Historical Insight: **What only one person accomplish in a lifetime? Pirquet, an exceedingly curious pediatrician with acute powers of observation and deduction, not only solved the riddle of serum sickness and developed the concept of allergy, but also made contributions to the study of nutrition and aging.**

Clemens Freiherr von Pirquet: Explaining immune complex disease in 1906

Arthur M. Silverstein

Institute of the History of Medicine, Johns Hopkins School of Medicine, 1900 East Monument St., Baltimore, MD 21205, USA. (arts@jhmi.edu)

"The conception that antibodies, which should protect against disease, are also responsible for disease, sounds at first absurd."

Clemens von Pirquet, 1906

Clemens von Pirquet, about 1903. From R. Wagner's *Clemens von Pirquet: His life and work*. (Courtesy of the Johns Hopkins Press.)

The history of any significant biomedical discipline is characterized by a number of curious factors. First, its founding fathers have often come from a variety of older, often unrelated, fields. Thus, chemist Louis Pasteur discovered immunization with attenuated pathogens; zoologist Ilya Metchnikoff discovered innate phagocytic immunity; bacteriologists Emil Behring and Shibasaburo Kitasato discovered antibodies and serotherapy; histologist and hematologist Paul Ehrlich advanced a theory of antibody formation; pathologist Karl Landsteiner discovered blood groups and an autoimmune disease; and physiologists Charles Richet and Maurice Arthus discovered anaphylaxis and antigen-induced necrotizing skin lesions, respectively[1].

The second phenomenon common to most biomedical disciplines is the cyclical character of many discoveries. It would appear that some phenomena must be rediscovered every generation or so, to assure their retention in the collective memory of the field. Thus, we saw in an earlier historical contribution to *Nature Immunology*[2] that in 1892, Paul Ehrlich elucidated the nature of the passive transfer of maternal antibody from mother to fetus across the placenta, and from mother to newborn *via* the milk. In the same experiments he worked out the principle features of the kinetics of the antibody response. These demonstrations, almost completely forgotten, were repeated many decades later, with substantially similar results. Sometimes, of course, newer contexts demand the return to these older questions, or newer techniques permit further clarification of mechanisms; however, the brilliance of many of these earlier observations should not be forgotten, nor should they go unappreciated.

In this offering, I shall call attention to a remarkable leap of imagination by pediatrician Clemens Freiherr (Baron) von Pirquet. In 1903, based upon purely clinical observations, the 29-year-old clinical assistant had an idea that would lead directly to an explanation not only of the role of the immune response in the incubation time of many diseases, but would explain also the pathogenesis of serum sickness, in terms of antigen-antibody complexes, a pathogenetic mechanism that would assume great importance 50–60 years later.

A magnificent conceptual leap

Pirquet was born near Vienna in 1874. He studied theology in Innsbruck and philosophy at Louvain in Belgium, where he obtained his bachelor's degree. It was then that he decided upon medicine, which he studied first at the University of Vienna, then at Königsberg and finally at Graz, where he graduated in 1900. Exposure to the great pediatrician Theodor Escherich (of *E. coli* fame) may have convinced him to specialize in pediatrics, and he did an internship and residency in that discipline at the Kinderspital in Vienna[3].

At the turn of the century, the major preoccupation of the pediatrician was infectious diseases. In addition, immunology was very much in the air in Vienna. Louis Pasteur had discovered vaccination with attenuated organisms; Emil Behring had discovered antidiphtheria serotherapy, for which he had received the first Nobel Prize in medicine; and Paul Ehrlich had advanced a popular theory of antibody formation and function[4]. In 1891, Ehrlich had suggested that the delay in the formation of antibodies after immunization or infection might account for the disappearance of rash and fever in diseases like measles[5]: in other words, the immune response, in addition to preventing disease prophylactically, might also cure a disease once it had started.

Then in 1903, Pirquet, the 29-year-old pediatrics resident went one better than Ehrlich (although it is not clear that he was aware of the earlier suggestion). In a rarely employed method to claim scientific priority for a concept, Pirquet sent a sealed letter that outlined his theory to the Academy of Sciences in Vienna[6]. It was only to be opened at the request of the author, and in fact this was done in 1908, and the letter was read at a session of the Academy.

