JLB

Review

The historical milestones in the understanding of leukocyte biology initiated by Elie Metchnikoff

Jean-Marc Cavaillon[1]

Unit Cytokines and Inflammation, Department Infection and Epidemiology, Institut Pasteur, Paris, France

RECEIVED FEBRUARY 20, 2011; REVISED MAY 3, 2011; ACCEPTED MAY 6, 2011. DOI: 10.1189/jlb.0211094

ABSTRACT

Progress in science is made with key discoveries, correct analyses, wrong statements, and disputes within the scientific community. Despite scientific controversies, Elie Metchnikoff has allowed the theory of phagocytes to triumph. Starting his career as a zoologist, Metchnikoff became a pathologist, beautifully defining the role of monocytes, macrophages, and neutrophils during inflammation and innate immunity. The discoveries of immune cells were made by other outstandings scientists, such as Paul Ehrlich, whose key contributions to humoral immunity led him to share the Nobel Prize with Metchnikoff. Ludwig Aschoff grouped certain cells under the term RES, according to their propensity for absorbing and storing vital stains. This classification was not always a source of accurate discoveries, and research on the exact function of RES cells led to some wanderings. This is illustrated by studies about the nature of the antibody-producing cells, which were first thought to belong to the RES, before being identified as plasmocytes and lymphocytes. *J. Leukoc. Biol.* 90: 413–424; 2011.

Introduction

Leukocyte biology remains a very dynamic field of investigation; new cells are still regularly discovered, as illustrated by the recent identification of the nuocytes [1]. In contrast, other cells may not exist anymore or at least have been forgotten. For example, this is the case of the clasmatocytes described in 1902 by Alexander Maximow (1874–1928), who characterized macrophages of the connective tissues [2]. Born in St. Petersburg, Russia, and dying in Chicago, Ill., USA, Maximow introduced the unitarian theory of hematopoiesis, upon which, the modern concept of blood cell origin and differentiation is based. In 1909, he introduced the term "stem cell", although he postulated that the lymphocyte was a common stem cell of the various blood elements [3]. The end of the 19th century and the first half of the 20th century have indeed been the most active period for the identification and characterization of cells, particularly those involved in innate and adaptive immunity. Interestingly, in a few cases, identification may not have been associated immediately with the correct characterization. For example, the most important contribution of Karl Wilhelm von Kupffer (1829–1902) was the 1876 discovery of stellate liver cells, which bear his name. However, he incorrectly believed that these cells were an integral part of the liver blood vessel's endothelium. It was only 22 years later that Tadeusz Browicz (1847–1928), a Polish pathologist, correctly identified them as macrophages. Similarly in 1868, Paul Langerhans (1847–1888) stained a sample of human skin with gold chloride and identified the cells, which bear his name, as nerve cells [4]. For many years thereafter, Langerhans cells were thought to be related to melanocytes [5]. In the late 1960s, it was speculated that they belonged to an intraepithelial phagocytic system. However, in 1979, they were unequivocally established to be hematopoietic cells, and their role as APCs was demonstrated in the early 1980s [5]. If errors accompanied these discoveries, the period was also rich in controversies. Establishing a new concept in that time was no easy task. Louis Pasteur (1822–1895), himself, experienced significant challenges imposing his demonstration against spontaneous generation. Concomitantly, the birth of immunology was accompanied by great controversy and numerous disputes, particularly between France and Germany, even if one of the main actors was a Russian scientist, Elie Metchnikoff (1845–1916), the father of innate and cellular immunity, who had joined Pasteur in 1888. He wrote in October 1913: . . . *The controversy over phagocytosis could have killed me, or permanently weakened me sooner. Sometimes, (I remember such attacks of Lubarsch in 1889, and those of Pfeiffer in 1894) I was ready to get rid of life.* The controversy was mainly about the respective roles played by humoral immunity and cellular immunity in fighting infection. Accordingly, in the 1890 Berlin meeting, the 1891 London meeting (**Fig. 1A**), and the 1894 Budapest meeting, struggles regarding the two theories were arduous and severe. While the famous British surgeon, Joseph Lister (1827–1912),

Abbreviations: MIF=migration inhibitory factor, RES=reticuloendothelial system

1. Correspondence: Unit Cytokines and Inflammation, Department Infection and Epidemiology, Institut Pasteur, 28 rue Dr. Roux, 75015 Paris, France. E-mail: jean-marc.cavaillon@pasteur.fr

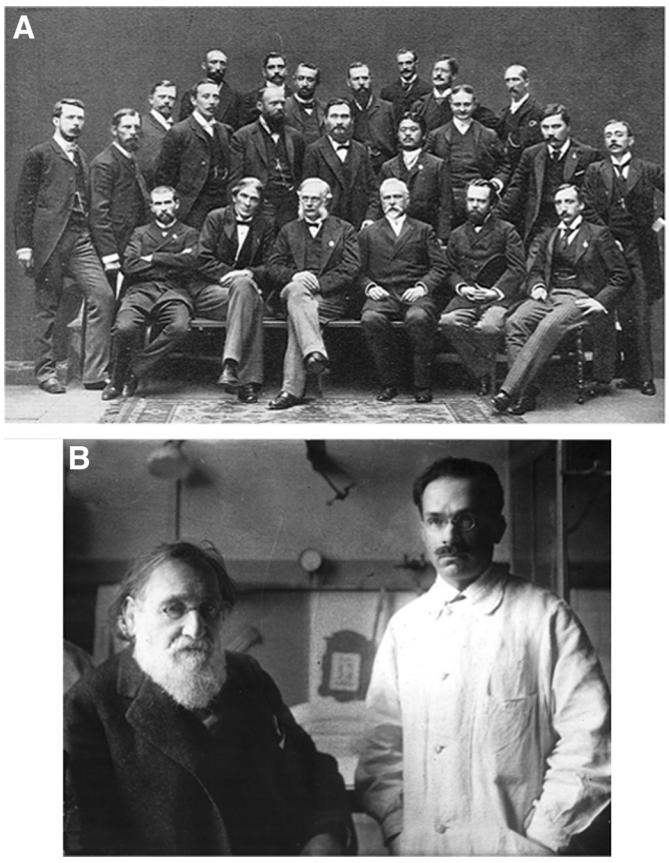

Figure 1. (A) Elie Metchnikoff at the 7th Congress of Hygiene and Demography (London, 1891), standing, second row, right behind Sir Joseph Lister and right to Dr. Shibasaburō Kitasato. (B) Elie Metchnikoff in his laboratory with Alexandre Besredka.

was supporting the work of Pasteur's group, Robert Koch (1843–1910), Hans Buchner (1850–1902), Emil von Behring (1854–1917), with his work on antitoxins, and Richard Pfeiffer (1858–1945), with his report on the extracellular killing of *Vibrio cholerae,* were providing strong evidences in favor of the humoral immunity. However, after the Budapest meeting, Emile Roux (1853–1933), a close friend of Metchnikoff, wrote to Pasteur: *I wrote this message just getting out of the meeting. Metchnikoff in a reply full of passion and focus on truth has allowed the theory of phagocytes to triumph. I think he has put the belief in many minds.*

It is fascinating that a leader of the humoral immunity theory, Paul Ehrlich (1854–1915) was one of the very first scientists to identify the immune cells, whereas the closest pupil of Metchnikoff, Alexandre Besredka (1870–1940; Fig. 1B), who succeeded him as the head of his laboratory, was the first scientist to obtain antiendotoxin antibodies in 1905 [6]. The controversy between the humoralists and cellular immunologists has been thoroughly discussed by Arthur Silverstein [7, 8]. Finally, a very wise Nobel committee decided to award the 1908 Nobel Prize to both Ehrlich and Metchnikoff, recognizing implicitly that humoral and cellular immunity contribute to the fight against pathogens.

ELIE METCHNIKOFF AND HIS KEY CONTRIBUTION[2]

Numerous books, including the very one written by his second wife, Olga [9], and many articles relay the life of Metchnikoff [10–15] (a list of books and articles about Metchnikoff, as well as the complete list of his publications are available at: http://www.pasteur.fr/infosci/biblio/resources/histoire/metchnikoff.php#2a). Olga Metchnikoff's biography of her husband was translated into English, and on April 30, 1922, *The New York Times* devoted a full-page analysis. It emphasized that as a wife and for a time, a scientific companion, she *was armed with the facts of his life, possessed this intimate knowledge and the scientific capability necessary to interpret his epoch-making discoveries in terms of common understanding.*

Elie Metchnikoff was born in the Russian Empire near Kharkoff, present-day Ukraine. After his major discovery about phagocytosis in Sicily, he went looking for a permanent position. Metchnikoff could have worked in Berlin, but following his visit with Koch in 1887, in which he was coldly received, Koch's absolute rejection of the concept of cellular immunity pushed him elsewhere. In contrast, his visit to Louis Pasteur was far more supportive: *On arriving at the laboratory for antirabies vaccines, I saw an old rather undersized man, with a left hemiplegia, very piercing gray eyes, a short beard and moustache, and slightly gray hair [. . .] He received me very kindly, and immediately spoke to me about the question that interested me the most, the struggle of the organism against microbes [. . .].* Metchnikoff decided to join the Institut Pasteur, which was newly created to continue Pasteur's successful research on the rabies vaccine, to welcome patients, and to initiate teaching in microbiology. Metchnikoff was offered a director position of one of five units created when the institute was opened in 1888. Later, he was appointed deputy-director of Institut Pasteur from 1904 to 1916. At first, Metchnikoff and his wife were a bit nervous about settling in Paris. Part of their uneasiness was undoubtedly a result of their lacking impression of life in France, yet the literature of Zola and other realistic French writers could explain it [11]. He never asked for French nationality, but according to Olga, the 28 years spent in France were the best of his life, and he would be forever grateful to Pasteur and to France for their welcome.

