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The Phagocyte, Metchnikoff, and the Foundation of Immunology

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ABSTRACT Since the ability of some cells to engulf particulate material was observed before Metchnikoff, he did not "discover" phagocytosis, as is sometimes mentioned in textbooks. Rather, he assigned to particle internalization the role of defending the host against noxious stimuli, which represented a new function relative to the previously recognized task of intracellular digestion. With this proposal, Metchnikoff built the conceptual framework within which immunity could finally be seen as an active host function triggered by noxious stimuli. In this sense, Metchnikoff can be rightly regarded as the father of all immunological sciences and not only of innate immunity or myeloid cell biology. Moreover, the recognition properties of his phagocyte fit surprisingly well with recent discoveries and modern models of immune sensing. For example, rather than assigning to immune recognition exclusively the function of eliminating nonself components (as others did after him), Metchnikoff viewed phagocytes as homeostatic agents capable of monitoring the internal environment and promoting tissue remodeling, thereby continuously defining the identity of the organism. No doubt, Metchnikoff's life and creativity can provide, still today, a rich source of inspiration.

INTRODUCTION

The life and work of Elie Metchnikoff are a rich source of inspiration to anybody interested in the biology and pathophysiology of myeloid cells. He made the fundamental discoveries that subsequently shaped the development of the field and that represent, still today, the basis of our knowledge. First and foremost, he defined these cells by their function (i.e., "phagocytosis"), a definition that suits better than any other designation the nature of these cells, including perhaps the term "myeloid" itself. Metchnikoff described for the first time a number of crucial features of phagocytic cells, including (i) phagocyte-mediated host protection; (ii) active internalization of live, in addition to dead, organisms; (iii) uptake of senescent or damaged host cells; (iv) destruction of internalized particles; (v) bacterial killing by virtue of enzymes ("cytases"); (vi) vacuolar acidification; (vii) distinction between microphages (polymorphonuclear leukocytes) and macrophages; (viii) inflammatory recruitment of phagocytes; (ix) chemotaxis; and (x) diapedesis. For these reasons, Metchnikoff is unanimously considered the founding father of the field of phagocyte biology.

However, since the ability of some cell types to actively engulf particulate material was observed in both invertebrates and vertebrates before Metchnikoff, he did not "discover" phagocytosis, as is sometimes mentioned in textbooks. His contribution to biology is far greater and extends beyond the field of phagocyte biology. By assigning to particle internalization the function of defending the host against noxious stimuli (this represented a new function relative to the previously recognized task of intracellular digestion), Metchnikoff envisioned for the first time the presence of an active body defense system and created the theoretical framework that led to the birth of immunology, an entirely new science. In this sense, Metchnikoff can be rightly viewed as the father of all immunological sciences and not only of innate immunity, cellular immunology, or phagocyte biology. Indeed, before Metchnikoff, immune

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phenomena were explained by "nonimmunological" mechanisms (1). For example, Louis Pasteur believed that the resistance to infection he had observed in animals vaccinated with attenuated microbes was linked to the consumption by the latter of specific growth factors required for the reproduction of bacteria inside the body. Pasteur himself soon realized the fallacy of his interpretation when he observed growth of pathogens in the blood of immune animals (2). Metchnikoff's concept of immunity as an active body function derived directly from his embryological studies. Indeed, he brought a fresh biological perspective to the field of medical pathology, which made it possible to assign to inflammatory phenomena a new functional significance. In addition, the recognition properties of Metchnikoff's phagocyte fit surprisingly well with recent discoveries and modern models of the "immune self" (3–5). For example, rather than assigning to the immune system exclusively the function of eliminating nonself components (as others did for many years after him), Metchnikoff also endowed his phagocyte with the ability to check for the presence of unwanted or damaged endogenous components, i.e., to detect the "altered" self. We will review here the fascinating story of how myeloid cells and their evolutionary ancestors inspired Metchnikoff's theoretical achievements. The historical and philosophical aspects of his discoveries have been the subject of extensive research by Alfred Tauber and coworkers (6, 7), to whom we owe some of the concepts presented here. In addition, Metchnikoff's life and discoveries have been the subject of several excellent accounts (8–16).

FATE OF THE PHAGOCYTE THEORY

Metchnikoff became a famous scientist in the 1890s after his theory had struck popular imagination by depicting armies of phagocytes moving against infectious agents to destroy them and save the body from deadly diseases. His phagocyte theory provided a vivid representation of immunity at work and a simple explanation for the resistance of some individuals to contagious infections despite exposure during epidemics. Moreover, the powerful and eccentric personality of Metchnikoff, who was heavily influenced by Mitteleuropean 19th-century romanticism, has lent itself to a number of picturesque portraits. He was depicted sometimes as a "mad scientist" battling relentlessly to defend his theory from the attacks of his detractors (17). Indeed, his phagocyte or "cellular" theory (presented in 1883) came immediately under ferocious criticism even before alternative explanations were offered. This occurred only in the late 1880s with the formulation of the humoral theory of immunity, according to which soluble factors present in serum and secretions (later identified as antibodies)—and not cells—were exclusively responsible for immunity. Great German scientists, such as Emil von Behring, Richard Pfeiffer, and Paul Ehrlich, championed the humoral theory, while French immunologists took sides with Metchnikoff, who was working at the Institut Pasteur at the time. The debate took belligerent tones and was influenced by the heated atmosphere of nationalism that followed the Franco-Prussian War (1870–71). Finally, after Almroth Wright showed that humoral factors (i.e., opsonins) could increase the susceptibility of bacteria to phagocytosis (18), the 1908 Nobel Committee declared a sort of ceasefire by awarding the Nobel Prize jointly to Metchnikoff and Ehrlich. Clearly, cellular and humoral theories were not mutually exclusive. Important observations were made in the 1920s and '30s showing that blockade of the phagocyte system (which had been renamed the "reticulo-endothelial system") by India ink or quartz particles reduced antibody formation (19–24). Despite this, the popularity of the cellular (or phagocyte) theory steadily declined during the 20th century as a result of difficulties in demonstrating immune specificity in the action of phagocytes. At the same time, biochemistry was taking the center stage in biology, and immunochemistry was increasingly successful in demonstrating the structural basis of antigen-antibody interactions. The success of "cellular immunology" in the '70s was mostly associated with excitement about the role of lymphocytes, and Metchnikoff's contributions continued to be relegated to history libraries. Only quite recently, after the rediscovery of the centrality of innate immunity in human health and disease and throughout evolution, was Metchnikoff's phagocyte vindicated as the initiator and orchestrator of immune responses. Yet Metchnikoff's image still suffers today from the same stereotype that originally made him famous as the vociferous protagonist of the humoral/cellular controversy. Not many realize that he was the first to bring into the context of medical sciences an evolutionary perspective that has had a major impact lasting until today. As a comparative embryologist, Metchnikoff had a different scientific background from that of Ehrlich and von Behring, who were physicians, or from that of other medically oriented, contemporary microbiologists and pathologists. Both biology and immunology owe a lot to Metchnikoff's original reinterpretation of Darwinian evolutionist principles: in recognizing the limits of a purely morphological approach, he focused on how a given activity (specifically, particle internalization) acquires new functional meanings through phylogenesis. He concluded that, in higher animals, particle internalization had lost its evolutionarily ancient function (nutrition) to fulfill the new tasks of tissue remodeling and elimination of potentially noxious agents. Studying a conserved activity or structure (such as a gene) from the perspective of its functional adaptations during evolution would seem today an obvious strategy. However, few realize that Metchnikoff was the first to fully demonstrate the power of this approach. In addition, as an embryologist, he was quick to extrapolate his conclusions from phylogeny to ontogeny. For example, he noted that the same activity (phagocytosis) was used for different purposes (respectively, resorption of the tadpole tail and antimicrobial defenses) during metamorphosis and adult life (25). Metchnikoff would have been delighted, but by no means surprised, to learn about the different functions of the *Drosophila* Toll pathway in the embryo (establishment of dorsoventral patterning [26]) and in the adult fly (host defense against infection [27]). To him, Toll and Toll-like receptor activities would have represented the perfect articulation of the basic function of his phagocyte, namely, to shape the identity of life during individual development and to preserve it later.