The theory was brilliant in its simplicity and in its implications. Citing clinical observations on systemic reactions to horse antitoxins, on reactions to vaccination against smallpox and on the course of a variety of acute infections, Pirquet suggested that the symptoms of exanthematous diseases like measles, which appear about a week after infection, far from ending because of the immune response, are actually initiated by this host response. It is the antibody[7] interacting with the pathogen that causes the symptoms, said Pirquet. He ended the letter by saying that he would shortly publish the observations upon which the theory was based, in collaboration with Dr. Bela Schick[8].

Here was a theory that would guide all of Pirquet's most significant contributions to immunity and immunopathology. As we shall see below, it would provide the basis for his explanation of the pathogenesis of serum sickness, it would underlie his interpretation of the dynamics of the immune response and it would be the foundation of his general theory of the nature of allergic reactions. Later work would demonstrate, of course, that Pirquet was correct in his interpretation of the role of the immune response in the pathogenesis of measles; it was shown that the pathogenesis is due to the development of cytotoxic T cells that clear the virus from infected dermal and other cells, resulting in rash and fever[9]. In the immunologically compromised host there is no rash, but predominantly a giant cell pneumonia[10]. However, Pirquet overgeneralized his theory: the rash and fever that accompany some other exanthems, such as smallpox, are due to the direct cytopathogenic action of the pathogen itself.

Serum sickness

In 1905, Pirquet and Schick published their monograph on serum sickness[11]. It was a study of the side-effects of administration of large quantities of foreign serum that contains antitoxins, a technique that was used for the treatment of diphtheria and tetanus. As clinical pediatricians, the authors devoted three-quarters of the 120 pages to clinical descriptions and put the greatest emphasis on the duration of the time lag between administration of the serum and the onset of symptoms. In particular, they stressed the fact that the symptoms appeared much more rapidly after a second exposure to the foreign serum than after the first administration. Their clinical descriptions are not limited merely to fever and rashes, they include reports of kidney damage with proteinuria, lymphadenopathy and joint symptoms.

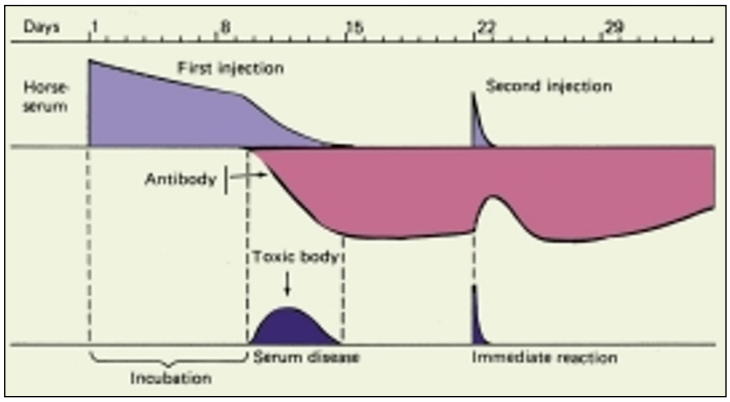

Figure 1. Von Pirquet's concept of the steps in the development of serum sickness in man. From A. M. Silverstein's *A History of Immunology* (1989). (Modified from the original and reproduced with permission from Academic Press.)

Citing their own data and the results of others, Pirquet and Schick determined the following. (i) The formation of circulating antibodies is delayed after administration of large amounts of foreign serum. (ii) There is a similar delay in the onset of the symptoms of serum sickness. (iii) A second, later, injection of serum leads to a drop in the amount of circulating antibodies and a more rapid onset of symptoms. (iv) The reaction is specific, as using a different serum for the second injection does not incite the same accelerated response. (v) Although small doses of serum stimulate antibody formation, they do not result in clinical symptoms.

At this point, the authors were unwilling to stipulate that the inciting antibody was indeed a "precipitating" antibody, as discovered not long before by Kraus[12], but stated that there was clearly "a chemical interaction between the horse serum and the antibodies of the vital (that is, immune) reaction[13]". The full mechanism proposed by Pirquet and Schick was made crystal clear in Pirquet's 1910 book *Allergie*[14]. In this treatise, Pirquet insisted that the responses to antigenic stimulus may be divided into two categories: one was immunity, or the classical protection against infectious disease, and the second he called by his newly coined term "allergy", or altered reactivity, in which the immune response itself mediates clinical disease. The latter category included not only serum sickness, but anaphylaxis, the Arthus reaction, hay fever, asthma and, later, autoimmune diseases[15].

