Perspectives

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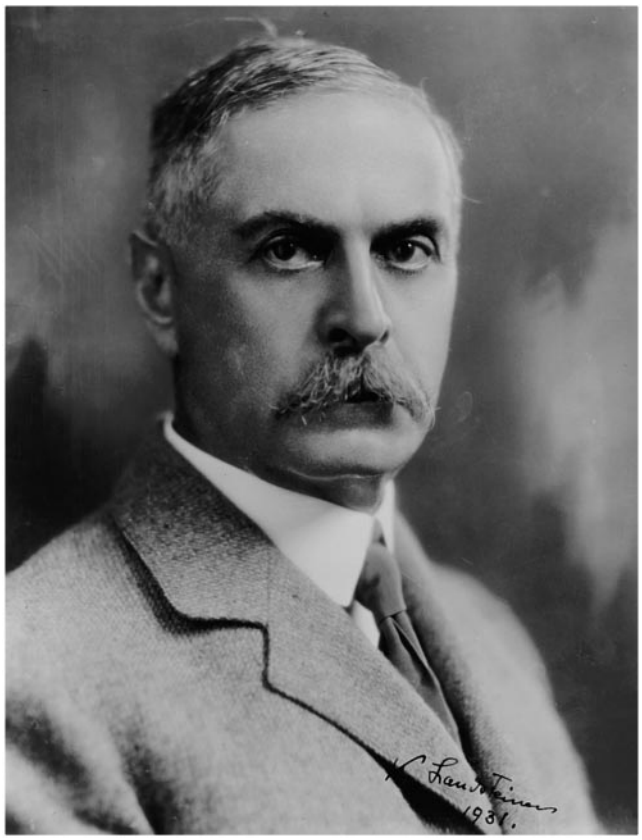
Karl Landsteiner and the First Human Marker Locus

Ray Owen

Division of Biology, California Institute of Technology, Pasadena, California 91125-0001

JUST a century ago, the year of the rediscovery of Mendelism, Karl Landsteiner published a short note on what we would now call antibody action in human blood serum, with a comment on the agglutination of the red cells of some people by the serum of others (Landsteiner 1900). The following year he elaborated the observation (Landsteiner 1901), and it is to that second short paper that reference is most appropriately made when dating the discovery of the normal human blood groups. As strict constructionists of the millennium would argue, the century began in 1901, not 1900.



Karl Landsteiner, 1931. Photo courtesy of the National Academy of Sciences.

Landsteiner outlined the background of this discovery in his 1930 Nobel Lecture (Landsteiner 1931), and it has been dealt with in detail in the Obituary Notices of Fellows of the Royal Society (Rous 1947) and the Biographical Memoirs of the National Academy of Sciences (Heidelberger 1969). Using serological methods, he had been impressed that "the proteins in various animals and plants are different and are specific for each species." He wondered "whether . . . individuals within a species show similar . . . differences. As no observations whatever were available pointing to such behavior, I chose the simplest among the possible plans of investigation . . . allowing blood serum and red blood corpuscles of different human individuals to interact" (Landsteiner 1931, p. 403).

It had, in fact, been noted earlier by others that such interactions often resulted in agglutination or lysis of the red cells and severe incompatibility in attempted blood transfusion. The predominant opinion of the day was that immune reactions regularly reflected a history of disease. It was against this background that Landsteiner chose to emphasize his studies of "apparently healthy men."

In the 1901 paper he tabulated the results of complete cross-testing of the sera and cells of six people working in his lab, including himself (Table 1). He noted first, as you can see from the diagonal, that the serum of none of the six individuals reacted with the person's own cells—a clear observation of self-tolerance. [Only 3 years later, he made a key contribution to the subject of autoimmune disease in studies of paroxysmal hemoglobinuria (Donath and Landsteiner 1904)]. Second, the serum of Dr. Pletschnig reacted with Dr. Sturli's cells, and Sturli's serum reacted with Pletschnig's cells. Viewing the results as antigen-antibody reactions, he could suggest that at least two classes of antibodies were involved, what we would now call anti-A and anti-B, reacting with corresponding cellular antigens A and B. Dr. Sturli, whose cells can be said to have A antigen, has anti-B in his serum, and Dr. Pletschnig has B on his cells, anti-A in his serum. "Dr. St.," like Landsteiner