METCHNIKOFF'S PERSONALITY

In the case of Metchnikoff, it is difficult to understand the nature of his discoveries without referring to his life and personality. For example, pessimistic or optimistic feelings about his personal life heavily influenced his scientific ideas. During the first half of his existence, he was often affected by misanthropy and depression and saw in living creatures—including himself—contrasting and disharmonious features that worried him. This was a different attitude from that of many naturalists of his time, who were inclined to admire the functional perfection of the organisms they were studying. The awareness of disharmony in nature generated a strong need in Metchnikoff to find some counterbalancing force that could be leveraged by science to ultimately solve the problems affecting mankind. Once he was convinced, after 1881, that he was on the right track toward defining such a harmonizing principle (which he later identified in the phagocyte), he became more optimistic and found new motivations for existence. Science was a religion to him and totally shaped his life. In this attitude he was not alone, and a similar romantic orientation can also be found in many of his contemporaries and in the scientists who preceded him, including Pasteur and his coworkers. Metchnikoff showed, from adolescence, a compulsory need to learn all he could about biology and nature using direct observation, books, visits to scientists, and attendance at scientific meetings throughout Europe. He continuously attempted to integrate the enormous body of knowledge he was acquiring into a unifying theory, and failure to find a satisfactory explanation for scientific evidence invariably threw him into a state of anxiety and despair. The need to resolve this state of unhappiness, which originated from frustration with his work, was the driving force behind the development of the phagocytosis theory, in which he finally found intellectual satisfaction. Since this achievement was his raison d'être, he always vigorously defended the theory against any criticism. It is not surprising that he wrote in 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" (15).

Yet he managed to maintain a friendly and respectful attitude toward his critics, as shown by his letters to Ehrlich, who visited him at Institut Pasteur in 1903 (15). Satisfaction with his scientific work was a necessary, but insufficient, condition for Metchnikoff's happiness in life, and a second "requirement" had to be met, namely the love and dedication of a feminine figure, which he found first in his mother and then in his wives. His two suicide attempts were driven by the loss of his first wife and by a serious disease of his second. His second wife, Olga, gave him happiness and all the support he needed to carry on his work until the end of his life. Her biography of Metchnikoff is a pleasant read that vividly and faithfully pictures his complex personality and the mental processes behind his discoveries (28).

Elie Metchnikoff (also spelled Ilya Mechnikov) was born in 1845 in his family estate near the village of Ivanovka in the Governorate of Kharkov (Kharkiv), Little Russia (Ukraine), then a province of the Russian Empire. He was the last of five children. His father (Fig. 1) was a middle-class aristocrat of Moldavian descent and a retired Imperial Guard officer, who apparently had only a minor role in Elie's education. His mother, Emilia Lvovna née Nevakhovich (Fig. 1), the beautiful and intelligent daughter of a converted Jewish entrepreneur, had instead a great influence on Metchnikoff, who constantly referred to her even when an adult. Elie spent his childhood in the family estate. As a child, he was very active, sensitive, demanding, and manipulative (his mother defined his temperament as

FIGURE 1 Metchnikoff's parents. Reproduced from reference 10, with permission.

"neurotic"). As we will see, this personality persisted throughout his life: he often showed frustration with the smallest complication and had difficulties in coping with problems at work, such as academic restrictions and less-than-ideal research facilities, which led to resignation from his position several times during his life.