Metchnikoff resembled a character of Dostoyevsky, with a tortured personality, a pessimistic approach to life, and sometimes quite depressed; indeed, a romantic life. Throughout his life, he had numerous health problems, particularly with his eyes, which sometimes prevented him from using the microscope, and with his heart, which caused him a great deal of suffering from the age of 33. Metchnikoff studied natural sciences in Kharkoff, becoming an embryologist and a zoologist with the help of admiration and influence stemming from Darwin's concept of evolution [14]. He was 16 or 17 when he wrote an adverse criticism in the *Journal de Moscou* of a geology book written by a Kharkoff professor. Despite his own sensitivity to criticisms, Metchnikoff's critiques led to an unpopular reputation. During his whole career, he was involved in violent

2. The text of this paragraph has been previously published partially in the *Endotoxin Newsletter*, vol. 17, Fall 2008 (International Endotoxin Society).

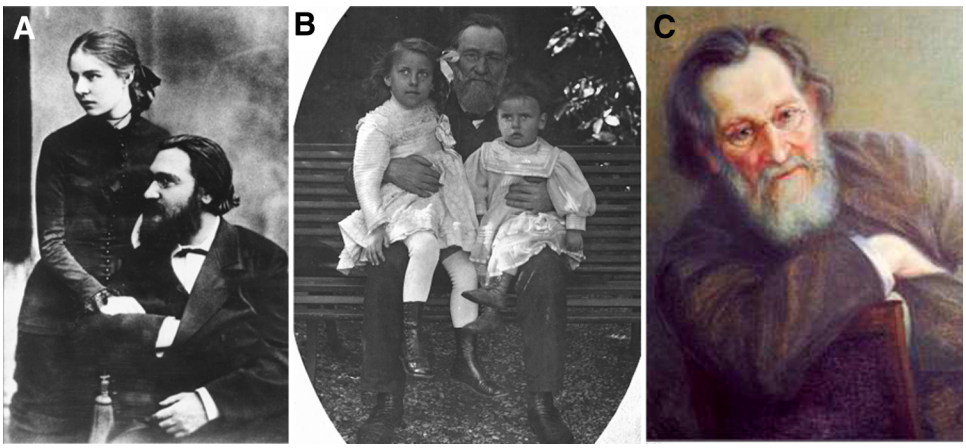

Figure 2. (A) Elie Metchnikoff and his wife Olga, born Belokopytova. (B) Elie Metchnikoff with Lily, his goddaughter and her brother. (C) Elie Metchnikoff's portrait painted by Olga.

polemics. At age 22, he was appointed professor of zoology at the University of Odessa but soon after, could not deal with the local authorities, resigned, and accepted a post as professor at St. Petersburg University in 1869. The same year, he married Ludmila Vassilievna Fedorovna as his first wife, but her poor health and infection with tuberculosis led them to travel to Maderia, where they were considering opening a bookshop. She died in Madeira in 1873. Her death left Metchnikoff desperate, leading to an attempted suicide by swallowing a large dose of opium. This prompted a move back to Odessa, where he was poor and open to any jobs that could allow him to earn some more money, including giving lessons of zoology to young students such as Olga Belokopytova (1858–1944). In 1875, they married; she was 17; he was 30 (**Fig. 2A**). In 1880, she had typhoid fever. The combination of his wife's illness and his tiring stuggle with cardiac problems left him depressed. Again, he attempted suicide by injecting himself with blood from a patient with relapsing fever. This was an attempt to end life and simultaneously to know whether this disease could be transmitted by the blood. He almost succeeded and was left severely sick. Although he never had children with Olga, he was in fact the father of his goddaughter, Lily Remy, the child of one of his friends (personal communication from Annick Perrot, past-curator of the Institut Pasteur Museum) (Fig. 2B). Once in the Institut Pasteur, Olga assisted Metchnikoff in his lab. Olga's talented artistry, as evidenced by her paintings, including those of her husband (Fig. 2C), finally led to her devotion to nonscientific activities.

In 1882, Metchnikoff, with Olga, two of his wife's sisters, and three of her brothers, moved to Messina prior to the end of his studies and during his travels in Italy. He aimed to pursue studies in comparative embryology of marine fauna and made his major discovery while his wife, sisters-in-law, and brothers-in-law were attending a circus show. Metchnikoff stuck rose thorns into starfish larvae and was surprised to see that many phagocytic cells in the hemolymph surrounded the "foreign object". He also observed the process in Daphnia, which he infected with yeast. Within the water fleas, cells were able to move, ingest, and destroy the yeast cells. His main contribution was the understanding that phagocytosis was a defense mechanism, that phagocytes were an active participant of inflammation, and that inflammation should no longer be seen only as a deleterious event. He worked hard on this concept, wrote articles, and gave lectures. Rudolf Virchow (1821–1902) was visiting Messina and told Metchnikoff: *This may be true, however, be aware that in the universities one teaches the opposite.* While visiting Vienna, Metchnikoff presented his observation to Carl Claus (1835–1899), a German zoology professor, who invited him to publish his results in his journal. Then, the first publication about "intracellular digestion" and the first appearance of the concept of phagocytosis were published in 1883 [16]. Metchnikoff did not create the words phagocyte and phagocytosis, but Claus suggested them from their Greek derivation for "devouring cells" [17]. The word "phagocyte" appeared the following year for the first time in an article title still written in German [18]. *The consequence of my stay in Messina was that, from a zoologist, I become a pathologist and a bacteriologist*, said Metchnikoff. In 1901, he gathered in a book, entitled *Immunity in Infective Diseases*, years of observations and reflection. Wonderful and precise drawings can be found (**Fig. 3**).

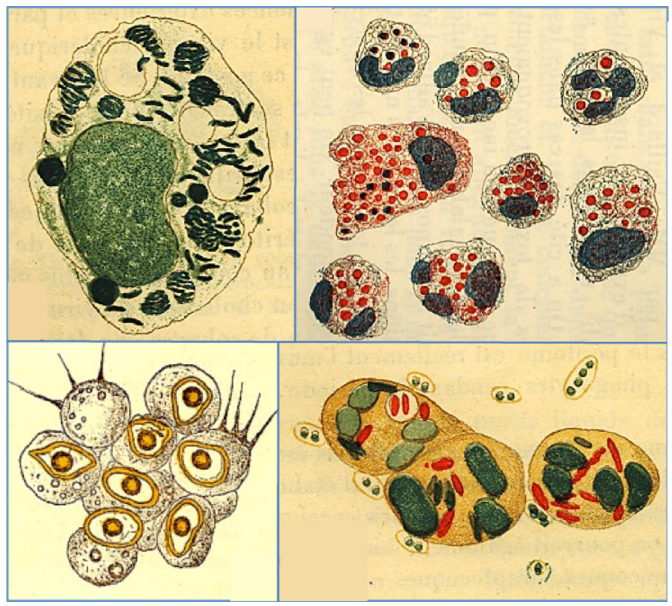

Figure 3. Drawings of phagocyting leukocytes [19].

Metchnikoff was not the first one to have observed the phenomenon of phagocytosis: Wiliam Osler (1849–1919), a Canadian medical doctor, had reported in 1876 the presence of carbon particles within cells harvested from the lung of coal miners [20]. The same year, Koch identified *Anthrax bacilli* in white blood cells, yet Koch had interpreted his finding to mean invasion of host cells by bacterial pathogens. An American physician, George Miller Sternberg (1838–1915), claimed that he was the first in 1881 to suggest that white blood cells might intake and destroy bacteria. However, he had not visualized the phenomenon nor did he study it later [17].

During the end of his career, Metchnikoff worked on aging and particularly on intestinal flora. Convinced that aging was the consequence of a chronic poisoning by intestinal microbes whose toxins diffuse into the body, thus damaging normal tissues, he hypothesized that a hygienic diet would correct these deleterious events. Having heard that old people in Bulgaria were mainly eating yogurt, he imported some, leading Metchnikoff to the discovery of *Lactobacillus bulgaricus*. An active defender of the use of probiotics, he was convinced that if he lived longer than 70 years, it was thanks to his diet. Naturally, he asked his doctor to look at his gut after his death.

Metchnikoff always prepared his lectures with meticulous care and was cautious to never overpass his allocated time. In contrast, as written by Metchnikoff in a letter sent to his wife in 1894 from Budapest, where he was attending a meeting, his colleague from Institut Pasteur, Dr. Roux, talked for one-half hour when he was given a 15-min talk. So, the fact that many congress speakers surpass their allocated time is not a new behavior to appear in the 21st century. After his trip to Stockholm to receive the Nobel Prize on May 14, 1909, he traveled to Russia and met Leon Tolstoi (**Fig. 4A**). Admirers of each other's work, they spent the day sharing their view in the consideration of life's social and philosophic aspects. For Metchnikoff, *Science is the only way that suffering humanity will find to ensure its future, because only Science can really know.* No doubt that Tolstoi's vision to reach happiness was quite different.