TABLE 1

Concerning the blood of six apparently healthy men

	Blood corpuscles of					
Sera	Dr. St.	Dr. Plecn.	Dr. Sturl.	Dr. Erdh.	Zar.	Landst.
Dr. St.	−	+	+	+	+	−
Dr. Plecn.	−	−	+	+	−	−
Dr. Sturl.	−	+	−	−	+	−
Dr. Erdh.	−	+	−	−	+	−
Zar.	−	−	+	+	−	−
Landst.	−	+	+	+	+	−

himself, has neither antigen and both antibodies; we call them Group O today. For both cell and serum reactions, "Dr. Erdh." is like Sturli, and "Zar." is like Pletschnig. The table therefore neatly distinguished three normal human blood groups, A, B, and O, and displayed each group in duplicate.

Sturli was assigned the job of adding numbers of persons to the test panels, and the following year he published, with von Decastello, recognition of the fourth and least common group, which we now call AB. Although Landsteiner believed that the four human blood groups were normal human qualities, not due to a past history of disease, he kept cautious on the point and for years remained puzzled by the remarkable fact that "every serum contains those agglutinins which act upon the agglutinogens not present in the cells" (Landsteiner 1931, p. 404). His quiet proposal, in 1902–1903 with Richter, that blood grouping might be useful in paternity cases suggests that he viewed the classification as genetic, but he did not press the point. It was not until 1910 that von Dungern and Hirzfeld concluded from extensive family studies that the agglutinogens A and B of the cells reflected Mendelian factors, and the pattern of inheritance suggested two genes, *A* and *B*, each with a recessive allele so that in the dihybrid diagram

A− B− = AB blood group
aa B− = B
A− bb = A
aa bb = O

The later evolution of this concept into our present perception of ABO as a multiple-allelic series has been ably presented to readers of Genetics by one of the editors of this section of our journal (Crow 1993).

Application of blood grouping to transfusion came rather slowly. The main hazard, perceived from early attempts, was ordinary blood clotting, not incompatibility; when blood was taken from a prospective donor, it clotted during the transfusion process, to conspicuously ill effect on the recipient. Shortly after 1900 surgeons developed extraordinary methods of joining an artery of the donor with a vein of the recipient so that the blood was not exposed to clotting during transfer. The method was difficult, and although in 1907 the typing of bloods to match for transfusions was reported by Ottenberg, it was not until 1915 that the use of an anticoagulant (citrate) solved the clotting problem, just in time for the extensive use of transfusions in the First World War, and with it the clear value of cross-matching.

As we note shortly, Landsteiner was active in many areas relating mainly to immunology. His interests in individuality and red cell antigens continued through his life, and it is in that context that we celebrate his contributions to genetics. Erlich and Morgenroth had early shown that when blood of one goat was injected into another goat, immune antibodies that reacted with the donor's, not the recipient's, cells appeared and that these antisera recognized a complexity of individual differences among goats. By 1910, Todd and White (1910) had published similar studies of cattle and chickens, work indicating that any individual within a species had an almost unique individuality. Landsteiner wondered why, given a match for ABO, human transfusions did not readily reveal such individuality. It was not until well after he was established at the Rockefeller Institute that, with Levine, he tried injecting rabbits with human blood and using the immune sera to detect differences among people. This led to the next marker for human genetics, the M-N alternative, later to prove so complicated (Landsteiner and Levine 1927; Race and Sanger 1975). The same experiments revealed the P groups.

Another approach to detecting human differences was conceived by Landsteiner in the late 1930s: inject cells from related animals (they used Rhesus monkeys) into guinea pigs and rabbits, and see if the resulting antisera distinguished human characteristics. This led to the recognition of the Rh system, named for the Rhesus donors.