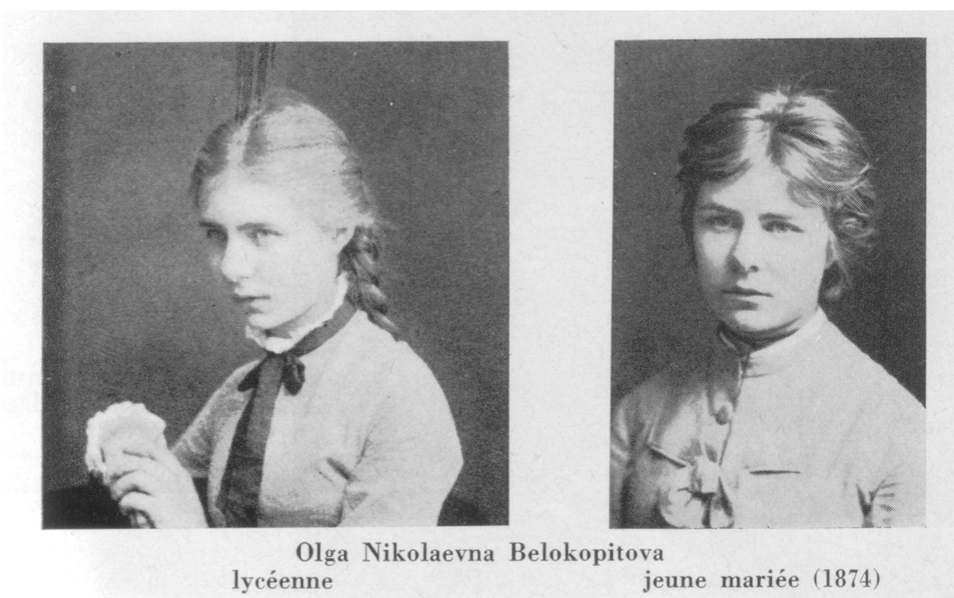
THE EMBRYOLOGIST

Metchnikoff was tutored at home under the attentive supervision of his mother, who loved him dearly and chose for him the best local teachers. As a child, he showed great interest in the animals and plants he observed in his native land and in illustrations. Elie entered the Kharkov Lycée (a school with progressively oriented teaching) when he was 11 and soon concentrated on natural history, botany, and geology, brilliantly completing his studies at 17. He was extremely intelligent, active, and talented, with a prodigious memory and imagination. In order to read the original works of German philosophers and scientists, he learned German when he was 14 and at 18 became acquainted with Darwin's *Origin of Species* and Rudolf Virchow's contributions to the cell theory. At 16, he published in the *Journal de Moscou* a critical review of a geology book written by a professor of Kharkov. After exiting the Lycée with a gold medal, Metchnikoff hastened to complete his studies at Kharkov University (which he disliked) and published, at 18, his first research article on *Vorticella*. In this paper, he compared the pseudopod, which functions as a stalk in this protozoan, with the vertebrate skeletal muscle (he concluded that there was no analogy, provoking a ferocious reaction from Professor Kuehne, a celebrated physiologist). At age 19, soon after completing his university studies, the young scientist felt a strong need to visit research laboratories throughout Europe. He visited the Universities of Giessen, Gottingen, and Munich and the marine stations of Heligoland Island and Naples, where he could find collections and fresh samples of different kinds of invertebrates. He met in Naples another young Russian, Alexander Kowalevsky, who was 5 years older and had already started studies in comparative embryology. His friendship with Kowalevsky was an important factor in Metchnikoff's decision to concentrate on invertebrate embryology, which he viewed as an ideal tool to identify similarities between different species. In 1865, while in Giessen, Metchnikoff observed particle internalization in protozoa (called "infusoria" at the time) and in the primitive gut of the flatworm *Geodesmus bilineatus*, confirming previous observations conducted by Lieberkuehn in sponges 10 years earlier. After these observations in Giessen, Metchnikoff continued his studies on the development of a wide range of invertebrate species, focusing on primary embryonic layers, and—notably—did not show any further interest in particle internalization. Only in the late 1870s, for reasons that will be apparent below, did he resume his interest in particle internalization, which ultimately inspired his great discovery.

In 1867, Metchnikoff returned to Russia and soon moved to St. Petersburg to defend his doctoral thesis on primary embryonic layers in invertebrates. He was also awarded there the Baer Prize, which he shared with Kowalevsky (Carl von Baer was an authoritative Estonian embryologist who taught from 1834 to 1862 in St. Petersburg). At age 22, Metchnikoff was appointed as docent at the University of Odessa, but he soon entered into conflict with academic authorities and moved to St. Petersburg University. In 1868, he again visited Italy (where he found Kowalevsky), touching Naples, Reggio Calabria, Messina, and Trieste. Back in St. Petersburg, he became fond of the young daughters of the professor of botany, Beketov, and started to conceive the rather peculiar idea of training one of them to conform to his feminine ideals, in order to subsequently marry her. Having failed, he married a friend of the Beketovs, Ludmila Vassilievna Fedorovitch, who was roughly as old as him and was already seriously ill with tuberculosis. Indeed, when they married in 1869, the bride could not walk or stand because of breathlessness and was taken to church on a chair. Metchnikoff's marriage was very unhappy because of the illness of his wife and serious financial difficulties. She died in Madeira in 1873, which left Metchnikoff in a state of deep depression and despair, resulting in a suicide attempt with opium. After recovering, Metchnikoff lived and worked in Odessa, where he continued to suffer from depression, pessimism, and misanthropy. He had joined the faculty of the University of Odessa in 1870 and continued to teach there until 1882. During his early life and until 1881, Metchnikoff was pessimistic not only about his life but also about the nature of humans in general. Moreover, as mentioned above, he saw a number of biological incongruities in many creatures. Luckily, in Odessa, he soon fell in love with one of the daughters of his neighbors, the beautiful 15-year-old Olga Belokopytova (Fig. 2 and 3), to whom he offered to give private lessons in zoology. He married Olga in 1875 and lived with her happily for the rest of his life. Olga immediately showed a strong devotion to her husband and helped him with his work by preparing illustrations and translating articles.

After marrying Olga, particularly from the late 1870s to the early 1880s, he started to take a novel evolutionary approach toward the solution of the problem at which he had worked for the first half of his scientific life, namely, the development of the primary embryonic layers in invertebrates. He focused on the function and fate of the mesoderm, which he believed to have a crucial role in gastrulation in invertebrates. In those years, a controversy had been going on between him and Ernst Haeckel concerning the hypothetical progenitor of multicellular organisms. Metchnikoff hypothesized that the first metazoan (which he named "*parenchymella*") was similar to the larvae of the most primitive invertebrates, the sponges, which are made up of a solid internal mass (parenchyma) of larger cells surrounded by smaller, externally flagellated cellular elements. Incidentally, the term "*parenchymella*" is still used today to designate the

FIGURE 2 Olga Metchnikoff. Reproduced from reference 10, with permission.