Metchnikoff had always been associated with the teaching activities that started in 1889 at Institut Pasteur under the direction of Dr. Roux. His concern for his students was warm, sometimes paternal, and always active and vibrant. Often, his students became his friends and collaborators for many years thereafter. An interesting testimony is given by the profile of Metchnikoff engraved by an anonymous student on a windowpane in the room where the first courses on microbiology were being delivered (Fig. 4B). Despite scientific controversies and memorable debates during the international congresses, he maintained good relationships with his German colleagues, as illustrated with the numerous letters exchanged with von Behring or Ehrlich. Metchnikoff and Roux paid tribute to Ehrlich in a 1914 paper published in French in a German journal [21]. They were extremely laudatory about Ehrlich's demonstration about the transmission of humoral immunity through the placenta and the milk; they wrote: *This work is a true masterpiece by the ingenuity of the experiments and the significance of results.* Adding: *We cannot read the works of Prof. Ehrlich without being struck by the extent and the variety of his knowledge*; and *the whole character is so sympathetic and interesting.* Even Metchnikoff had the opportunity to welcome Ehrlich in 1903 and Koch in 1904 at the Institut Pasteur (Fig. 4C).

Metchnikoff's lab was quite popular, and many scientists joined him; most notably, they included: Alexandre Besredka (Fig. 1A), who prior to his move from Odessa, was asked to perform medical studies illustrating how scientists were aware that only a close collaboration with medical doctors could lead to key achievements. Indeed, among the five unit heads chosen by Pasteur at the creation of his institute, two were medical doctors—Drs. Roux and Jacques-Joseph Grancher (1843–1907), the physician who injected the first rabies vaccine in the young Joseph Meister. Besredka performed numerous studies looking for the best routes of immunization and attempted to define the best approaches to avoid shock after serotherapies with horse sera by proposing a desensitization process. He later succeeded Metchnikoff as the head of his laboratory. Michel Weinberg (1868–1940), a medical doctor and biologist also from Odessa, worked on helminths and antibodies against secretory products and set up a serodiagnostic test for echinococcosis. Constantin Levaditi (1874–1953), another physician, cytologist, and immunologist from Romania, worked on mast cells and on the pathogenesis of various diseases (syphilis, tuberculosis, herpes, chickenpox, smallpox, po-

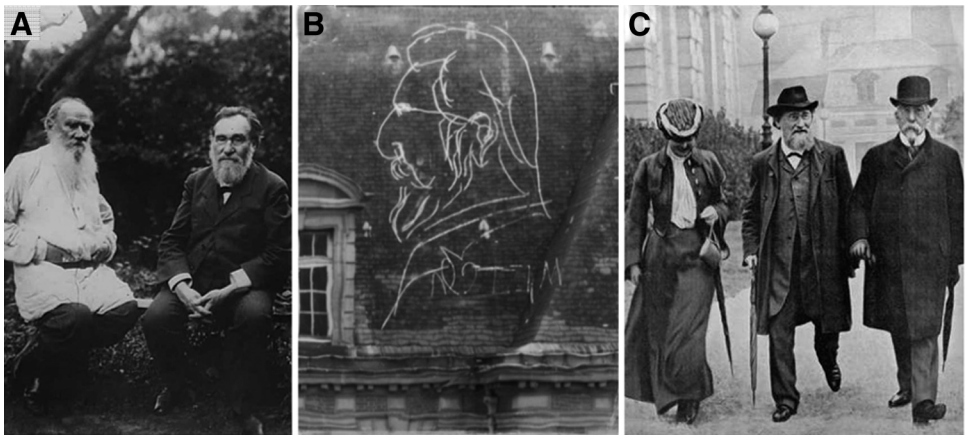

Figure 4. (A) Elie Metchnikoff with Leon Tolstoi (May 1909). (B) Profile of Elie Metchnikoff engraved by an anonymous student on a windowpane in the room where the first courses about microbiology were being delivered. (C) Robert Koch visiting the Institut Pasteur, accompanied by Elie Metchnikoff (1904).

liomyelitis); he recommended the treatment of syphilis with bismuth. Félix Mesnil (1868–1938), a biologist and past-student of the most prestigious French "Grandes Ecoles" (Ecole polytechnique and Ecole normale supérieure), with Alphonse Laveran, discovered a new parasite, finally named *Leishmania donovani*. Jean Cantacuzène (1863–1934), a medical doctor from Bucharest who worked twice in Metchnikoff's lab from 1892 to 1894 and from 1896 to 1901, studied anti-*V. cholerae* immunity and the role of phagocytosis of Spirillosis parasites. In Bucharest, he opened an institute that bears his name, similar to Institut Pasteur—The Cantacuzino Institute. Jules Bordet (1870–1961), a medical doctor from Belgium and Metchnikoff's most prestigious pupil, was awarded the Nobel Prize in 1919. In 1898, while in Metchnikoff's lab (1894–1901), he discovered hemolytic sera and showed that the mechanism of action on foreign RBCs was similar to that by which an antimicrobial serum acts on microbes. Indeed bacteriolysis was first reported 4 years earlier by Richard Pfeiffer (1858–1945). Bordet called his discovery "alexine", but it is Paul Ehrlich who coined the word "complement", which was finally used. In Brussels, Bordet created the "Institut Pasteur du Brabant".

On December 1915, Metchnikoff suffered serious cardiac problems, which Dr. Fernand Widal diagnosed as myocarditis. His health worsened, and in July 1916, he was transferred to Pasteur's apartment. He said to Roux: *See how my life is linked to the Institut Pasteur. I worked for many years, and now I am treated here during my illness. To complete the link, the right thing would be my cremation in the big oven where the experimental animals are burnt, and to keep my ashes in a jar above one of the cabinets of the library*. Dr. Roux thought that it was a macabre joke, but Olga later confirmed Metchnikoff's wishes (**Fig. 5**).

Figure 5. Metchnikoff's urn in the old library of Institut Pasteur.

FURTHER CHARACTERIZATION OF MACROPHAGE AND PHAGOCYTOSIS

A key mechanism that accompanied phagocytosis is opsonization. This phenomenon was discovered by Sir Edward Almroth Wright (1861–1947). He was very famous for his 1896 discovery of an effective vaccine for typhoid fever that limited the British army's suffering during World War I. (In 1915, there were 1000 cases in the British army, and 69,000 cases in the French army.) Additionally, in 1903, he discovered the phenomenon of opsonization and coined the word from Greek "opsono" (opsono), meaning, *I prepare victuals for . . . .* In his paper, he offered the greatest definition ever proposed: *The body fluids modify bacteria in a manner which renders them a ready prey to phagocytes* [22].

After Metchnikoff and Wright, the decline of cellularism occurred [8, 23]. The decline was a result of its lack of specificity, the difficulty of cell technology, in contrast with an easy access to serum, an easy demonstration of the antibody activity, and a rapid rise of immunochemistry and biochemistry. Metchnikoff had proposed a central role for phagocytic cells, but during the next five decades, very limited progress had been made to elucidate the biological role of phagocytes, and no specificity of their action could be demonstrated despite the works of Sanford S. Elberg, K. Faunce, Jr., and Emanuel Suter (cited by Silverstein [8]).

In the first decades of the 20th century, efforts were mainly devoted to identify the origin of macrophages. Demonstrations of the origin of tissue macrophages had first been achieved in vitro. In 1914, from blood cells of a leukemic patient, Awrorow and Timofejewskij [24] concluded that the lymphocyte is a stem cell, from which the enlarged mononuclear cell arises, and from it, the other types of transformed cells found in their plasma cultures develop, including macrophages. In 1922, Carrel and Ebeling [25] reported long-term cultures of chicken blood. Alexis Carrel (1873–1944) was awarded the Nobel Prize in 1912 for his work on vascular surgery and surgical grafts. He became particularly controversial after publishing his idea about eugenics and euthanasia, as well as supporting the eugenics policies of the Third Reich. In their report, Carrel and Ebeling [25] observed that granulocytes disappeared from their cultures after a few passages, and typical macrophages were seen at the periphery of the culture, sometimes in the immediate vicinity of the transition forms and fibroblasts. It is unclear whether what they called fibroblasts were true fibroblasts or macrophages shaped like fibroblasts [25].

In 1924, Sabin, Doan, and Cunningham [26, 27] had been carrying on a series of observations, again with chicken blood, in which they differentiate the various types of blood cells by means of vital dyes and on this basis, attempt to group the blood cells more in accordance with what they consider to be their origin. This method permits investigators to distinguish monocytes from clasmatocytes and claim that although monocytes and clasmatocytes are phagocytic, there is nevertheless a distinct difference between the two types of cells. Some of the transformed leukocytes resemble clasmatocytes, a term used in its usual sense to define tissue macrophages by these authors.

In 1925, Lewis [28] reported similar observations, finally achieved with normal human blood.

The first in vivo demonstration, in which monocytes could become macrophages, came in 1930 [29]. The relation of monocytes to macrophages was studied in the transparent tails of living amphibian larvae (*Rana catesbiana)* by injecting egg cream and yolk into the tissue with or without carmine. Only stained monocytes were observed to migrate within the tissue. For mammals, the authenticity of change from blood monocyte to tissue macrophage depended on the observations of Ebert and Florey made in 1939 [30]. Using the rabbit's ear chamber, they observed the migration of marked blood monocytes entering into the damaged tissues, increasing in size, accumulating refractive droplets within their cytoplasm, and assuming appearances comparable with those of tissue macrophages.

