has taught us that, unfortunately, this hope has not been fulfilled.

## MODERN TRANSFUSIONS

Despite the widespread understanding of red cell antigens and their clinical significance, fatal haemolytic reactions to transfusion still occur. They are estimated to be in the range of one in 250 000 to one in 1000 000 transfusions. From

statistics, we know that, in 1997, approximately 12 million red cell units were transfused in the USA of which an estimated maximum of 12 could have been fatal (Dzik, 2002; Regan & Taylor, 2002).

Extensive blood donor screening and other measures have reduced the risk of human immunodeficiency virus infection to approximately 1 in 680 000 units and the risk for hepatitis B virus (HBV) infection to 1 in 63 000 units (AuBuchon *et al.*, 1997). The estimated risk of transfusion-transmitted HCV is now in the range of one in 103 000 infusions (AuBuchon *et al.*, 1997). Transfusion errors, therefore, remain the most important risk of blood transfusion, and ABO incompatibility errors probably account for half of the fatalities. Non-fatal errors occur in one in 12 000 to one in 19 000 transfusions with clinical manifestations of delayed reactions to transfusion. Some patients have overt haemolytic reactions because of antibodies to minor red cell antigens that are undetectable by routine antibody assays before transfusion. An error incidence of 335 per 5.5 million units of red cells transfused has been suggested in the UK. Non-infectious hazards of transfusion, therefore, account for over 95% of reported adverse events.

Recent estimates of per unit risk of cardiac toxicity or mistransfusion errors exceed the known risk of viral infection by as much as 10 000-fold. Therefore, concerns over blood safety should, in reality, be seen as outweighed by the risks to the patient from pure procedural errors (Dzik, 2002; Regan & Taylor, 2002).

#### LANDSTEINER'S DISCOVERY IN CONTEXT

We need to understand the concepts of immunology at the end of the 19th century in order to recognize the impact of Landsteiner's discovery of natural antibodies directed against human red cells in healthy serum on the paradigms of immunity existing at that time (Mazumdar, 1975; Mazumdar, 2002).

The obvious questions after Landsteiner's blood group discovery focused around which diseases caused the production of these antibodies and against which pathogens an individual had to defend himself. At the end of the 19th century, cellular and humoral immunity had only one purpose, to defend the body against foreign invaders such as bacteria. No use other than to provide the basis for survival was seen. The consequences of immunity to bacteria were best described by the experiments conducted by Pfeiffer in Berlin. When cholera organisms were injected intraperitoneally into immunized guinea pigs and collected sequentially, the bacteria clearly changed in morphological appearance and finally dissolved (Pfeiffer & Isayev, 1894). These results entered the scientific literature under the name 'Pfeiffer's phenomenon', which became the basic law of immunology and the fundament on which many more experiments by different groups in Europe were built.

Max von Gruber, the first chairman of the Department of Hygiene at the University of Vienna, repeated Pfeiffer's original experiments with cholera cultures he had obtained from Pfeiffer but he mixed the *Vibriae cholerae* with an immune serum before the i.p. injection. The slight change in

procedure resulted in bacteria clumping, a new observation. This was the first revelation of the basic mechanism of an animal's humoral defence against bacterial invasion, and was followed by Gruber and his English student, Durham, investigating the phenomenon further in a series of systematic, quantitative experiments. In 1896, Durham reported the bacteria clumping observation to the Royal Society in London and Gruber to the Royal and Imperial Society of Physicians in Vienna (Durham, 1896; Gruber, 1896a). Several attempts to give the observation a name finally resulted in 'serum antibody agglutinin'. Gruber spoke at the 13th Annual Meeting for Internal Medicine in Wiesbaden in the same year and demonstrated his talk by passing around agglutinated bacteria in serum (Gruber, 1896b). Gruber and Durhams' prime interest was to use the reaction to identify bacteria. Meanwhile M.F. Widal from the Medical Faculty in Paris took advantage of Pfeiffer's phenomenon for the clinical diagnosis of bacterial diseases in humans (Widal, 1896).

Another of Gruber's English students, however, also began to work on the diagnosis of typhoid fever by the agglutination reaction. His work resulted in a letter to *The Lancet* 4 months after a similar publication from Widal. A dispute over priority was finally resolved with the designation 'Gruber-Widal reaction', which is still the name used for the diagnostic agglutination test for typhoid today (Grünbaum, 1896).