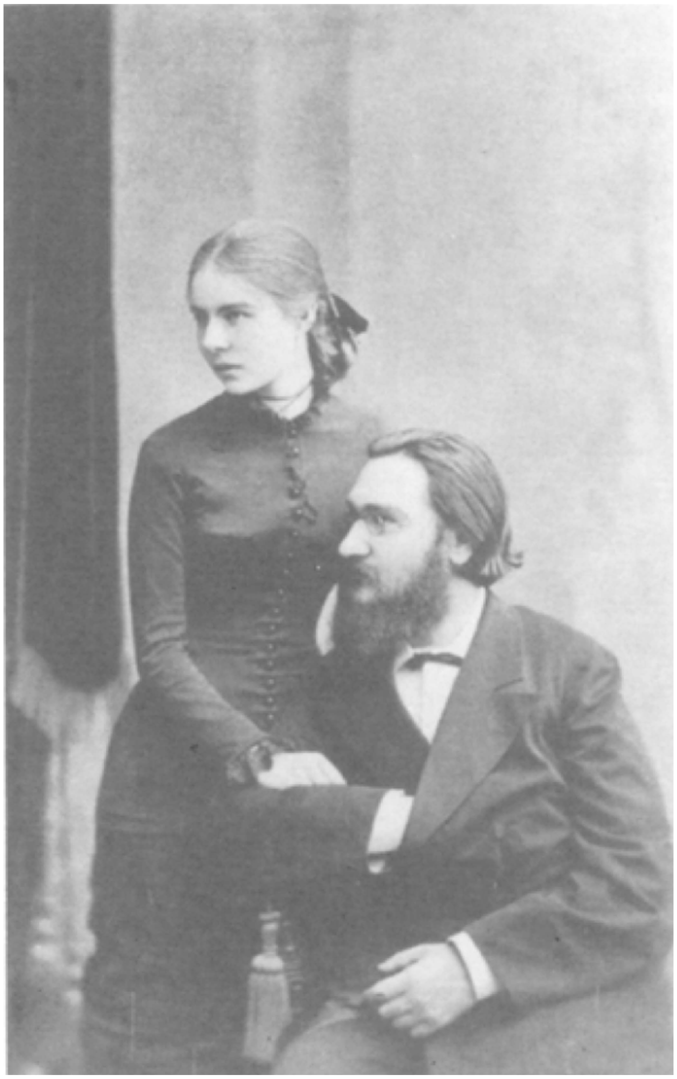


FIGURE 3 Elie and Olga Metchnikoff. Reproduced from reference 15, with permission.

larvae of *Demospongiae*, the largest and most ancient class of sponges, or *Porifera*. Haeckel had proposed instead that the first multicellular organism was similar to the invaginated gastrulas that Kowalevsky had observed in primitive chordates. He called this hypothetical metazoan progenitor "gastrea." The controversy with Haeckel had a role in the genesis of the phagocyte theory, because it helped Metchnikoff reach the following conclusions: (i) the inner *parenchymella* mass contained mobile, or "wandering ameboid," cells originating from the mesoderm and capable of taking up particulate material; (ii) the wandering cells served as nutritive cells in animals without a gut, such as sponges; and (iii) in higher invertebrates the mesoderm gave rise to the wandering ameboid cells, the locomotion apparatus, and the circulatory system. The entoderm gave origin to a well-developed intestine capable of extracellular digestion and absorption.

In 1880, Olga had typhoid fever and Metchnikoff went through a stressful situation, as described in her book: "Though worn out with devoted nursing, he tried to make up the time lost to research and overworked himself, with the result that cardiac trouble was followed by fits of giddiness and unconquerable insomnia. He fell into such a state of neuroasthenia that, in 1881, he resolved in a moment of depression to do away with his life" (28).

Curiously, having decided to die, he thought that he could use that circumstance to solve a scientific problem, namely, to determine whether relapsing fever was transmissible with blood. According to Olga, he did this also to "spare his family from an obvious suicide." At any rate, he injected himself with the blood from a patient with relapsing fever, contracted the disease, and almost died. Strangely, after recovering, he underwent a sort of physical and psychological resurrection: the eye problems that had tortured him for most of his life suddenly disappeared and he found new vitality. This resulted in a period of intense work that led to his most important discovery, which occurred in Messina, and to the formulation of the phagocytosis theory.

THE MESSINA DISCOVERY

After Olga's parents died in 1881 and 1882, Metchnikoff took care of his wife's family and properties. He managed to sell Olga's share of her father's land and to leave in the hands of her older brother the care of the remaining part of the property. At the same time, he also got rid of his share of the land property near Ivanovka. In 1882, having resigned from the University of Odessa because of the political turmoil that followed the assassination of Czar Alexander II, he was finally free to realize his dream to reach the Sicilian town of Messina. He wrote about this town in 1908, after a terrible earthquake had destroyed it: "Thus it was in Messina that the great event of my scientific life took place. A zoologist until then, I suddenly became a pathologist. I entered a new road in which my later activity was to be exerted. It is with warm feeling that I evoke that distant past and with tenderness that I think of Messina, of which the terrible fate has deeply moved my heart" (28).

In her biography of Metchnikoff, Olga vividly describes their life in Messina (28):

> At Messina we settled in a suburb, the Ringo, on the quay of the Straits, in a small flat with a garden and a splendid view over the sea. We did not have much room, and the laboratory

> had to be installed in the drawing-room, but, on the other hand, Elie only had to cross the quay in order to find the fisherman that provided him with the material needed for his researches and with whom he frequently went out sailing. Metchnikoff loved Messina, with its rich fauna and beautiful scenery. The splendid view of the sea and the calm outline of the Calabrian coast over the Straits delighted him.

The conceptual path that led Metchnikoff to the Messina discovery is deeply rooted in his embryological studies. As outlined above, Metchnikoff believed that nutrition in primitive invertebrates, such as sponges, occurred by intracellular digestion and was performed by mesodermic ameboid cells capable of ingesting food particles, moving around the body, and feeding other cells. In higher invertebrates, starting with the echinoderms (e.g., starfish and sea urchins), a well-developed intestine took up nutritional functions. What could then be the function of mobile "devouring" cells in the higher animals? These cells were no longer performing their original nutritive function, yet they retained their ability to ingest particulate material. While in Messina, Metchnikoff took advantage of the abundant and varied local marine fauna to undertake a systematic research program. First, he set out to confirm that the mesodermic ameboid cells could actively internalize particulate material and digest it: "I found it an easy matter to demonstrate that these elements seized foreign bodies of very varied nature by means of their living processes, and certain of these bodies underwent a true digestion within the amoeboid cells" (29).