The antisera reacted with all human cells, but appropriately diluted they recognized the red cells of many but not all New Yorkers (Landsteiner and Wiener 1940). A year earlier Levine (Landsteiner's colleague in the M-N-P discoveries) had published a case study of a woman who had been admitted to Bellevue Hospital

in July 1937. She gave birth to a macerated fetus in early September, and, needing a transfusion, she was given 500 cc of whole blood from her husband, also of group O. Within 10 min she began to have severe symptoms, followed by more bleeding. A cross-match later revealed that her serum agglutinated her husband's cells. A total of 104 group O bloods were tested against her serum; only 21 were compatible. The reactions were found to be independent of M, N, and P. The antiserum lost activity on storage, however; the system was not given a name or subjected to family studies, so briefly it remained only an interesting clinical observation (Levine and Stetson 1939).

With the discovery of the Rhesus factor in 1940, Landsteiner and Wiener were able to relate it to the antigen recognized in the Levine and Stetson context. For some time the two were thought to be the same, and "Rh" became firmly established in the general literature as a basis for maternal-fetal incompatibility, dependent on the inheritance of an allele from the father, absent in the mother, and controlling an antigen that immunized her during pregnancy and childbirth. The system proved overwhelmingly complex and became the subject of heated controversy. Wiener and his supporters chose to regard it as dependent on a long series of multiple alleles, reflected in a symbolism that took Rh as its base, with alleles distinguished by upper- and lowercase, superscripts, numbers, and primes. R. A. Fisher, with R. Race, developed a hypothesis of three adjacent loci, designated D, C, and E, each polymorphic. Landsteiner died just as the controversy was initiated, so he did not become involved in it. It is unlikely that he would have taken part; his consistent philosophy from early in his scientific life was to focus on facts that forced one to an interpretation, and the facts to resolve the argument, from his viewpoint primarily of chemistry, were not at hand. Indeed, as later developments in molecular genetics have demonstrated, and as often is true in heated polemics, both sides were partly right. More recent knowledge of the biochemical genetics of the cellular antigens and the molecular biology of their controlling loci is beyond the scope of this *Perspectives*. A comprehensive and reasonably up-to-date reference is Cartron and Rouger (1995). We can expect that future developments in human genomics will greatly enlighten this area.

A further complication soon became evident: the antigens recognized in the Levine-Stetson system and in the Landsteiner-Weiner system and their corresponding antibodies were not the same. All newborn babies, whether Rh-positive or -negative with human anti-Rh sera, were positive with the guinea pig antibody. Extracts of human Rh-negative blood gave rise to Rh antibodies in guinea pigs. No matter how potent, human Rh antibodies do not clump Rhesus cells at all. Unusual human sera that appeared to test as anti-Rh, but from which the antibody could be absorbed by Rh-negative cells, began to be noticed. These sera, along with guinea pig antisera, defined a new locus, assigned the symbol LW (for Landsteiner-Wiener), that proved to be genetically independent of Rh. Thus LW came to designate a system dependent on the original Rhesus studies of Landsteiner and Wiener, while the symbol Rh came to designate a system that, in the original hands of Levine and Stetson, had nothing to do with Rhesus. Race and Sanger, in the final edition of their classic *Blood Groups in Man*, summarize their LW section: "If we were to do justice to the papers in this section, we should never finish the chapter" (Race and Sanger 1975, p. 232). I take the same way out for this *Perspectives* on Landsteiner.

Although his interests were extraordinarily broad and his contributions very extensive (his obituary lists 346 publications), Landsteiner's main base was in chemistry. He started medical school in 1885 at the age of 17 and soon engaged in experimental investigation, under Ernst Ludwig. Becoming "Doktor der gesamten Heilkunde" in 1891, he joined a clinic at University Hospital in Vienna and took up advanced study and research under several outstanding organic chemists of the day. He became interested in immunology and active in surgery, in pathological anatomy, and in a variety of medical and technological areas. He was among the first to prepare partially purified antibodies by dissociating antigen-antibody complexes and the first to use darkfield microscopy to visualize the spirochetes of syphilis and to transmit syphilis to monkeys. He and E. Popper transmitted poliomyelitis to monkeys, and he was importantly involved in the demonstration that polio is caused by a virus. His work was interrupted by World War I, during which he served as a medical officer, and conditions after the war made continued research there nearly impossible. So in 1919 he moved to The Hague, where conditions were more favorable, and in 1923 accepted a membership at the Rockefeller Institute in New York. He promptly became a U.S. citizen. There followed 20 very productive years, until his death in 1943. Aside from the red cell studies we have outlined, his other major interest over those later years was in the chemical and immunological basis of skin sensitization and allergy, seminal work conducted mainly with John Jacobs and Merrill Chase. But looking back on his life as a scientist, Landsteiner was proudest not of the work for which he received the prize, or of others of his many contributions, but of the insight he had provided into the primary specificity of serological reactions.

