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HISTORICAL PERSPECTIVE: EDITORIAL

Remembering Metchnikoff in the time of COVID-19

May 15, 2020 marked the 175th birthday of Elie Metchnikoff (1845–1916). The planned celebrations in Kharkiv were postponed because of the Covid-19 pandemic. To mark this significant milestone, *JLB* is pleased to present a special department, "Historical Perspectives." Here, we offer a brief perspective by Siamon Gordon on the contemporary relevance of Metchnikoff's work to the current Covid-19 infection, a discussion by Allan Mowat on the contribution this article has made to the field, and a translation of a fundamental article by Metchnikoff, as presented by Claudine Neyen.

The genesis of this current article began when Siamon Gordon traveled to the United States for 2 months, and took the opportunity to visit his alma mater at Rockefeller University, the home of Mϕ research for more than 50 years (1960–2010). Investigators at Rockefeller had taken up Metchnikoff's mantle by pursuing studies of the microbiome and Mϕs and expanding that work to include other cells engaged in host defenses. Dr. Gordon met Carol Moberg, a biographer of René Dubos, a French-born soil microbiologist (1901–1982) who discovered the first antibiotics, tyrothricin and gramicidin, both of which proved too toxic for clinical use. Nevertheless, that early work was imbued with the spirit of Metchnikoff, and led Dubos to study tuberculosis and the microbiome. Metchnikoff initiated the study of aging, the preventive use of yogurt to promote a healthy symbiotic microbial relationship in the gut, and foresaw the therapeutic potential of replacing its flora. Dubos opposed a simplistic germ theory of infectious disease, emphasizing that pathogenic microbes were necessary, but not sufficient to cause disease, which depended on their host environment. Subsequent work by James Hirsch, Zanvil Cohn, and Ralph Steinman, also pioneers in the field, delved into contemporary phagocyte investigation.

This special section explores the contribution of these early investigators in greater detail.

1 | INTRODUCTION

The current year marks the 175th birthday of Elie Metchnikoff (1845–1916) (See Fig. 1), grandfather of the Mϕ, its phagocytic capacity and role in natural resistance to microbes.[1] His research extended beyond the defense of the host to pathogens, to include his obsession with the resident bacteria of the gut, the microbiome, and the process of aging, both cofactors in the host-pathogen interaction that determines the outcome after infection by COVID-19. It therefore seemed of more than historic interest to trace the relevance of his ideas to the present pandemic. We have selected a review he wrote in 1909 on the microbiome and its possible therapeutic potential,[2] translated here into English, to assess its contemporary significance. At the same time, we explore aspects of host susceptibility to infections such as COVID-19, which perfectly illustrates the complex interplay between the host and pathogen, as evident from historic and contemporary studies on Mϕs.

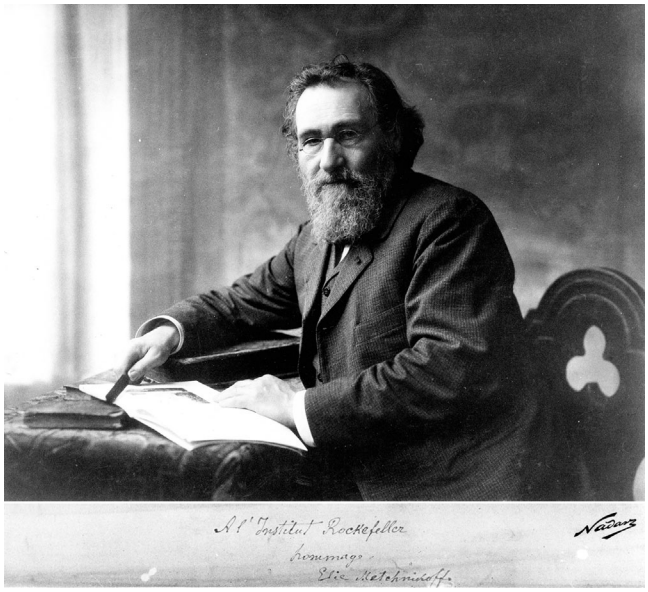

FIGURE 1 Photograph of Elie Metchnikoff (1845–1916), considered the grandfather of macrophages

2 | MACROPHAGES AND MICROBES

At the time Metchnikoff wrote his review, he was acknowledged as a fearless investigator in experimental pathology. He had started his research career in Odessa, in Czarist Russia, with expeditions to the Mediterranean coast, Messina and Naples, where he travelled with his wife's family and a microscope,[3] to study the development of invertebrate marine organisms, cast upon the shore. He observed the sessile cells called "Mϕs" (big eaters), contrasted with "microphages" (now