To explain his theory of the pathogenesis of serum sickness, Pirquet plotted the time course of the process in terms of serum (Pirquet's "allergen"), antibody and the formation of what he called the "toxic body", whose presence was defined by the existence of clinical symptoms. Both the horse serum and the precipitating antibody in the patient's serum were determined by periodic serum samples. The antigen was assessed using rabbit anti-horse serum in a precipitin test and serum antibody was measured by testing similarly with horse serum. His results are shown in **Fig. 1**.

It is remarkable how much information was presented in this simple diagram. First, Pirquet clearly demonstrated the dynamics of the primary and booster responses. During the initial incubation period, he showed the slow fall in the titer of circulating antigen. He then showed how suddenly, with the onset of antibody formation, antigen quickly disappears from the circulation: a phenomenon that would, in more recent times[16], be termed the "immune elimination" phase. He next revealed how it is precisely at this stage that newly formed antibody neutralizes antigen and "toxic bodies" form, which gives rise to the clinical disease. Pirquet almost brought himself to admit that these toxic bodies were in fact antigen-antibody precipitates, but finally backed off to suggest that the interaction of antigen with antibody leads to the formation of some sort of "toxic physiological product". How close this was to the modern concept that the immune complex fixes complement, which leads to the release of the toxic mediators of the reaction[17]. Indeed, Pirquet mentioned in passing the "chance" observation that the titer of serum complement decreases sharply during the process, but it was too early to recognize the significance of this finding.

The second portion of this figure was equally revealing. In it, Pirquet showed that a second injection of horse serum results in an immediate response that consists of the very rapid elimination of the antigen, a sharp reduction in circulating antibody titer and the concomitant formation of toxic body and disease symptoms. In another diagram, he would show that when the second injection of serum is delayed for several months, until circulating antibody has disappeared from the patient, the full reaction is not immediate, but "accelerated". This was attributed to the well known recall, or anamnestic reaction, in which immunological memory hastens the renewal of antibody formation.

The remainder of Pirquet's book was devoted to the application of his theory of the role of the immune response to the incubation times of various diseases and processes, especially that of tuberculosis. He repeated the story of measles and extended it to other

exanthematous diseases (not all of which panned out as indicated above). In addition, he made a reasonable case for treating the incubation period and clinical response to primary cowpox vaccination as an example of the role of the developing immune response. Similarly, he assigned to the immune response the positive reaction to tuberculin, and indeed Pirquet earlier had introduced and made popular the cutaneous tuberculin test[18].

Future activities

It is curious that the man who, in a brief seven years, had made such significant contributions to immunology and to the theory of disease immunopathogenesis, should have so completely left the field. After having coined the term allergy and helped to define its parameters, he could surely not have thought the field exhausted: this was the period of expanding interest in experimental anaphylaxist and in clinical allergies, and indeed were the gestational years for the discipline of clinical allergy.

In 1909, at the age of 35, Pirquet accepted an appointment as the first professor of pediatrics at the Johns Hopkins Medical School in Baltimore, having turned down a purely research position offered by Emile Roux at the Pasteur Institute in Paris. Pirquet remained in Baltimore for only about one year, leaving for a professorship in Breslau, Germany. Hopkins attempted to lure him back with highly lucrative offers, but finally Pirquet declined. The death of his former chief in Vienna, Escherich, opened up the possibility of an appointment to this most prestigious Chair in pediatrics, and in the end the appointment was his.

Pirquet remained in Vienna for the rest of his life. He taught several generations of pediatricians and was a popular and respected teacher and supervisor. He developed a strong interest in anthropometrics and published numerous formulae and tables for estimating developmental parameters and organ weights. From this he moved to an interest in nutrition and developed dietary regimens known collectively as the "Pirquet System of Nutrition". He returned to the concept of allergy in his final years, although not in its immunological sense. Rather, he wrote on how altered reactivity (or sensitivity) to diseases is dependent upon age[19]. He died on February 28, 1929.