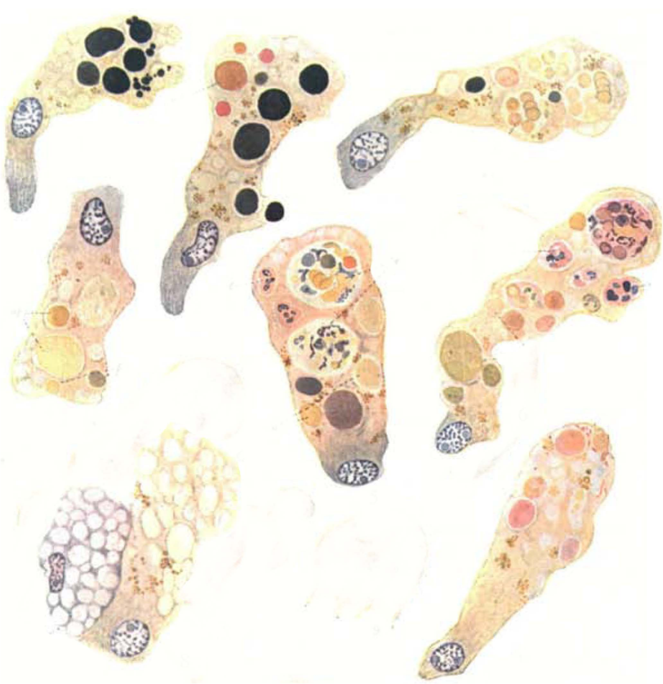
Second, he sought to investigate the role of these cells during ontogenesis. After observing, in the larvae of *Synaptae* (a family of echinoderms), that the ameboid cells "accumulate and unite into masses" in the numerous organs that undergo atrophy during metamorphosis, he concluded that these cells had a causal role in tissue resorption during development (29). Notably, while in Messina, Metchnikoff began to study with great interest Ernst Ziegler's treatise on pathological anatomy (29), in order to gather more information on a process whose details were apparently unknown to him until quite recently: "some time before my departure from Messina, I listened to the reading of Cohnheim's treatise on General Pathology and I was struck by his description of the facts and of his theory on inflammation. The former, especially his description of the diapedesis of white corpuscles across the vessel wall seemed to me of momentous interest. His theory, on the other hand, appeared to be extremely vague and nebulous" (29).

The accumulation of white corpuscles in the extravascular space described by Ziegler and Cohnheim must have been reminiscent to Metchnikoff of the "masses" of ameboid cells that he was observing at the time in the atrophied organs of echinoderm larvae. These elements (i.e., his novel concern with inflammation and the congregation of ameboid cells in atrophied organs) led Metchnikoff to conceive a crucial experiment, which was performed in December 1882 and is vividly described in a famous account (28):

> I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defense of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts. I said to myself that, if my supposition was true, a splinter introduced in the body of a star-fish larva, devoid of blood vessels or of a central nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done.
>
> There was a small garden in our dwelling, in which we had a few days previously organized a "Christmas tree" for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star fish larvae as transparent as water.
>
> I was too excited to sleep that night in the expectation of the results of my experiment, and very early the next morning I ascertained that it had fully succeeded.
>
> That experiment formed the basis of the phagocyte theory, in the development of which I devoted the next twenty-five years of my life.

Was the phagocytosis theory the result of a spark of intuition or the logical development of a conceptual trajectory lasting several years? No doubt, the theory was based on a continuous intellectual effort going back to at least the mid-1870s. Yet the Messina studies reveal a conceptual shift that marked the beginning of his transition from zoology to pathology. Indeed, two novel elements are obvious in the December 1882 experiment: (i) the use of a *noxious*, as opposed to a *harmless*, stimulus (i.e., a rose thorn as opposed to colored particles) to provoke a response in the mobile cells; and (ii) the choice of cell *congregation* around the stimulus rather than particle *internalization* or *digestion* as the readout of such a response. Clearly, Metchnikoff applied to the research model he used at the time (direct observation of live invertebrate larvae) an experimental design used in vertebrates by Cohnheim, Ziegler, and other medical pathologists (induction of inflammation by splinters or croton oil). Metchnikoff and other naturalists before him had been using grains of carmine, indigo, or red blood cells for many years to observe particle internalization in invertebrates (Fig. 4). It never occurred to anybody that this process might represent a defense reaction against the particulate agents under study, and the phenomenon was always interpreted as serving a nutritional purpose or not explained at all. Now Metchnikoff addressed a different subject, inflammation, which was outside the realm of his own specialty. And he entered this new arena à la Metchnikoff. He immediately confuted the prevailing theory, championed by Cohnheim, that inflammation originated from a pathological process in blood vessels or, as others asserted, in nerve terminations. He showed (this is the third key element of the December 1882 experiment) that inflammation could be induced in animals, such as starfish larvae, which are devoid of vascular and central nervous systems. This paved the way for the new concept that cell accumulation during inflammation was an active leukocyte (or "ameboid cell") function, rather than the passive result of circulatory dynamics, as Cohnheim believed. Moreover, Metchnikoff's idea that inflammation was beneficial to the host was not prevalent at the time and was violently criticized. Pathologists were convinced that bacteria hijacked white blood cells to find further nourishment and to disseminate in the body. Virchow, who was visiting Messina at the time, viewed Metchnikoff's ideas favorably but warned him that his theory was in contrast with the prevailing opinion on the effects of inflammation.

FIGURE 4 Columnar cells from a flatworm showing intracellular digestion in planariae. Reproduced from reference 44.



In the summer of 1883, Metchnikoff moved to Riva del Garda in northern Italy, where he wrote an article on his new ideas. Returning to Russia, Metchnikoff stopped in Vienna on the way and presented his theory to Claus, the local professor of zoology, who suggested the term "phagocyte" for the "devouring" cells, from the Greek *phagein* ("eat") and *kutos* ("hollow vessel or cell"). Ultimately, in 1883, Metchnikoff presented at a naturalists' meeting in Odessa his first paper on phagocytosis. When Metchnikoff's theory of inflammation came to the attention of professional pathologists, it provoked a violent and persistent reaction. The objections varied widely in nature, but the more difficult to answer came from reductionists (e.g., Baumgarten) claiming that Metchnikoff's theory lacked mechanistic physicochemical evidence and was based on vitalistic and teleological notions. In other words, to his critics, Metchnikoff had arbitrarily endowed the phagocyte with unexplained vitality and purpose to defend the body against infections. He managed, however, to successfully defend his theory by continuously providing new biological, if not physicochemical, evidence in favor of it.