NEUTROPHILS AND INFLAMMATION

In his lectures about the comparative pathology of inflammation gathered in a book published in 1892, Metchnikoff indicates that phagocytes should be considered as an active participant of inflammation, and inflammation should not be seen only as being deleterious. At the time, in 1865, the French dictionary of Medicine, Surgery, Pharmacy, "Accessory Sciences" (*sic*) and Veterinary Sciences, "morbid" was still the qualification given to define inflammation. Was Metchnikoff the first one to recognize that inflammation is a helpful physiologic response to aggression? The answer is no; John Hunter (1728–1793), a Scottish surgeon, in his book, published 1 year after his death, entitled, *A Treatise on the Blood, Inflammation and Gun-Shot Wounds*, wrote a brilliant definition of inflammation: *Inflammation in itself is not to be considered as a disease, but as a salutary operation, consequent either to some violence or some disease.* Metchnikoff also acknowledged the key role of leukocyte transmigration from the bloodstream: *Once arrived at the conclusion that inflammation in higher animals is a healthy reaction of the body and diapedesis, with all that accompanies it, is part of this response, several features of inflammation are simple and clear.* He illustrated his words with a drawing made by Arnold [31], who in 1873, mentioned that *Leukocytes pass through specialized openings between endothelial cells* (via "para-cellular" migration). However, transmigration of circulating cells was first reported by Augustus Volney Waller (1816–1870), an English neurophysiologist, who in 1846, published his early research interests focused on the use of microscopy to demonstrate leukocyte emigration using the frog tongue as a model [32]. In 1867, Julius Friedrich Cohnheim (1839–1884), a German pathologist, proved that white blood cells cross blood vessels to become pus cells [33].

In his books, Metchnikoff also clearly defines the role of neutrophils, which he called "microphages": *In the Vertebrates we meet with two great categories of white corpuscles, of which one group resembles those of the invertebrates in that they also possess a single large nucleus and an amoeboid protoplasm. These are the macrophages of the blood and of the lymph, and are intimately connected with the macrophages of such organs as the spleen, lymphatic glands, and bone marrow. Another group of white corpuscles in the Vertebrata is made up of small amoeboid cells, which are distinguished by having a nucleus, which, although single, is divided into several lobes. These are the microphages. Phagocytosis is exhibited not only by the macrophages but also, in a high degree, by the microphages which stand out as the defensive cells par excellence against microorganisms [. . .]. The microphages, on the other hand, appear to play their part, specially, in acute infections.* His view was particularly pertinent. Still 30 years ago, I was told that neutrophils were cells ready to die, unable to synthesize any proteins, despite Metchnikoff having recognized their key role in infection: *It has been said, and one continues today to affirm that polymorphonuclear leukocytes are cells predestined to death, unable to considerable activity. Instead, the leukocytes are precisely the most active cells of the body.*

The first description of neutrophils was made in 1865 by Max Johann Sigismund Schultze (1825–1874) [34], who first described the four different types of blood leukocyte corresponding to what are now recognized as the lymphocyte, the monocyte, the eosinophil, and the neutrophil. Ehrlich, a trained chemist, started his career as a histologist using chemical dyes for selective cell staining and identifying lymphocytes and eosinophils. About the latter, Metchnikoff recognized: *The best proven result was provided by Mr. Ehrlich, who showed that the eosinophil leukocytes are mainly produced by bone marrow.* Among the key discoveries made by Ehrlich was the first identification of mast cells [35]. Despite Henry H. Dale (1875–1968) discovering histamine in 1910, a discovery for which he was awarded the Nobel Prize in 1936, it was only in the mid-1950s of the 20th century that a link was made between mast cells and histamine [36] or a link between mast cells and inflammation [37]. Most interestingly was that the important role of mast cells in an anti-infectious response was probably reported even earlier, in 1949 [38].

The usefulness to create inflammation on purpose, was the major discovery of Gaston Ramon (1886–1963). In 1911, Ramon was hired by Dr. Roux as a veterinarian to oversee the preparation of antisera against tetanus and diphtheria toxins. When dealing with more than 100 horses, he noticed that when there was a small abscess or the presence of pus at the site of toxoid injection, levels of antibodies were higher [39]. Then, he intentionally induced inflammation at the site of injection with pus or his favorite substance, tapioca, to enhance antibody production. He was indeed the very first one to use the "dirty little secret of the immunologists", mentioned by Charles Janeway (1943–2003) [40], and had invented the concept of adjuvant. This discovery issued a key statement indicating a need to involve an inflammatory reaction at the antigen-injection site to enhance immune response [39]. In 1935, Ramon suggested the use of lanolin [41] after others had suggested the use of paraffin oil, olive oil, or cod liver oil. In 1937, Jules T. Freund (1890–1960), an Austria-Hungarian-born American immunologist, proposed to add inactivated *Mycobacterium tuberculosis* to an oil–water emulsion [42], ending in the popular CFA. Forty years later, Louis Chedid at Institut Pasteur and Edgar Lederer (1908–1988) at Paris-South University would reveal the smallest active adjuvant structure derived from the bacterial peptidoglycan, namely, the muramyl dipeptide [43].

THE ERRONEOUS CONCEPT THAT THE RES ACCOUNTED FOR ANTIBODY FORMATION

Karl Albert Ludwig Aschoff (1866–1942) grouped certain phagocytic cells into a classification under the term RES, which he coined in 1922 [44, 45]. The concept was introduced as early as 1914 by Kenji Kiyono (1855–1955) [46], after both had coined the word histiocyte (*histiozit*) in 1913 at Freiburg University [47]. Professor Kiyono, also known for his archeological studies about the origin of the Japanese nation, was awarded the Prize of the Japan Academy of Science for his work on RES. One of the most prominent characteristics of RES cells is their propensity for absorbing and storing, in granular form, vital stains introduced into the blood in solution. Cells were gathered in different groups: 1. endothelial cells of the blood and lymph vessels; 2. fibrocytes or ordinary connective tissue cells; 3. reticulum cells of the splenic pulp and of the cortical nodules and cords of LNs; 4. RES cells of the sinuses of LNs, of the blood sinuses of the spleen, of the capillaries of the bone marrow, Kupffer's stellate cells, of the suprarenal cortex, and of the hypophysis; 5. histiocytes of the connective tissues, the clasmatocytes; and 6. splenocytes and monocytes.

Aschoff proposed eliminating groups 1 and 2, which stain faintly or not at all and function differently from the other groups. He also proposed combining group 3 and 4 because of their common functions and to call them in a strict sense, RES. In 1929, Russell [48] showed that brain microglial cells were fulfilling the qualities of RES cells.

Attempts were made to understand antibody formation. In his book about immunity and infectious diseases [19], Metchnikoff makes great efforts to convince the reader that humoral and cellular theories are not exclusive: *One often thinks that the theory I have just summarized is in fundamental disagreement with the theory of side chains or receptors expressed by Mr. Ehrlich. I cannot agree with this opinion* [. . .]. *In all cases, it is clear that the theory of receptors should not be regarded as the antithesis of the theory of phagocytes.* Metchnikoff designated nomenclature to define the key actors of humoral immunity. He used the word "cytase" for the complement system or from time to time, the word alexine of his collaborator Jules Bordet. For Metchnikoff, the microbicidal activity contained in neutrophils (microcytase of microphages) and in macrophages (macrocytase) belongs to the same family. He described with the word "fixative" (or "sensitizing substance" or "immunizing substance") as what Ehrlich call "amboceptors", i.e., the antibodies. There was difficulty for Metchnikoff to decipher among all of the properties of an immune serum (agglutination, lysis, protection): *The preventive or anti-infective substance is not the same as the agglutinin. But do we have the right to treat it identical with the fixative'?* When Metchnikoff addressed the origin of antibodies, he offered the following scenario: *These are the elements of phagocytic organs, i.e., the phagocytes themselves that produce the preventive substance. But can we admit as long as the fixative substance also comes from the same source'?* [. . .] *The spleen is truly the main place where the fixative substance is elaborated before appearing in the blood* [. . .]. *We necessarily reach the conclusion that the 'fixative' is a close second phagocytic ferment produced in abundance during intracellular digestion. But instead of staying within the content of phagocytes, the fixative is partly secreted out of these elements, it passes into the blood plasma and other body fluids and eventually disappears from the body, probably due to its elimination by excretory organs.* The idea raised by Metchnikoff that macrophages could be the source of antibodies was perpetuated during the few following decades, and for some years, evidence has been presented implicating the cells of the RES in the formation of antibodies. Demonstrations were achieved by blocking the RES during immunization. Trypan blue was used as the blocking agent in 1924 by Gay and Clark [49], when they showed dramatic effects on anti-sheep RBCs in rats and rabbits. Similar demonstrations were reported in 1926 by Jungeblut and Berlot [50], using injection of India ink to block the RES in guinea pigs before a s.c. injection of diphtheria toxin. In 1929, Roberts [51] confirmed that *the effect of a reticulo-endothelial blockade in rabbits on the appearance of antibody in the circulating blood intimates a trend toward the inhibition of the rate and extent of hemolysin, agglutinin, and precipitin appearance.* Other reports published between 1922 and 1934 resulted in the same conclusion [52–54]. Most authors only made a relationship between RES blockade and antibody production, although they were careful not stating that the cells of the RES were the source of antibodies. Nowadays, one may consider that blocking RES was preventing the involvement of APCs. Using quartz particles to inhibit or paralyze the RES, Elvidge [55] ended his work published in 1933 with a less-ambiguous statement: *It is finally concluded that opsonin production is a function of cells of the reticulo-endothelial system.* The final demonstration that RES cells were the source of antibodies was reported in 1939 by Florence Sabin (1871–1953) [56]. She summarized her observations as such: *One may stimulate the phagocytic cells either of the liver and spleen or of the tissues and lymph nodes to produce antibodies [. . .]. The cells of the reticulo-endothelial system normally produce globulin and that antibody globulin represents the synthesis of a new kind of protein under the influence of an antigen.* She described an intracellular phenomenon that leads to antibody secretion: *If the material phagocytized is an antigen, it is rendered into suitable soluble form within the vacuole and then passed into the cytoplasm itself. There, its presence in some way increases the synthesis of globulin and modifies some of it into antibody globulin. With the shedding of parts of the surface films of these cells, both normal globulin and antibody globulin are carried into the blood plasma.* Of course, Sabin should not only be considered for this erroneous demonstration. While working at Johns Hopkins University Hospital (Baltimore, MD, USA), she made key analyses about the origin of blood cells, blood vessels, and connective tissue before moving to the Rockefeller Institute, where she made major contributions to the understanding of tuberculosis. Sabin also worked on the development of the lymphatic system in young pig and chick embryos, emphasizing that lymphatic vessels arise as an active growth of endothelial cells. She wrote an important review [57] about the lymphatic system, in which she recalled the work of Friedrich Daniel von Recklinghausen (1833–1910), a German pathologist who discovered that the lymphatic capillary was composed of cells. In 1925, Sabin became the first woman elected to the National Academy of Sciences of the United States. She worked on the clasmatocytes described by Maximow in connective tissue, for which Aschoff and Kiyono