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neutrophils), and their migration to engulf and digest foreign particles, including bacteria, initiating inflammation. He attributed his conversion from embryologist to comparative pathologist to this discovery in 1878, and to his appreciation of the significance of the process, later termed phagocytosis, to infection.[4] At personal invitation, he moved to the newly established Pasteur Institute in Paris, where he pursued wide ranging studies into Mϕs and infectious diseases for the remainder of his career.[5,6] These included tuberculosis, syphilis and typhoid, combining the flourishing field of microbiology with the nascent science of immunology. The shared Nobel award to Elie Metchnikoff and Paul Ehrlich in 1908,[7] was an attempt by the Swedish selection committee to resolve the dispute that had arisen between the cellular and humoral schools of immunology, a legacy from the Franco-Prussian war, as well as the combative scientific research of the time.

Toward the later stages of his career Metchnikoff became obsessed with the microbial contents of the gut[2] as well as the secret of longevity[8] in certain populations, a subject he termed gerontology. This led to travels to Bulgaria and self-experimentation with *Lactobacilli*, recommended later as "probiotics." The current translation by Claudine Neyen (this section) reveals his inimitable style of personal anecdotes combined with polemical, yet thorough scholarship. We owe our awareness of this review to René Dubos, a French microbiologist at the then Rockefeller Institute, who carried the torch for Metchnikoff to New York.

3 | THE GERM AND THE HOST

René Dubos (1901–1982)[9] had migrated to the United States from France in 1924, inspired by a Russian botanist and chemist, Sergei Winogradsky, to study micro-organisms within soil, their natural habitat. He completed a doctorate at Rutgers with Selman Waksman, a future Nobel laureate, who developed streptomycin as an antibiotic for *Mycobacterium Tuberculosis*. During early postdoctoral work with Oswald Avery at Rockefeller, Dubos discovered the induction of bacterial enzymes able to degrade complex polysaccharides, important virulence factors in pneumococcal infection; he regarded bacterial adaptation to their environment as an important principle of evolution. Subsequently, and independently, he discovered the earlier antibiotics gramicidin and tyrothricine, produced by *Bacillus brevis*; these, unfortunately, proved too toxic for clinical use.[10] After a stint at Harvard, Dubos returned to Rockefeller in 1944 to study tuberculosis, a disease from which his wife had died, an echo of Metchnikoff's first wife's consumptive illness. After pioneering studies on bacterial growth, in vitro and during infection of mice, he turned to the multiple microbial species that could be cultured from the gut under anaerobic conditions, reinforcing his lifelong admiration for Metchnikoff.[11] This led to an awareness of the host microenvironment as a major factor that determined the outcome of bacterial coexistence, resulting in either symbiosis or disease. In his words, denying the germ theory of Pasteur, whom he admired as his biographer, Dubos insisted, "The germ is necessary, but not sufficient to cause the disease." He became a pioneer of environmental ecology, decades before present day awareness.

4 | LEUKOCYTES AND MICROBIAL INFECTION

Between 1960 and 2010, the Dubos laboratory morphed into the foremost laboratory that established the cellular properties of neutrophils (James G. Hirsch), Mϕs (Zanvil A. Cohn) and later, Dendritic cells (Ralph M. Steinman). A similar shift from microbe to the infected host had been initiated by Howard Florey at the Dunn School in Oxford, after development of Penicillin, later followed by Edward Abraham's Cephalosporin.[12] Since antibiotics had (at least for a while) cured the problem of many extracellular microbial pathogens, Florey used the same strategy to assign the different white blood cells implicated in host resistance to infection, to new doctoral students, namely lymphocytes (James Gowans),[13] Mϕs (George Mackaness)[14] and, initially, neutrophils (Henry Harris).[15] Throughout this period, the group at Rockefeller, now a University, continued and built on the Metchnikoff tradition of characterizing mouse and human leukocyte cellular biology during infection, by microscopy, cell isolation, fractionation and biochemistry. Hirsch[16,17] studied degranulation and killing of bacteria, both extra- and intracellular pathogens, such as Toxoplasma and Legionella, whereas Cohn turned to tuberculosis, leprosy and eventually HIV, as well as *Trypanosoma Cruzi*, to define the phagosomal interactions with lysosomes.[18] Studies in the Hirsch-Cohn laboratory showed that myeloid leukocytes are not only "specialized professional" phagocytes,[19] but also potent secretory cells.[20] The mechanisms of opsonic phagocytosis, endocytosis and viral entry were explored, among other topics.[21] Finally, Steinman and Cohn[22] revealed the unique ability of Dendritic Cells to capture, process and present microbial and other antigens to naive T and B lymphocytes, acting as a bridge between innate and adaptive immunity. Steinman received a Nobel Prize, shared with Jules Hoffmann and Bruce Beutler on innate pathways of microbial recognition and effector responses; tragically, he had died a few days before the announcement, but the award was honored.