THE PATHOLOGIST

The Messina discovery had a marked influence on Metchnikoff himself, who became more optimistic and set out to find further proof of his hypothesis. In his first efforts as a pathologist, Metchnikoff worked with the small freshwater crustacean *Daphnia*, which was the victim of a fungal infection under natural conditions. He observed first that spindle-shaped fungal spores penetrated the intestinal wall and reproduced in the body. He also noticed that the phagocytes of the crustacean attacked the fungal cells. He turned to anthrax bacilli and found that the phagocytes could not attack the more virulent strains (or the spore form of the bacterium), while they could destroy the less virulent ones.

After a difficult period in Odessa, where he was appointed director of an institute established in 1886 to carry out Pasteur's vaccine treatment of rabies and other diseases, in 1888 he resigned and traveled through Europe. In Paris he met Pasteur, who showed appreciation for his ideas. Pasteur invited Metchnikoff to join the Institut Pasteur, where he remained to work for the rest of his life (Fig. 5 to 8).

At Pasteur, Metchnikoff was engaged, during the 1890s, in work aimed at disproving the arguments of

FIGURE 5 Elie Metchnikoff at 46 years of age. Reproduced from reference 10, with permission.

his critics and at confirming his theory of phagocyte-mediated immunity. He published two important books summarizing the work developed in this period: *Lectures on the Comparative Pathology of Inflammation* (1892) (30) and *Immunity in Infective Diseases* (1901) (29). In 1903, in collaboration with Emile Roux, he showed that syphilis could be transmitted to anthropoid monkeys (reproducible animal models of the disease were not available at the time) and could be prevented by the topical application of mercurials (e.g., calomel) after inoculation with infectious material. These experiments were confirmed in 1906 on a volunteer, the medical student Paul Maisonneuve. This represented the first successful attempt to prevent syphilis after exposure and the beginning of chemotherapy for the disease, although the discovery was soon overshadowed by the introduction of Ehrlich's arsphenamine (Salvarsan) in 1910.

In those years, Metchnikoff was also attempting to extend the boundaries of his phagocyte theory in an effort to ameliorate the consequences of senescence. He proposed that toxins produced by the intestinal bacteria responsible for the putrefaction of food residues were absorbed into the body and damaged host cells. Phagocytes, in turn, in an effort to limit the consequence of chronic cell damage, were ultimately responsible for the body changes (including graying of hair) associated with senescence. He proposed that the process of senescence could be slowed down by a diet that could replace, at least in part, the endogenous gut flora, leading to a healthier state that he called "orthobiosis." He successfully promoted, to this end, the use of fermented milk products, such as yogurt, because of their high content in lactobacilli. Yogurt consumption thereafter became widely popular, giving rise to a new industry. From 1913, Metchnikoff suffered from several bouts of heart failure, which remained compensated until 1916. He died on July 16, 1916, in the apartment originally occupied by Pasteur at the Institut Pasteur. Milestones in the life of Metchnikoff are reported in Fig. 9.

THE IMPORTANCE OF SELECTIVE EATING

In conclusion, Metchnikoff pioneered research on immunity, evolutionary developmental biology, aging, intestinal microbiota, and probiotics, to cite only a few research areas. However, his major scientific contribution lay in the novel approach he successfully used to answer basic biological questions. As an embryologist, he went to the heart of the matter and set out to identify the mechanisms that determine the shape and identity of living creatures. This was a formidable undertaking in

FIGURE 6 Elie Metchnikoff and Alexandre Besredka, Institut Pasteur, 1914. Besredka was a medical doctor from Odessa who collaborated with Metchnikoff at the Institut Pasteur from 1897. Reproduced from reference 10, with permission.

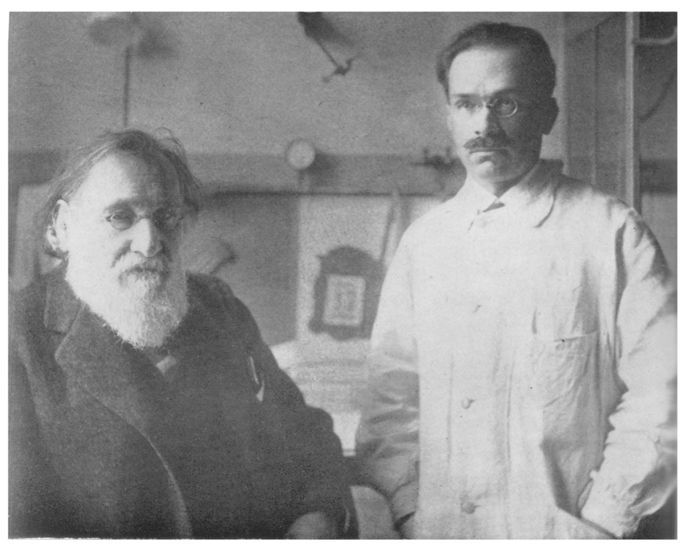


FIGURE 7 Elie Metchnikoff portrait painted by Olga. Reproduced from reference 15.

an era in which the principles of modern genetics were still very far from being defined. Focusing on the role of primary embryonic layers in the development of invertebrate animals, he looked at how these structures "reinterpreted" their basic functions in different settings (e.g., in higher versus lower invertebrate physiology). In this approach, a crucial choice was to focus on nutrition, which Metchnikoff considered the most ancient and fundamental biological activity of all. It was at this point that the ameboid myeloid cell ancestors bewitched him. To him, they became not only an essential marker of the mesoderm but, more importantly, a tool to discern how an old function (nutrition by intracellular digestion) could be adapted to new needs during evolution. This was an extraordinary intuition and a new way to approach a biological problem. By observing them in a wide variety of higher animals, Metchnikoff realized that ameboid cells had the ability to move freely around the body and interact with other cell types, unbound by any obligations to perform a specific function. He believed that these "communication skills," which derived from their primitive activity of feeding other cells after intracellular digestion, enabled phagocytes to modulate the otherwise conflicting activity of other cell types, thereby integrating or harmonizing the function of different body components into a coherent "plan." The ameboid cells retained their primitive "eating" functions, but now with the new task of eliminating unwanted, damaged, or senescent cells of the body or tissue debris (in a function that Metchnikoff called "physiological inflammation"). This conclusion anticipated by more than a century our current knowledge of the ability of phagocytes to recognize and ingest apoptotic cells and, in general, of the ability of the innate immune system to detect damaged endogenous elements (31). Moreover, his proposal that phagocytes promote tissue trophism and growth has been confirmed by the ability of macrophages to stimulate angiogenesis, neuronal patterning, bone morphogenesis, metabolism, and wound healing (32). The role in the preservation of organism integrity assigned by Metchnikoff to the phagocyte is

FIGURE 8 Robert Koch visiting the Institut Pasteur, accompanied by Elie Metchnikoff (1904). Reproduced from reference 15, with permission.