had clearly established the relationship with the monocytes. Charles A. Doan (1896–1990), a renowned hematologist, professor of medicine, and director of the Ohio State University Hospital, had invited Sabin to attend a hematologic symposium to be held in Columbus, Ohio, USA, to present her theory of the relationship of the RES in antibody formation. In a letter dated September 1, 1939, Doan was proposing additional experiments to Sabin, enabling the possibility of procuring some of Dr. Michael Heidelberger's dye protein, which she used in an attempt to cinematographically record exoplasmic shedding of the phagocytic cells when antibodies are first appearing in circulating blood. He added: *I still cannot quite get away from the feeling that monocyte and clasmatocyte are distinct entities and that they respond specifically and separately under many of the variety of irritants or stimulants which come to the tissues, both naturally and experimentally introduced. On the other hand, if the evidence would justify the conclusion that two morphologically distinctive types do represent simply a difference in the metabolic state of activity of a common strain of cells, I should be more than happy to accept and support such a point of view.* Following continual collaboration, Sabin and Doan showed that bovine tubercle bacilli were able to stimulate the maturation of monocytes and were destroyed by clasmatocytes [58]. When in 1955 the Reticuloendothelial Society was created, Doan was nominated as the first president. In 1964, the Society launched the *Journal of the Reticuloendothelial Society*, which in 1984, became the *Journal of Leukocyte Biology*.

Still, the concept of RES gathering endothelial cells and mononuclear phagocytes within the same family was misleading, and in the early 20th century, links between the different cells were regularly considered. For example, in 1922, Florence Sabin, in her review about the origin of the blood cells [59], wrote that the endothelium gives rise to clasmatocytes: *A single cell of the endothelium of the inner row enlarges, protrudes into the lumen, develops the vacuoles that are characteristic of clasmatocytes, and may even engulf a red blood cell.* In 1934, in a paper revisiting the concept of RES, Roger Denio Baker (1902–1994), a professor of pathlogy at Duke, [60] asked the question: *Is the term phagocytic system not just as good a one to use as RES, since phagocytosis seems to be the most apparent feature of the RES?* Although he answered negatively, arguing that *polymorphonuclear neutrophils are also exceedingly phagocytic*, he pointed out that *just as phagocytosis is not a function specific for the RES, so is staining with vital dyes not specific.* He concluded that the name RES was improper but so commonly used that it was impossible to change. He was quite right, as even nowadays, the term RES is still used despite its ambiguous definition.

POST-METCHNIKOFF CHARACTERIZATION OF MACROPHAGES

The development of new technologies after World War II led to progress in studies of macrophages. In the early 1960s, the seminal works of George Mackaness (1922–2007) and James G. Hirsch (1922–1987) led to renewed efforts to study phagocytosis and macrophages. Studying the phagocytosis of *Staphylococcus aureus*, Mackaness showed that this bacterium was relatively resistant to phagocytosis by macrophages, unless a specific immune serum was provided. The greater efficiency observed with polymorphonuclear phagocytes led him to suggest that the antibacterial mechanisms of the two cell types were fundamentally different [61]. He also showed that inactivation of *Listeria monocytogenes* was better achieved in convalescent mice, thanks to the presence of resistant macrophages, as a result of immunological activation occurring during the primary infection [62]. This work led him to introduce the concept of macrophage activation. Electron microscopy observations of peritoneal macrophages from mice immunized with *L. monocytogenes* revealed structural differences with macrophages from normal mice [63]. Studying *Brucella abortus* or *M. tuberculosis* infection, in addition to Listeria, Mackaness [64] showed that the acquired resistance was dependent on the immunological reactivity of the host and specific antibodies against microbial antigens. Another major contribution of Mackaness was the discovery of cellular cooperation [65]. He demonstrated that the acquired resistance was dependent on the activation of macrophages through a product resulting from specific interaction between sensitized lymphoid cells and the microorganism. Hirsch also made important contributions to the understanding of the mechanisms linked to phagocytosis and opsonophagocytosis. In 1956, he characterized a bactericidal substance isolated from polymorphonuclear cells, he called, "phagocytin", reminiscent of the "microcytase" of Metchnikoff. Phagocytin, identified in rabbit neutrophils, was bactericidal on Gram-negative and -positive bacteria but was not bacteriolytic. Extracts from human and guinea pig neutrophils were less efficient, and the activity was absent from mouse and rat cells [66]. Hirsch also reported that group A streptococci exert an antiphagocytic effect through the action of the hyaluronic acid capsule and M protein. Factors present in human plasma but absent in rabbit plasma are able to counteract this antiphagocytic effect [67]. Thanks to microscopic and "cinemicrophotographic" studies, Hirsch further detailed the fusion between the granule membrane and the invaginated cell membrane overlying the ingested particle with discharge of granule content directed to the phagocytic vacuole [68, 69]. His experimental approach also allowed him to identify a similar mechanism with eosinophils [70].

Baker and his criticisms about the concept of RES [60] were paving the route to the further classification proposed by Ralph van Furth. If I may give a personal remembrance, I listened to van Furthis talk at the 9th International RES Congress, held in Davos, Switzerland, in 1982, organized by Ernst Sorkin (Davos) and Sigurd Normann (Gainesville, FL, USA). After his presentation, I was convinced that these cells were the most fascinating cells to study. Ten years prior, van Furth had proposed a new classification, explaining the lineage of macrophages [71]. With Zanvil Cohn (1926–1993), van Furth identified blood monocytes as the precursors for tissue macrophages and bone marrow as the source of monocytes [72]. In 1993, van Furth published a far more elaborated figure depicting the origin and kinetics of mononuclear phagocytes and the nature of the involved hematopoietic factors [73]. The laboratory of Cohn was *the* place to study macrophages [74]. Among many key discoveries was the role played by specific phagocyte granules, the lysosomes, which discharge their contents into the phagosome containing the ingested microorganism, leading to the digestion of the microbes

[75]. Siamon Gordon, another student of Cohn, characterized fusion cells or heterokaryons, which are obtained with macrophages and melanocytes or fibroblast cell lines after the action of viruses [76, 77]. Gordon also contributed to the characterization of the synthesis and secretion of lysozyme and plasminogen activator by activated macrophages [78, 79]. Cohn and his colleagues also showed that macrophages were releasing other factors such as metabolites of oxygen and arachidonic acid. Finally, it was in his laboratory where Ralph Steinman discovered the DCs in 1973 [80].

In addition to the mediators identified in Cohn's lab, macrophages were shortly identified as a source of cytokines, such as IL-1, previously known as "lymphocyte-activating factor" [81], and TNF [82]. Furthermore, in the 1960s and 1970s, macrophages were shown to be themselves under the influence of soluble factors that could modulate their functions. Indeed, the capacity of the antigen to prevent migration of peritoneal cells from tuberculin-sensitive guinea pigs discovered by David et al. [83] led Bloom and Bennett [84] to show that such activity was present in supernatants of purified peritoneal lymphocytes obtained from tuberculin-hypersensitive guinea pigs. The activity was a result of a macrophage MIF, which in 1966, was one of the first cytokines to be described. Most interestingly, it was the same MIF shown by Stanley Cohen as a product of virus-infected fibroblasts that led him to coin the word "cytokine" [85, 86]. In 1975, a "macrophage-arming factor" was described by two independent groups [87, 88], until the "macrophage-activating factor" was identified to be IFN-γ in 1983 [89]. During the 1970s and the 1980s, many other macrophage-specific discoveries were reported regarding surface Fc and complement receptor expression, their maturation in response to the M-CSF, their role in the phagocytosis of senescent neutrophils, their role in antigen presentation, or their capacity to be rendered tolerant to endotoxin.