Landsteiner joined Gruber's institute in January 1896. He remained there for 2 years and was undoubtedly involved in studies on the agglutination of bacteria.

From Behring's discovery of antibodies against diphtheria toxin in 1890, it slowly became apparent that immunity was not only a reaction against injuries caused by viable pathogens but a much more general response to exposure to foreign materials. Landsteiner was the first to show that specific bactericidal and agglutinating antibodies can be obtained by injecting inactivated bacteria, and that the body's response was not as a consequence geared to protecting against an infection, but more a general reaction (Landsteiner, 1897).

Landsteiner's interpretation of the origin and role of human agglutinins was one of three contenders. Paul Ehrlich, the director of the Frankfurt Royal Institute for Experimental Therapy, considered (using his own terminology) heteroagglutinins to result from the body's defence against bacterial invasion, autoagglutinins to be associated with blood diseases and isoagglutinins to result from the immunological individuality in a normal person (Mazumdar, 1975). Philipp Eisenberg, who worked in the same clinic as Landsteiner in the Vienna University Hospital, reviewed all these possibilities in 1901 (Eisenberg, 1901), including his own results of 150 human sera tested for isoagglutinins and haemolysins on normal human red cells. He had already noted that cells were never agglutinated by their own serum. Eisenberg was convinced that the agglutinins or antibodies were not specific for any bacterial infection and were, therefore, not useful for any diagnostic purposes. He believed the agglutinins were the result of the body's resorption of lysed red cells. It was 4 weeks after

Eisenberg's review in the *Wiener klinische Wochenschrift* that Landsteiner's paper on the agglutination of normal human blood, describing blood groups for the first time, appeared in the same journal.

Paul Ehrlich was the strongest opponent to Landsteiner's interpretation of agglutinins. In 1901, Ehrlich, talking at the 73rd Annual Meeting of the *Gesellschaft Deutscher Naturforscher und Ärzte* (an association of German scientists and doctors) in Hamburg, rejected a useful role for agglutinins in the organism and explained them away as by-products with no meaningful function for life (Ehrlich, 1901). He asked what sense it made that 'things are in the blood circulation directed against quite heterogeneous materials which under normal circumstances can never come into the picture' and 'what good is it to the goat if it has in its blood something directed against the red cells or against spermatozoae of other animals'. For Ehrlich, antibodies had no particular purpose, and were part of a chain of relations going in different directions and participating in different physiological processes. Ehrlich believed a number of different receptors for antibodies existed on each cell and, consistent with his 'side-chain theory', the receptor-antibody reaction was necessary for the metabolic survival of the cells or for carrying nutritious material to other tissues.

#### LANDSTEINER'S EARLY LIFE

When his son Karl was born in Baden near Vienna on 14 June 1868, Leopold Landsteiner was a well-known personality in Vienna, a city of culture in which both the arts and sciences flourished. Leopold Landsteiner, doctor of law, was a famous journalist and is often regarded as the co-founder of modern Austrian journalism. He had worked as a correspondent for German newspapers in Paris and been a lecturer in political sciences at the University of Lille. In 1848, the year of the revolution, he had returned to Vienna and worked for a few newspapers before becoming the founder of several newspapers himself. Leopold died at the age of 57 years when Karl was only 7 years old. Karl was, therefore, primarily brought up by his mother, Fanny Hess, who came from a Jewish merchant family from Prossnitz in Moravia (Speiser, 1961; Gottlieb, 1998; Mazumdar, 2002).

The boy proved to be an outstanding pupil and attended the Wasa Gymnasium in the ninth district of Vienna, an excellent school for classical higher education of which Stefan Zweig was another famous pupil. The graduation examination included a translation from Latin into German (Livius XXIII 12) and Greek (Homer, *Odyssee* XIX, verses 220–260), as well as a German to Latin translation.