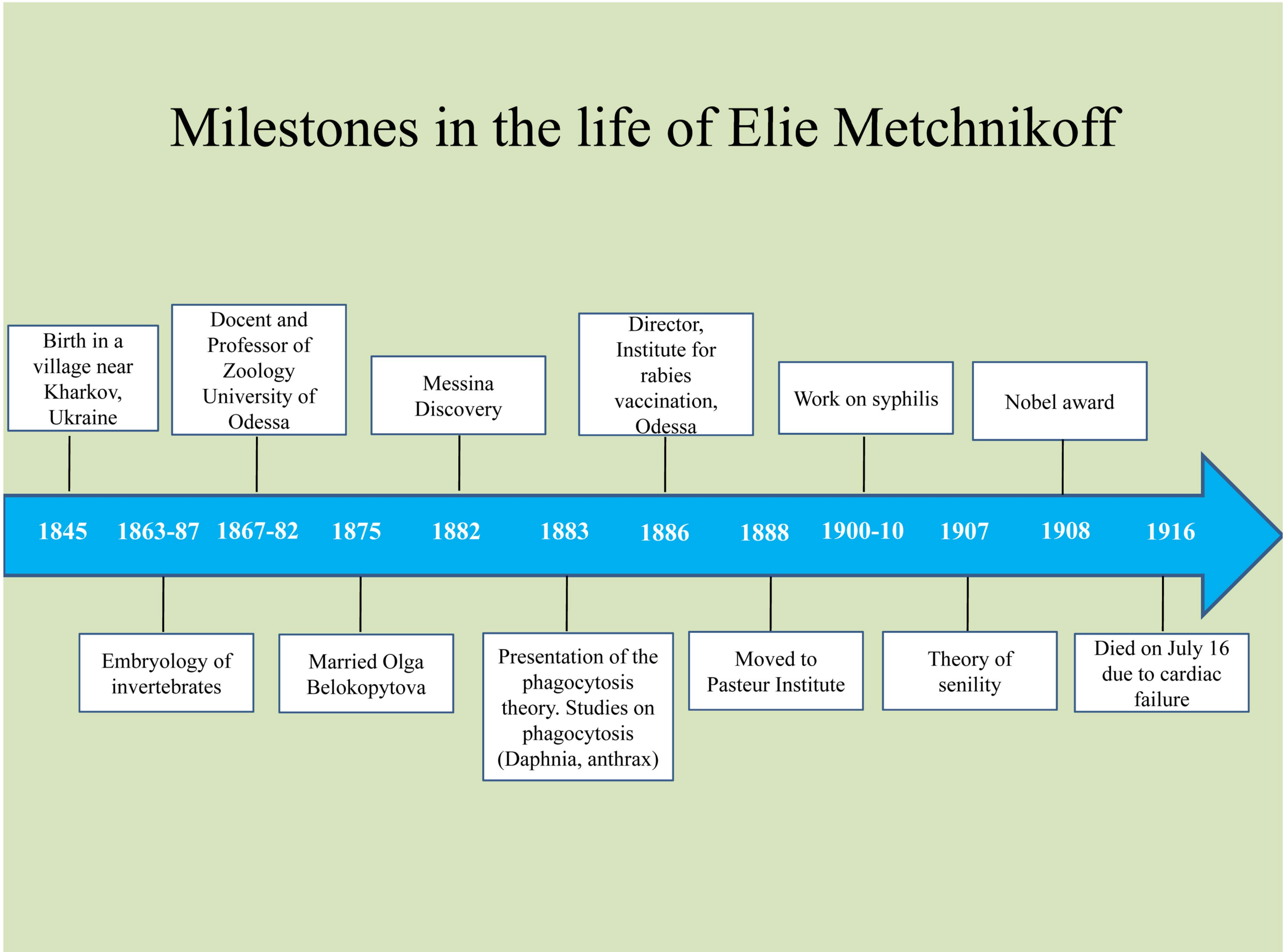


FIGURE 9 Milestones in the life of Elie Metchnikoff.

equally evident in its ability to reach infection sites and destroy pathogens (in a function he called "pathological inflammation"). This activity represented, to Metchnikoff, the adaptation of a "selective attack apparatus," to use Alfred Tauber's words (33), used by primitive animals to detect the presence of food, or, in other words, a transition from an "eat-to-feed" to an "eat-to-defend" function. Intriguingly, recent studies point to similarities and interconnections in the signal transduction pathways involved in sensing of nutrients and pathogens (34, 35), with potentially profound implications for the pathogenesis of chronic metabolic diseases (35). For example, classic sensors of microbial molecules, such as Toll-like receptors 2 and 4, can respond, in both macrophages and adipocytes, to the presence of nutritional lipids (36–39). It is also intriguing that macrophages and adipocytes show similar transcriptional profiles and ability to generate proinflammatory responses under certain conditions (41, 42). Moreover, autophagy can be triggered in response to nutrient starvation or invasion of the cytosol by intracellular pathogens (43). In summary, Metchnikoff's vision of the immune system and of myeloid cell biology is surprisingly modern, as indicated by recent discoveries. No doubt, his total commitment to science and his creativity in tackling fundamental biological questions will continue to provide a powerful source of inspiration.

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REFERENCES

1. **Silverstein AM.** 1989. *History of Immunology*. Academic Press, San Diego, CA.

2. **Mechnikov I.** 1908. On the present state of the question of immunity in infectious diseases. *Nobel lecture*, December 11, 1908.