Although it appeared rapidly obvious that macrophages present in different tissues were sister cells belonging to the same family and were not identical, it took more time to realize that even within a given compartment, subpopulations of cells could be described. Monocyte and macrophage heterogeneity has been widely reviewed [90–94]. Nowadays, macrophages are classified as M1, M2, and even as M2a, M2b, and M2c [95, 96]. To what degree this classification is correct and how enabling this classification helps to understand the great plasticity of these cells are left to the appreciation of the reader. In true life, their chance to be simultaneously under many signals is probably greater than to be only under one of them, which ends with a M1 or M2 phenotype. The influence of the microenvironment within the tissues or compartments where they reside probably plays a greater role than a classification made after in vitro experiments. For example, if the liver microenvironment of Kupffer cells, scarce in arginine, is mimicked in vitro by a culture with a far-lower level of arginine than in most culture media, Kupffer cells in response to LPS will produce PGE2 and very low levels of TNF, in total opposition with cells cultured in a regular medium [97]. Similarly, the microenvironment within the lungs, rich in GM-CSF [98], is responsible for the inability of alveolar macrophages to develop endotoxin tolerance in contrast to any other mononuclear phagocytes [99].

A WORD ABOUT LYMPHOCYTES

I would not like to leave the reader with the idea that RES cells are the source of antibodies. Lymphocytes were well-identified since the work of Schulze [34], Ehrlich [35], and Maximow [3], yet no key role was assigned, and wrong hypotheses were suggested [3]. One owes Arthur Silverstein [100] praise, after recalling to the immunologist community that James B. Murphy (1884–1950), an experimental pathologist working at the Rockefeller Institute, was the very first one to prove that the lymphocytes were active participants in infection and cancer. In 1914 and 1915, he demonstrated their role in the defensive mechanism against tuberculosis [101] and in the resistance to the growth of inoculated tumor cells [102].

By 1931 [103], efforts to determine the tissues in which antibodies originated were under way. In 1935, LNs [104] and spleen [105] were recognized correctly as the main organs where antibody production occurred. Although plasma cells were known, in 1931, Franklin R. Miller wrote a paper clearly demonstrating that plasma cells were not derived from lymphocytes [103]. In 1943, Mogens Bjørneboe (1910–2006) specialized in immunization from the State Serum Institute of Copenhagen, and Harald Gormsen (1909–1996), professor at the Forensic Institute in Copenhagen University, specialized in pathological anatomy, considered that their findings were contrary to lymphocytic genesis of the plasma cells [106]. Nevertheless, they were the first to publish a close correlation between antibody production and plasma cell proliferation, especially in the spleen and the liver, despite the admission that most investigators thought antibody formation was ascribed to the RES [106]. They further confirmed that plasma cells were antibody producers, which could be found in nonlymphoid tissues such as adipose tissue or renal sinus [107]. They stated: *It is however a possibility that both lymphocytes and plasma cells produce antibodies and it may be mentioned that according to many investigators lymphocytes and plasma cells are closely related, though several recent investigations seem to indicate that plasma cells at least in spleen, lymph nodes, and bone marrow descend directly from reticulum cells.* In 1947, Astrid Fagraeus (1913–1997) [108] performed the first in vitro antibody production from small fragments of rabbit spleen, LN, thymus, bone marrow, and liver. She established that the antibody formation capacity was particularly assigned to the spleen red pulp rich in plasma cells, whereas other organs rich in RES were significantly inferior in their capacity for antibody production. The following year, she further extended her observation and concluded: *The conclusion is drawn that antibodies under the conditions of the experiments are formed by cells of the RES, passing through a chain of development, the final link of which is the mature plasmocytes* [109]. Such a statement illustrates the strength and magnitude of the RES concept, even for those who appropriately identified the plasma cells as the source of antibodies.

These pioneering works were further confirmed by Harris et al. [110], who prepared lymphocyte extracts from immunized animals and in 1945, showed that the antibody titer in the extract was higher than in lymph plasma. They concluded their nice work by writing, *lymphocytes are instrumental in the formation of antibodies.* Albert H. Coons (1912–1978) et al. [111] in 1941

offered the immunologist community a major technical revolution with the development of fluorescent antibodies. John Marrack had previously, successfully modified antibodies with tetrazotized benzidine to produce colored antibodies, but this was inherently insensitive. The immunostaining technique allowed Coons to detect antigen in tissues and to show that plasma cells were indeed containing antibodies; in 1955, Coons et al. [112] showed that antibodies against the antigen were present in groups of plasma cells in the red pulp of the spleen, the medullary areas of LNs, the submucosa of the ileum, and the portal-connective tissues of the liver. He and coworkers [113] also found that the secondary response was accompanied by a far larger number of stained cells. Another important technical advance was proposed in 1963 by Jerne and Nordin [114], who describe the possibility to numerate "plaque-forming cells" in agar, i.e., the number of lymphoid cells releasing antibodies isolated from a hematopoietic organ. The method was further improved by Mishell and Dutton [115], who demonstrated the first immune response in vitro. It was not shown that B lymphocytes are the precursors of antibody-producing cells until 1970, when B lymphocytes were found to express Ig molecules on their cell surface [116, 117].

Of course, one cannot end the story on the beginning of the lymphocyte saga without mentioning the work of Sir James L. Gowans (1924–). In the mid-1950s/early 1960s, he demonstrated the circulation of lymphocytes within the blood and the lymph compartments [118, 119] and with others, made the link between these cells and the antibody formation [120], which showed their involvement in immune tolerance [121]. He brightly wrote: *The demonstration that a population of small lymphocyte can carry the property of immunological tolerance is strong evidence that they are involved in the inductive phase of antibody formation. The simplest view would be that small lymphocytes interact with antigen (or with antigen which has been 'processed' by reticulo-endothelial phagocytes), become fixed in lymphoid tissue and give rise to a dividing cell line of the kind identified by Nossal and Mäkela* [i.e., a plasma cell]. *The cell line generates the cells, which eventually synthesize antibody. Although there is strong morphological evidence that small lymphocytes are the ultimate precursors of antibody forming cells, it must be emphasized that it has not yet been unequivocally demonstrated.* Gowans was at the birth of a tremendous amount of discoveries, offering the basis of understanding of the natural history of lymphocytes [122].

LET METCHNIKOFF CONCLUDE

Progress in science is made with key discoveries, correct analyses, and disputes against the scientific community, which tendentially, is very conservative and reluctant to change when dogmas are rooted in their minds. This is true for our great Metchnikoff, who correctly analyzed the observation of phagocytosis, which started with a rose thorn and gave rise to the whole concept of innate and cellular immunity; fought with vigor to convince his opponents; and also believed that senile decay was an effect of intestinal putrefaction. He made great efforts to convince that humoral and cellular immune responses were not excluding each other. Undoubtedly, he can be considered as the father of both cellular and innate immunity. Even if in the 1970s, the rebirth of cellular immunity was rather associated with the biology of lymphocytes, the rebirth of innate immunity with the discovery of TLRs by the end of the 20th century offered a new chance for macrophages and phagocytosis to become a subject of regained interest. Elie Metchnikoff beautifully understood and defined the role of monocytes/macrophages and neutrophils during inflammation and innate immunity. In an address delivered at the Institut Pasteur on December 29, 1890, he said: *The broad fact that the invasion of the organism by microbes most often induces, on the one hand, an inflammatory reaction with its associated emigration of leucocytes, and that, on the other hand, the phagocytes are capable of including and destroying the invaders, leads us to admit that the afflux of phagocytes to the invaded region and their bactericidal properties are mechanisms which serve to ward off bacterial attack and to maintain the integrity of the organism.* What he ascertained in the 19th century remains true in the 21st century.

ACKNOWLEDGMENTS

I thank very warmly Mrs. Annick Perrot, past-curator of the Institut Pasteur Museum, who provided me with information, books, and pictures (including those in this text) and the team of the library of the Institut Pasteur for their kindness, availability, and help. I thank Dr. Brian Mozeleski for English editing.

REFERENCES

1. Neill, D. R., Wong, S. H., Bellosi, A., Flynn, R. J., Daly, M., Langford, T. K., Bucks, C., Kane, C. M., Fallon, P. G., Pannell, R., Jolin, H. E., McKenzie, A. N. (2010) Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* **464,** 1367–1370.
2. Maximow, A. (1902) Experimentelle untersuchungen ber die entzündliche Neubildung von Bindegewebe. In *Beiträge zur Pathologischen Anatomie und zur Allgemeinen Pathologie* (E. Ziegler, ed.), Suppl. Heft 5, Jena, Germany, G. Fischer.
3. Maximow, A. (1909) Der Lymphozyt als gemeinsame Stammzelle der verschiedenen Blutelemente in der embryonalen Entwicklung und im postfetalen Leben der Säugetiere. *Folia Haematol. (Frankf.)* **8,** 125–134.
4. Langerhans, P. (1868) Uber die nerven der menschliden haut. *Pathol. Anat.* **44,** 325–337.
5. Ginhoux, F., Merad, M. (2010) Ontogeny and homeostasis of Langerhans cells. *Immunol. Cell Biol.* **88,** 387–392.
6. Rietschel, E. T., Cavaillon, J-M. (2003) Richard Pfeiffer and Alexandre Besredka: creator of the concept of endotoxin and anti-endotoxin. *Microbes Infect.* **5,** 1407–1414.
7. Silverstein, A. M. (2003) Cellular versus humoral immunology: a century-long dispute. *Nat. Immunol.* **4,** 425–428.
8. Silverstein, A. M. (2009) *A History of Immunology*, 2nd Ed., Burlington, MA, USA, Academic.
9. Metchnikoff, O. (1920) *La vie d'Elie Metchnikoff*, Paris, Hachette.
10. Hirsch, J. G. (1959) Immunity to infectious diseases: review of some concepts of Metchnikoff. *Bacteriol. Rev.* **23,** 48–60.
11. Vaughan, R. B. (1965) The romantic rationalist. A study of Elie Metchnikoff. *Med. Hist.* **9,** 201–215.
12. Tauber, A. I. (2003) Metchnikoff and the phagocytosis theory. *Nat. Rev. Mol. Cell Biol.* **4,** 897–901.
13. Tauber, A. I., Chernyak, L. (1991) *Metchnikoff and the Origins of Immunology. From Metaphor to Theory.* Oxford, UK, Oxford University Press.
14. Silverstein, A. M. (2003) Darwinism and immunology: from Metchnikoff to Burnet. *Nat. Immunol.* **4,** 3–6.
15. Kaufmann, S. H. E. (2008) Immunology's fundation: the 100 year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff. *Nat. Immunol.* **9,** 705–712.
16. Metchnikoff, E. (1883) Untersuchungen uber die intracellulare Verdauung bei Wirbellosen Thieren. *Arbeit Zoologischen Instituten, Universitat Wien* **5,** 141–168.
17. Ambrose, C. T. (2007) Osler and the black corpuscles: profiles of three early students of phagocytosis. *J. Med. Biogr.* **15** (Suppl. 1), 39–45.
18. Metchnikoff, E. (1884) Uber eine sprosspilzkrankheit der daphnien; Beitrag zur über den kamp der phagocyten gengen krankheitserreger. *Arch. Pathol. Anat. Physiol. Klin. Med.* **96,** 177–195.
19. Metchnikoff, E. (1901) L'immunité dans les maladies infectieuses. Paris, Masson.
20. Ambrose, C. T. (2006) The Osler slide, a demonstration of phagocytosis from 1876 reports of phagocytosis before Metchnikoff's 1880 paper. *Cell. Immunol.* **240,** 1–4.