In 1885, Karl Landsteiner began his medical studies at the Alma mater Vindobonensis, at the Vienna Medical School. He graduated, with a short break to complete his military service, in February 1891 and began his training at the Second Clinic for Internal Medicine, one of the medical departments at the University hospital of Vienna. At the time Kahler, who is known for the first description of multiple myeloma (*morbus Kahler*), was head of this department. The years between 1891 and 1893 found Landsteiner intensifying his studies in chemistry in

Germany with Emil Hermann Fischer, who received the Nobel Prize in chemistry in 1902, and in Switzerland.

When he took up his first post as an assistant in 1896 under Max von Gruber in Vienna University's Institute of Hygiene, Landsteiner was immediately exposed to the disputes on the fundamental issues of immunity and immunology raging between Gruber and Paul Ehrlich. The very poor working conditions at the Institute of Hygiene eventually caused Gruber to leave in 1902 to take up the departmental chair in Munich.

Landsteiner was only at the institute for a short time before he transferred to the Department of Pathological Anatomy in the autumn of 1897, working as an unpaid assistant for the first year. This department was headed by A. Weichselbaum (the discoverer of the bacterial cause of meningitis). Landsteiner applied for the position of prosector in Trieste in 1899 but was unsuccessful and stayed at the Department of Pathological Anatomy until the end of 1907. He spent what was undoubtedly one of the most productive periods of his life there, interacting with such extraordinary minds as Erdheim, Gohn, Paltauf, Löwenstein, Billroth and the paediatrician Escherich, who discovered the bacteria *coli commune*. Landsteiner himself worked quietly, and rapidly gained more recognition abroad than in his own country. During this time, he published 75 papers (of which 52 were of a serological nature, 12 dealt with bacteriology and virology, and 11 with pathological issues) and performed more than 3600 postmortem examinations, accounting for about one fifth of all the autopsies in the department (Speiser, 1961).

Just before his graduation from university, Landsteiner and his mother had converted to Catholicism. Anti-Semitism rendered a university career impossible for Jews because professorships were only open to Catholics in the Austrian-Hungarian Empire. Unfortunately, Landsteiner was never to become a full professor despite his conversion to Catholicism that had apparently been sufficient for others. Contradicting Ehrlich's hypothesis on immunity created a hostile environment for him in Vienna that might have featured in his failure to gain promotion. Ironically this failure, for whatever reason, probably contributed to his decision later to leave Austria, and in the end saved his own and his family's lives from the Nazi gas chambers.

Landsteiner's mother, to whom he had been extraordinarily close, died in 1908. At the beginning of that year, he had become head of the Department of Pathological Anatomy and prosector of the Wilheminen Hospital, which is still one of the largest communal hospitals in Vienna, with over 1500 beds. His period at the hospital lasted for 12 years until 31 March 1920, and 91 of his total of 360 publications were published during this time.

At the age of 38 in 1916, Landsteiner married Helene Wlasto. She was a member of the Greek Orthodox Church but converted to Catholicism soon after their marriage. They had one child, Ernst Karl, who was to become a surgeon in Providence, Rhode Island.

After the end of World War I, life in Vienna was intolerable with starvation and hypothermia commonplace. Conditions were such that, in January 1920, Landsteiner

was informed that he was to continue his duties and obligations as the prosector at the Wilheminen Hospital but with little remuneration. The Austrian authorities either through their own financial difficulties, anti-Semitism or for other unclear reasons finally forced him to retire with a pension insufficient to satisfy the simplest necessities of life.

His financial status was no doubt a factor in driving him to seek a job abroad but the stifling environment that prevented him from satisfying his intellectual curiosity through the pursuit of research was a more important impetus for him. He eventually secured a prosectorship at the RK Ziekenhuis, Catholic Hospital in The Hague, Holland, where the quality of his life improved but his financial situation remained poor. Some of the 12 publications he published during this time were in Dutch. He waited in vain for a call to return to Vienna but eventually accepted an invitation to go to the USA. Through contacts with Dutch scientists and their friendship with Simon Flexner, who was the director of the Rockefeller Research Institute and had earlier confirmed Landsteiner's polio-transmission studies, he finally joined the Rockefeller

Institute in New York and left his modest house by the sea in Scheveningen to follow 'the call of the New World'. The Landsteiner family became American citizens in June 1929.