3. **Tauber AI.** 1994. *The Immune Self: Theory or Metaphor?* Cambridge University Press, Cambridge, United Kingdom.

4. **Tauber AI.** 1999. The elusive immune self: a case of category errors. *Perspect Biol Med* **42:**459–474.

5. **Tauber AI.** 1991. The immunological self: a centenary perspective. *Perspect Biol Med* **35:**74–86.

6. **Tauber AI, Chernyak L.** 1991. *Metchnikoff and the Origins of Immunology: from Metaphor to Theory*. Oxford University Press, Oxford, United Kingdom.

7. **Gourko H, Williamson DI, Tauber AI.** 2000. Introduction, p 3–32. *In* Gourko H, Williamson DI, Tauber AI (ed), *The Evolutionary Biology Papers of Elie Metchnikoff*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

8. **Hirsch JG.** 1959. Immunity to infectious diseases: review of some concepts of Metchnikoff. *Bacteriol Rev* **23:**48–60.

9. **Vaughan RB.** 1965. The romantic rationalist: a study of Elie Metchnikoff. *Med Hist* **9:**201–215.

10. **Lépine P.** 1966. *Elie Metchnikoff et l'immunologie*. Pierre Seghers, Paris, France.

11. **Metchnikoff E.** 1959. Souvenirs. Recueil d'articles autobiographiques, 328 pages, traduits du russe par L. Piatigorski et commentes par A. Gaissinovitch, Moscou.

12. **Besredka A.** 1921. *Histoire d'un idee*. Paris, France.

13. **Levaditi C.** 1945. Centenaire d'Elie Metchnikoff. *Presse Medicale* **30:**363–364.

14. **Ramon G.** 1945. Eloge d'Elie Metchnikoff à l'occasion du centième anniversaire de sa naissance. *Bull Acad Med* **129:**294–301.

15. **Cavaillon JM.** 2011. The historical milestones in the understanding of leukocyte biology initiated by Elie Metchnikoff. *J Leukoc Biol* **90:**413–424.

16. **Gordon S.** 2008. Elie Metchnikoff: father of natural immunity. *Eur J Immunol* **38:**3257–3264.

17. **De Kruif P.** 1954. *The Microbe Hunters*, 2nd ed. Harcourt, Brace and Jovanovich, San Diego, CA.

18. **Wright AE, Douglas SR, Sanderson JB.** 1903. An experimental investigation of the rôle of the blood fluids in connection with phagocytosis. *Proc R Soc Lond* **72:**357–370.

19. **Jungeblut CW, Berlot JA.** 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.

20. **Roberts EF.** 1929. The reticulo-endothelial system and antibody production. I. The appearance of antibody in the circulation. *J Immunol* **16:**137–149.

21. **Cannon P, Baer R, Sullivan TL, Webster JR.** 1929. The influence of blockade of the reticulo-endothelial system in the formation of antibodies. *J Immunol* **17:**441–463.

22. **Tuft L.** 1934. The effect of the reticulo-endothelial cell blockade upon antibody formation in rabbits. *J Immunol* **27:**63–80.

23. **Siegmund H.** 1922. Speicherung Dutch reticuloendothelien, cellulite Reaktion und Immunitat. *Klin Webnschr* **1:**2566–2567.

24. **Elvidge AR.** 1933. The reticulo-endothelial system and the source of opsonin. *J Immunol* **24:**31–64.

25. **Metchnikoff E.** 2000. The struggle for existence between parts of the animal organism, p 207–216. *In* Gourko H, Williamson DI, Tauber AI (ed), *The Evolutionary Biology Papers of Elie Metchnikoff*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

26. **Anderson KV, Jürgens G, Nüsslein-Volhard C.** 1985. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the *Toll* gene product. *Cell* **42:**779–789.

27. **Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA.** 1996. The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in Drosophila adults. *Cell* **86:**973–983.

28. **Metchnikoff O.** 1924. *The Life of Elie Metchnikoff 1845–1916* (Lankester ER, transl). Constable, London, United Kingdom.

29. **Metchnikoff E.** 1905. *Immunity in Infective Diseases* (Binnie FG, transl). Cambridge University Press, Cambridge, United Kingdom.

30. **Metchnikoff E.** 1968. *Lectures on the Comparative Pathology of Inflammation* (Starling FA, Starling, EH, transl). Dover, New York, NY.

31. **Seong SY, Matzinger P.** 2004. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat Rev Immunol* **4:**469–478.

32. **Pollard JW.** 2009. Trophic macrophages in development and disease. *Nat Rev Immunol* **9:**259–270.

33. **Tauber AI.** 2003. Metchnikoff and the phagocytosis theory. *Nat Rev Mol Cell Biol* **4:**898–901.

34. **Hotamisligil GS, Erbay E.** 2008. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* **8:**923–934.

35. **Efeyan A, Comb WC, Sabatini DM.** 2015. Nutrient-sensing mechanisms and pathways. *Nature* **517:**302–310.

36. **Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS.** 2006. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* **116:**3015–3025.

37. **Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, Araújo EP, Vassallo J, Curi R, Velloso LA, Saad MJ.** 2007. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* **56:**1986–1998.

38. **Davis JE, Gabler NK, Walker-Daniels J, Spurlock ME.** 2008. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity (Silver Spring)* **16:**1248–1255.

39. **Ajuwon KM, Spurlock ME.** 2005. Palmitate activates the NF-κB transcription factor and induces IL-6 and TNFα expression in 3T3-L1 adipocytes. *J Nutr* **135:**1841–1846.

40. **Akira S, Uematsu S, Takeuchi O.** 2006. Pathogen recognition and innate immunity. *Cell* **124:**783–801.

41. **Khazen W, M'bika JP, Tomkiewicz C, Benelli C, Chany C, Achour A, Forest C.** 2005. Expression of macrophage-selective markers in human and rodent adipocytes. *FEBS Lett* **579:**5631–5634.

42. **Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK.** 2006. Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. *Endocrinology* **147:**5340–5351.

43. **Levine B, Mizushima N, Virgin HW.** 2011. Autophagy in immunity and inflammation. *Nature* **469:**323–335.

44. **Arnold G.** 1909. Intra-cellular and general digestive processes in Planariae. *Quart J Micro Sci* **54:**207.