21. Roux, E., Metchnikoff, E. (1914) L'Oeuvre de P. Ehrlich. *Berliner Klinische Wochenschrift* **71,** 529–531.
22. Wright, A. E., Douglas, S. R. (1903) An experimental investigation on the role of the blood fluids in connection with phagocytosis. *Proc. R. Soc. Lond.* **72,** 357–370.
23. Silverstein, A. M. (1991) The dynamics of conceptual change in twentieth century immunology. *Cell. Immunol.* **132,** 515–531.
24. Awrorow, P. P., Timofejewskij, A. D. (1914) Kultivierungsversuche von leukämischem Blute. *Virchows Arch.* **216,** 184–214.
25. Carrel, A., Ebeling, A. H. (1922) Pure cultures of large mononuclear leucocytes. *J. Exp. Med.* **36,** 365–377.
26. Cunningham, R. S., Sabin, F. R., Doan, C. A. (1924) The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit. *Proc. Soc. Exp. Biol. Med.* **21,** 326.
27. Sabin, F. R., Doan, C. A., Cunningham, R. S. (1924) The separation of the phagocytic cells of the peritoneal exudate into two distinct types. *Proc. Soc. Exp. Biol. Med.* **21,** 330.
28. Lewis, M. R. (1925) The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. *Am. J. Pathol.* **1,** 91–100.
29. Clark, E. R., Clark, E. L. (1930) Relation of monocytes of the blood to the tissue macrophages. *Am. J. Anat.* **46,** 149–185.
30. Ebert, R. H., Florey, H. W. (1939) The extravascular development of the monocyte observed in vivo. *Br. J. Exp. Pathol.* **20,** 342–356.
31. Arnold, J. (1873) Ueber diapedesis. *Virchows Arch.* **58,** 203–230.
32. Waller, A. (1846) Microscopic examination of some of the principal tissues of the animal frame, as observed in the tongue of the living frog, toad. *Edinburgh and Dublin Philosophical Magazine (London),* 271–287.
33. Cohnheim, J. (1867) Ueber entzundung und eiterung. *Path. Anat. Physiol. Klin. Med.* **40,** 1–79.
34. Schultze, J. S. (1865) Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes. *Arch. Mikrosc. Anat.* **1,** 1–42.
35. Ehrlich, P. (1877) Beiträge zur kenntnis der anilinfärbungen und ihrer verwendung in der mikroskopischen Technik. *Arch. Mikrosc. Anat.* **13,** 263–277.
36. Benditt, E. P., Bader, S., Lam, K. B. (1955) Studies of the mechanism of acute vascular reactions to injury. I. The relationship of mast cells and histamine to the production of edema by ovomucoid in rats. *AMA Arch. Pathol.* **60,** 104–115.
37. Mota, I. (1994) The discovery of the relationship between mast cells, histamine and IgE. *Immunol. Today* **15,** 242–245.
38. Smith, R. O., Wood Jr., W. B. (1949) Cellular mechanisms of antibacterial defense in lymph nodes; pathogenesis of acute bacterial lymphadenitis. *J. Exp. Med.* **90,** 555–566.
39. Ramon, G. (1926) Procédés pour accroitre la produciton des antitoxines. *Ann. Inst. Pasteur (Paris)* **40,** 1–10.
40. Janeway Jr., C. A. (1989) Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* **54,** 1–13.
41. Ramon, G., Richou, R., Lemétayer, E. (1935) Sur l'injection à l'animal d'expériences de la toxine diphtérique en mélange avec différentes substances, en particulier avec la lanoline. *C. R. Seances Soc. Biol. Fil.* **118,** 108–111.
42. Freund, J., Casals, J., Hosmer, E. P. (1937) Sensitization and antibody formation after injection of tubercle bacilli and paraffin oil. *Proc. Soc. Exp. Biol. Med.* **37,** 509–513.
43. Audibert, F., Chedid, L., Lefrancier, P., Choay, J., Lederer, E. (1977) Relationship between chemical structure and adjuvant activity of some stynthetic analogues of N-acetyl-muramyl-l-alanyl-d-isoglutamine (MDP). *Ann. Immunol. (Paris)* **128C,** 653–661.
44. Aschoff, L. (1922) Das retikulo-endotheliale system und seine Beziehungen zur Gallenfarbstoffbildung. *Munch. Med. Wochenschr.* **69,** 1352–1356.
45. Aschoff, L (1924) Das reticulo-endotheliale system. *Ergebnisse der Inneren Medizin und Kinderheilkunde (Berlin)* **26,** 1–118.
46. Kiyono, K. (1914) Zur Frage der histiozytären Blutzellen. *Folia Haematol. (Frankf.)* **18,** 149–170.
47. Aschoff, L., Kiyono, K. (1913) Zur Frage der grossen Mononucleären. *Folia Haem* **15,** 385–390.
48. Russell, D. S. (1929) Intravital staining of microglia with trypan blue. *Am. J. Pathol.* **5,** 451–458.
49. Gay, F. P., Clark, A. R. (1924) The reticutoendothelial system in relation to antibody formation. *JAMA* **88,** 1296–1297.
50. Jungeblut, C. W., Berlot, J. A. (1926) The role of the reticulo-endothelial system in immunity. I. The role of the reticulo-endothelial system in the production of diphtheria antitoxin. *J. Exp. Med.* **43,** 613–622.
51. Roberts, E. F. (1929) The reticulo-endothelial system and antibody production. I. The appearrance of antibody in the circulation. *J. Immunol.* **16,** 137–149.
52. Cannon, P., Baer, R., Sullivan, T. L., Webster, J. R. (1929) The influence of blockade of the reticulo-endothelial system in the formation of antibodies. *J. Immunol.* **17,** 441–463.
53. Tuft, L. (1934) The effect of reticulo-endothelial cell blockade upon antibody formation in rabbits. *J. Immunol.* **27,** 63–80.
54. Siegmund, H. (1922) Speicherung Dutch reticuloendothelien, cellulite Reaktion und Immunität. *Klin. Wehnschr.* **1,** 2566–2567.
55. Elvidge, A. R. (1933) The reticulo-endothelial system and the source of opsonin. *J. Immunol.* **24,** 31–64.
56. Sabin, F. R. (1939) Cellular reaction to a dye-protein with a concept of the mechanism of antibody formation. *J. Exp. Med.* **70,** 67–82.
57. Sabin, F. R. (1916) The method of growth of the lymphatic system. *Science* **44,** 145–158.
58. Sabin, F. R., Doan, C. A. (1927) The relationship of monocytes and clasmatocytes to early infection in rabbits with bovine tubercle bacilli. *J. Exp. Med.* **46,** 627–644.
59. Sabin, F. R. (1922) On the origin of the cells of the blood. *Physiol. Rev.* **2,** 38–69.
60. Baker, R. D. (1934) The reticuloendothelial system: a reconsideration. *South. Med. J.* **27,** 913–917.
61. MacKaness, G. B. (1960) The phagocytosis and inactivation of staphylococci by macrophages of normal rabbits. *J. Exp. Med.* **112,** 35–53.
62. MacKaness, G. B. (1962) Cellular resistance to infection. *J. Exp. Med.* **116,** 381–406.
63. North, R. J., MacKaness, G. B. (1963) Electron microscopical observations on the peritoneal macrophages of normal mice and mice immunised with *Listeria monocytogenes.* II. Structure of macrophages from immune mice and early cytoplasmic response to the presence of ingested bacteria. *Br. J. Exp. Pathol.* **44,** 608–611.
64. MacKaness, G. B. (1964) The immunological basis of acquired cellular resistance. *J. Exp. Med.* **120,** 105–120.
65. MacKaness, G. B. (1969) The influence of immunologically committed lymphoid cells on macrophage activity in vivo. *J. Exp. Med.* **129,** 973–992.
66. Hirsch, J. G. (1956) Phagocytin: a bactericidal substance from polymorphonuclear leucocytes. *J. Exp. Med.* **103,** 589–611.
67. Hirsch, J. G., Church, A. B. (1960) Studies of phagocytosis of group A streptococci by polymorphonuclear leucocytes in vitro. *J. Exp. Med.* **111,** 309–322.
68. Hirsch, J. G., Cohn, Z. A. (1960) Degranulation of polymorphonuclear leucocytes following phagocytosis of microorganisms. *J. Exp. Med.* **112,** 1005–1014.
69. Hirsch, J. G. (1962) Cinemicrophotographic observations on granule lysis in polymorphonuclear leucocytes during phagocytosis. *J. Exp. Med.* **116,** 827–834.
70. Archer, G. T., Hirsch, J. G. (1963) Motion picture studies on degranulation of horse eosinophils during phagocytosis. *J. Exp. Med.* **118,** 287–294.
71. Van Furth, R., Cohn, Z. A., Hirsch, J. G., Humphrey, J. H., Spector, W. G., Langevoort, H. L. (1972) The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull. World Health Organ.* **46,** 845–852.
72. Van Furth, R., Cohn, Z. A. (1968) The origin and kinetics of mononuclear phagocytes. *J. Exp. Med.* **128,** 415–435.
73. Van Furth, R. (1993) Cell biology of mononuclear phagocytes. In *Hematopoietic Growth Factors and Mononuclear Phagocytes* (R. Van Furth, ed.), Basel, Switzerland, Karger, 1–9.
74. Steinman, R. M., Moberg, C. L. (1994) Zanvil Alexander Cohn, 1926–1993. *J. Exp. Med.* **179,** 1-30.
75. Cohn, Z. A., Wiener, E. (1963) The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion. *J. Exp. Med.* **118,** 1009–1020.
76. Gordon, S., Cohn, Z. (1970) Macrophage-melanocyte heterokaryons. I. Preparation and properties. *J. Exp. Med.* **131,** 981–1003.
77. Gordon, S., Ripps, C. S., Cohn, Z. (1971) The preparation and properties of macrophage-L cell hybrids. *J. Exp. Med.* **134,** 1187–1200.
78. Gordon, S., Todd, J., Cohn, Z. A. (1974) In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. *J. Exp. Med.* **139,** 1228–1248.
79. Gordon, S., Unkeless, J. C., Cohn, Z. A. (1974) Induction of macrophage plasminogen activator by endotoxin stimulation and phagocytosis: evidence for a two-stage process. *J. Exp. Med.* **140,** 995–1010.
80. Steinman, R. M., Cohn, Z. A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* **137,** 1142–1162.
81. Gery, I., Waksman, B. H. (1972) Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s). *J. Exp. Med.* **136,** 143–155.
82. Männel, D. N., Moore, R. N., Mergenhagen, S. E. (1980) Macrophages as a source of tumoricidal activity (tumor necrosis factor). *Infect. Immun.* **30,** 523–530.
83. David, J. R., Al-Askari, S., Lawrence, H. S., Thomas, L. (1964) Delayed hypersensitivity in vitro. I. The specificity of inhibition of cell migration by antigens. *J. Immunol.* **93,** 264–273.
84. Bloom, B. R., Bennett, B. (1966) Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* **153,** 80–82.
85. Cohen, S. (2004) Cytokine: more than a new word, a new concept proposed by Stanley Cohen thirty years ago. *Cytokine* **28,** 242–247.
86. Cohen, S., Bigazzi, P. E., Yoshida, T. (1974) Commentary. Similarities of T cell function in cell-mediated immunity and antibody production. *Cell. Immunol.* **12,** 150–159.
87. Piessens, W. F., Churchill, W. H., David, J. R. (1975) Macrophages activated in vitro with lymphocyte mediators kill neoplastic but not normal cells. *J. Immunol.* **114,** 293–299.
88. Dimitriu, A., Dy, M., Thomson, N., Hamburger, J. (1975) Macrophage cytotoxicity in the mouse immune response against a skin allograft. *J. Immunol.* **114,** 195–199.
89. Nathan, C. F., Murray, H. W., Wiebe, M. E., Rubin, B. Y. (1983) Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* **158,** 670–689.
90. Stout, R. D., Suttles, J. (2004) Functional plasticity of macrophages: reversible adaptation to changing microenvironments. *J. Leukoc. Biol.* **76,** 509–513.
91. Gordon, S., Taylor, P. R. (2005) Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* **5,** 953–964.