That was the chemist's approach, which laid the groundwork for immunochemistry. Recognizing that simple substances (haptens) could be coupled to proteins and that immune antisera to these modified proteins included antibodies directed against the introduced haptens, Landsteiner worked out the technology of studying just those antibodies. Their specificity could be related to particular defined groups on the antigen

and evaluated quantitatively with precipitin reactions and hapten inhibition of precipitation. These earlier studies were summarized in German (in 1933) and published in a second, English edition that became a bible for many of us in immunology and immunogenetics (Landsteiner 1936). A revised edition was published 2 years after Landsteiner's death, edited by his son Ernest K. Landsteiner and acknowledging help from Merrill Chase and Alexander Wiener. The books dealt in depth with the serological specificity of proteins, cell antigens, antibodies, simple chemical compounds, specific nonprotein cell substances, and antigen-antibody reactions. The 1945 edition includes an appendix by Linus Pauling, on molecular structure and intermolecular forces, and thereby hangs a tale I've found interesting.

The tale is told by Thomas Hager in his biography of Pauling (Hager 1995) and is based on the author's extensive studies of the Pauling papers and conversations with Linus himself and others. In 1936, Pauling lectured at the Rockefeller Institute on hemoglobin. Responding to a note from Landsteiner (about whom he knew mainly because Landsteiner had received a Nobel Prize 5 years earlier), Pauling listened while Landsteiner raised puzzling questions about antibodies: How did their great diversity arise, and what was the molecular basis for their specificity? What were the physical-chemical forces that led to their specific reaction with antigens? How could the immune system tailor these antibody proteins to recognize a myriad of molecules the body had never before encountered?

Pauling could not enlighten any of these questions, but they piqued his interest. He bought a copy of Landsteiner's book, the brand new 1936 edition, read it on the train, and arrived in Pasadena an enthusiast. He soon had a rough draft of a manuscript about how an antibody might be formed and the physical-chemical basis of its specific reaction with antigen. But he was distracted by other events and set the draft aside. When he gave a lecture at Cornell University in November 1937, Landsteiner journeyed to Ithaca, and a brief visit turned into what Pauling recalled as "the best course of instruction in a complicated field that anyone ever received," an intensive 4-day tutorial. The two remained in mutually stimulating contact until Landsteiner's death.

They were, however, quite different. Landsteiner, from his start, shunned the airing of hypotheses. New ideas came to him very frequently, and he was constantly suggesting to his juniors and colleagues trial experiments which "would take no time." But he was "incorrigibly doubtful and when a discovery declared itself he would instantly conclude that it could not be real." "Experiments which revealed anything were done many times over, and not until the data on a point under determination were, in his term, 'thick' would he publish" (Rous 1947, pp. 306–307).

Pauling, in contrast, readily pressed generalizations. As he put it, "I found that Landsteiner and I had a much different approach to science. Landsteiner would ask, 'what do these experimental observations force us to believe about the nature of the world?' and I would ask, 'what is the most simple, general and intellectually satisfying picture of the world that encompasses these observations and is not incompatible with them?'" (Hager 1995, pp. 239–240). I would guess that it takes both kinds to press on with science. Do we tend to teach the Landsteiner approach, but to remember better the Pauling place in history?

So, if we had word from Landsteiner today, it might be as Tennyson's Ulysses:

> I am become a name;
> For always roaming with a hungry heart
> Much have I seen and known, cities of men
> And manners, climates, councils, governments,
> Myself not least, but honor'd of them all.

He deserves to be remembered as more than a name and more than the discoverer of the first human marker locus.

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