1. Just as immunology was markedly ecumenical during its early days, so has it expanded similarly during this age of immunobiology. Much important work in the field is being done by transplant surgeons, internists, hematologists, neurologists, endocrinologists, physiologists and pharmacologists who often do not belong to an immunological society, do not publish in immunological journals and do not even consider themselves immunologists.
2. Silverstein, A. M. *Nature Immunol.* **1**, 93–94 (2000).
3. More details about Pirquet's life are found in Wagner, R. *Clemens von Pirquet: His life and work* (Johns Hopkins Press, Baltimore, 1968).
4. Pirquet, however, opposed Ehrlich's theory and aligned himself with Max Gruber, Ehrlich's most vocal opponent. See their challenge to Ehrlich in Gruber, M. & Pirquet, C. *Münch. med. Wochenschr.* **50**, 1193–1196, 1259–1263 (1903).
5. Ehrlich, P. *Deutsch. med.Wochenschr.* **17**, 976–979 (1891) (Translation: Erlich, P. in *Collected Papers* Vol. 2, 21–26.) Ehrlich always looked for clinical implications in all of his research results.
6. This sealed letter is translated in full in Wagner, R. *Clemens von Pirquet: His life and work*, 52–54 (Johns Hopkins Press, Baltimore, 1968).
7. Antibodies were then the only known products of the immune response. It was thought that precipitins, agglutinins, hemolysins, and so on were separate substances.
8. Not long after, Pirquet and Bela Schick published on the theory of incubation time, see Von Pirquet, C. & Schick, B. *Wien. klin.Wochenschr.* **16**, 758–759, 1244–1247 (1903). (Schick, 1877–1967, was a fellow student in pediatrics who later developed the Schick test to demonstrate immunity to diphtheria.)
9. See Griffin, D. E. in *Measles Virus* (eds ter Meulen, V. & Billeter, M. A.) 117–134 (Springer, New York, 1995).
10. Mitus, A. *et al. N. Eng. J. Med.* **261**, 882–889 (1959); Markowitz, L. E. *et al. J. Infect. Dis.* **158**, 480–483 (1988).
11. von Pirquet, C. & Schick, B. *Das Serumkrankheit* (Deuticke, Leipzig, 1905). (Translated by Schick as *Serum Sickness*, Williams & Wilkins, Baltimore, 1951.)
12. Kraus, R. *Wien. klin.Wochenschr.* **10**, 736–738 (1897).
13. von Pirquet, C. & Schick, B. *Serum Sickness*, 103 (Williams & Wilkins, Baltimore, 1951).
14. von Pirquet, C. *Ergebn. Inn. Med. Kinderheilk.* **5**, 459–539 (1910). Reprinted as von Pirquet, C. *Allergie* (Springer, Berlin, 1910). (Translation: *Arch. Intern. Med.* **7**, 258–288, 383–440, 1911. Reprinted as *Allergy*, American Medical Association, Chicago, 1911.)
15. Several generations of immunologists would insist that immunity and allergic diseases depend upon completely different mechanisms and systems and this view still finds adherents today. A counter-argument is made by Silverstein, A. M. & Rose, N. R. *Semin. Immunol.* **12**, 173–178 (2000).
16. Dixon, F. J., Maurer, P. H. & Deichmiller, M. P. *J. Immunol.* **72**, 179–186 (1954).
17. See, for example, the modern studies on immune complex disease that include the fluorescent antibody studies, which show the deposition of immune complexes and complement at the sites of pathology: Theofilopoulos, A. N. & Dixon, F. J. *Adv. Immunol.* **28**, 1–90 (1979); Cochrane, C. G. & Koffler, D. *Adv. Immunol* **16**, 185–264 (1973); Unanue, E. R. & Dixon, F. J. *Adv. Immunol.* **6**, 1–90 (1967).
18. von Pirquet, C. *Berlin klin.Wochenschr.* **44**, 699–700 (1907).
19. von Pirquet, C. *Wien klin.Wochenschr.* **42**, 65–67 (1929). This was published posthumously and in full as *Die Allergie des Lebensalters. Die Bösartigen Geschwülste* (Thieme, Leipzig, 1930).