92. Strauss-Ayali, D., Conrad, S. M., Mosser, D. M. (2007) Monocyte subpopulations and their differentiation patterns during infection. *J. Leukoc. Biol.* **82,** 244–252.
93. Hume, D. A. (2008) Differentiation and heterogeneity in the mononuclear phagocyte system. *Mucosal Immunol.* **1,** 432–441.
94. Mosser, D. M., Edwards, J. P. (2008) Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* **8,** 958–969.
95. Gordon, S. (2003) Alternative activation of macrophages. *Nat. Rev. Immunol.* **3,** 23–35.
96. Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M. (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **25,** 677–686.
97. Callery, M. P., Mangino, M. J., Flye, M. W. (1991) A biologic basis for limited Kupffer cell reactivity to portal-derived endotoxin. *Surgery* **110,** 221–230.
98. Dranoff, G., Crawford, A. D., Sadelain, M., Ream, B., Rashid, A., Bronson, R. T., Dickersin, G. R., Bachurski, C. J., Mark, E. L., Whitsett, J. A., et al. (1994) Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* **264,** 713–716.
99. Fitting, C., Dhawan, S., Cavaillon, J-M. (2004) Compartmentalization of tolerance to endotoxin. *J. Infect. Dis.* **189,** 1295–1303.
100. Silverstein, A. M. (2001) The lymphocyte in immunology: from James B. Murphy to James L. Gowans. *Nat. Immunol.* **2,** 569–571.
101. Murphy, J. B., Ellis, A. W. (1914) Experiments on the role of lymphoid tissue in the resistance to experimental tuberculosis in mice. *J. Exp. Med.* **20,** 397–403.
102. Murphy, J. B., Morton, J. J. (1915) The lymphocyte in natural and induced resistance to transplanted cancer: II. Studies in lymphoid activity. *J. Exp. Med.* **22,** 204–211.
103. Miller, F. R. (1931) The induced development and histogenesis of plasma cells. *J. Exp. Med.* **54,** 333–347.
104. McMaster, P. D., Hudack, S. S. (1935) The formation of agglutinins within lymph nodes. *J. Exp. Med.* **61,** 783–805.
105. Rich, A. R. (1935) Acute splenic tumor produced by non-bacterial antigens. *Proc. Soc. Exp. Biol. Med.* **32,** 1349–1351.
106. Bjørneboe, M., Gormsen, H. (1943) Experimental studies on the role of plasma cells as antibody producers. *Acta Pathol. Microbiol. Scand.* **20,** 649–692.
107. Bjørneboe, M., Gormsen, H., Lundquist, F. (1947) Further experimental studies on the role of the plasma cells as antibody producers. *J. Immunol.* **55,** 121–129.
108. Fagraeus, A. (1947) Plasma cellular reaction and its relation to the formation of antibodies in vitro. *Nature* **159,** 499.
109. Fagraeus, A. (1948) The plasma cellular reaction and its relation to the formation of antibodies in vitro. *J. Immunol.* **58,** 1–13.
110. Harris, T. N., Grimm, E., Mertens, E., Ehrich, W. E. (1945) The role of the lymphocyte in antibody formation. *J. Exp. Med.* **81,** 73–83.
111. Coons, A. H., Creech, H. J., Jones, R. N. (1941) Immunological properties of an antibody containing a fluorescent group. *Proc. Soc. Exp. Biol. Med.* **47,** 200–202.
112. Coons, A. H., Leduc, E. H., Connolly, J. M. (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exp. Med.* **102,** 49–60.
113. Leduc, E. H., Coons, A. H., Connolly, J. M. (1955) Studies on antibody production. II. The primary and secondary responses in the popliteal lymph node of the rabbit. *J. Exp. Med.* **102,** 61–72.
114. Jerne, N. K., Nordin, A. A. (1963) Plaque formation in agar by single antibody-producing cells. *Science* **140,** 405.
115. Mishell, R. I., Dutton, R. W. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126,** 423–442.
116. Pernis, B., Forni, L., Amante, L. (1970) Immunoglobulin spots on the surface of rabbit lymphocytes. *J. Exp. Med.* **132,** 1001–1018.
117. Raff, M. C., Sternberg, M., Taylor, R. B. (1970) Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature* **225,** 553–554.
118. Gowans, J. L. (1957) The effect of the continuous re-infusion of lymph and lymphocytes on the output of lymphocytes from the thoracic duct of unanaesthetized rats. *Br. J. Exp. Pathol.* **38,** 67–78.
119. Gowans, J. L. (1959) The recirculation of lymphocytes from blood to lymph in the rat. *J. Physiol.* **146,** 54–69.
120. McGregor, D. D., Gowans, J. L. (1963) The antibody response of rats depleted of lymphocytes by chronic drainage from the thoracic duct. *J. Exp. Med.* **117,** 303–320.
121. Gowans, J. L., McGregor, D. D., Cowen, D. M. (1962) Initiation of immune responses by small lymphocytes. *Nature* **196,** 651–655.
122. Gowans, J. L. (1996) The lymphocyte—a disgraceful gap in medical knowledge. *Immunol. Today* **17,** 288–291.

KEY WORDS:
monocytes/macrophages · granulocytes · lymphocytes · reticuloendothelial system · neutrophils