At the Rockefeller Institute, Landsteiner constantly fought for funding. He only had a small room until his Nobel Prize award brought a larger laboratory and more staff. His main interest at this time was in the chemical and immunological basis of skin sensitization and allergy. Together with M. W. Chase, he showed that skin sensitivity to certain chemicals is induced by allergens formed by combinations of these components with host proteins. Experiments in which lymph node cells derived from a sensitized animal were transferred into a normal recipient were the basis of further studies on the cellular aspects of compact dermatitis and other cell-mediated immune responses.

At the age of 71 years, Landsteiner retired as an active member of the Rockefeller Institute staff. He suffered a heart attack at the bench and died 2 days later on 26 June 1943, 6 months after the death of his wife.



**Fig 2.** (A and B) A 1000 Schilling note featuring Dr Karl Landsteiner. (B) The 1000 Austrian Schilling note (€72.7), in circulation until the introduction of the Euro, 1 January 2002, depicted the areas of physiology and medicine to which Landsteiner devoted his work: 1. Landsteiner seated behind a microscope. 2. Landsteiner introduced dark-field microscopy to visualize the spirochetes of syphilis. 3. A model of a virus: Landsteiner successfully experimentally transmitted poliomyelitis from humans to monkeys and showed that the agent causing polio belongs to a group of filterable microorganisms. 4. A model of an immunoglobulin molecule, for his contributions to immunity. He was amongst the first to prepare partially purified antibodies by dissociating antigen-antibody complexes. 5. Human blood groups ABO. 6. Red cells: apart from the discovery of blood groups, a major constituent of his experimental immunization studies. The facsimile of the 1000 Schilling note was kindly provided by Peter Buchegger, Austrian National Bank.

## LANDSTEINER'S LEGACY

Landsteiner's discovery that red cells from some people are agglutinated by serum from others was the beginning of the haematological revolution.

Blood had always been regarded as the essence of life and fascinated Western ancient philosophers and medical thinking. The English physician William Harvey (1578–1637) wrote that, 'blood acts above all the powers of elements and is endowed with notable values and is also the instrument of the omnipotent creator'. 'It is', he believed, 'the fountain of life and the seat of the soul'.

The full advantage of blood as a source for medicinal products, from labile blood products to stable blood products, i.e. therapeutic concentrates, was made possible through Landsteiner's discoveries. In 2001, the pharmaceutical industry processed more than 12 million litres of plasma for manufacturing therapeutic factor concentrates, immunoglobulins, hyperimmunoglobulins, albumin and protease inhibitors.

Landsteiner made a significant contribution to the progress of medicine. He studied the antigenic properties of chemically modified proteins, revealing new specificities, and added to protein antigens by linking small organic molecules, which he named 'haptens' (Landsteiner & Jagic, 1904; Landsteiner, 1921). The introduction of haptens to immunological research opened the investigation into the issues of antibody combining sites, antigenic determinants and antibody–antigen binding forces. In 1904, only 3 years after his publication on the blood groups, Landsteiner, together with Donath, made a key contribution to the pathogenesis of paroxysmal haemoglobinuria (Donath & Landsteiner, 1904). Landsteiner began research work on syphilis in 1905, which led to the introduction of dark-field microscopy for the detection of *Treponema pallidum* and extensively studied human-to-animal syphilis transmission with the ultimate goal of developing antibodies for diagnostic purposes as well as explaining immunity (Landsteiner *et al*, 1907; Luger, 1991).

His concept of the specificity of serological reactions was summarized in his textbook. The English edition of this textbook became a 'bible' for many immunologists (Landsteiner, 1936).

Landsteiner's extensive discoveries were made without the tools used today in genomics and proteomics. His contributions were the basis for enormous progress in medicine for the benefit of humanity in the 20th century (Fig 2). A bright mind, driven by intellectual curiosity, is sufficient to make discoveries, even in meagre circumstances devoid of luxuries.

Baxter BioScience, Vienna,  
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After this article was submitted, a historical review, 'Red cell agglutination: the first description by Creite (1869) and further observations made by Landois (1875) and Landsteiner (1901)' by N. C. Hughes-Jones and Brigitte Gardner (*British Journal of Haematology*, 2002, 119: 889–893) was published. This review described the almost unknown contribution of Adolf Creite to red cell agglutination in 1869.

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