Imbued with these approaches, one of the present authors, SG, continued these approaches to Mϕ immunobiology after setting up his own laboratory in Oxford (1976–2008), focusing on novel plasma membrane receptors as markers of cell differentiation, uptake of bacteria, viruses and fungi, and of innate and adaptive immune activation.[23] The F4/80 adhesion GPCR made it possible to define the tissue distribution of Mϕs in the mouse and to identify an orthologue, EMR2, more widely expressed on myeloid leukocytes, in humans. Decades after using irradiated Sendai virus as a student to fuse Mϕs in vitro, the molecular basis and function of Mϕ fusion became a renewed topic of research.[24]

5 | MACROPHAGES AND COVID-19 INFECTION

Given this background, it was logical to apply the experimental legacy of Metchnikoff and Dubos, to the immunopathogenesis of COVID-19.[25] It would not have been possible to invent a disease to illustrate

better the concepts outlined above. From the host viewpoint, the infection can vary from asymptomatic to mild clinical disease and recovery, or in a subset of patients, progression to a life threatening, hyperinflammatory syndrome.[26] There is circumstantial evidence that Mϕs contribute to several of the comorbidities that increase the risk of severe infection, including aging and metabolic predisposing disorders such as obesity, diabetes and atherosclerosis. There is little evidence at present that tissue Mϕs express the ACE2 receptor required for direct infection. They are the major cells implicated in clearance of dying epithelial and endothelial cells and tissue debris by a range of scavenger and opsonic receptors. As sources of antiviral interferons, pro- and anti-inflammatory cytokines, chemokines, pro and anti-coagulants, and low molecular weight radicals and metabolites, they contribute to immune activation, inflammation, and resolution of infection. The basis of the dysregulated hyperinflammatory syndrome, mainly attributed to myeloid rather than lymphoid cells, is not yet clear, but potential clues have begun to emerge. One is an ineffective interferon response,[27] another the role of oxidized membrane-derived lipids, reported to activate inflammasome and caspase-mediated IL-1 beta release by viable Mϕs.[28] Tyrosine-phosphorylated receptors such as AXL play a role in recognition of phosphatidyl serine expression by infected cells, as well as promoting the entry of COVID[29] as co-receptors to ACE2.[30] Arachidonate metabolites such as maresins and resolvins,[31] act on Mϕs themselves through GPCRs to resolve inflammation, and their secretory products, urokinase (via plasminogen activation), collagenase, and elastase contribute to fibrinolysis and tissue repair.[20] Moreover, Mϕs interact with T and B lymphocytes and other cells, through cytokine, antibody, adhesion, Fc, and complement receptors, as well as non-opsonic sensors such as TLRs and lectins.[23]

Perhaps the most mysterious aspect of COVID-19 pathogenesis is the role of aging in susceptibility to severe infection. Akbar and colleagues have discussed the role of Inflammaging[32] and Dixit and members of his laboratory have described age-related metabolic interactions between Mϕs and innate lymphoid cells which promote inflammasome activation.[33] A contributory role for the microbiome is suggested by the leakiness of the gut epithelium with possible translocation of live bacteria or metabolic products with increasing age,[34] taking us back to Metchnikoff.

Finally, the widespread tissue distribution of Mϕs and their migration through blood and lymph, provide a Trojan horse mechanism,[35] independent of ACE2 expression, for monocytes and Mϕs to disseminate virus systemically to all major organs of the body, including lungs, spleen, gut, heart, brain, liver, and kidney, as well as bone marrow and lymph nodes. These circulating and migrating mononuclear phagocytes express an array of C-type lectins such as CD169(Siglec-1) that could serve such a function.[36]

6 | CONCLUSION

We have emphasized cellular and host properties that place Mϕs at the center of the host-pathogen interaction, and have considered selected aspects of COVID-19 pathogenesis that may apply to infectious diseases in general. The prescience and lives of pioneers such as Metchnikoff[37] and Dubos[9] are memorable and remain relevant to the present day. Both Homer and Virgil reminded us that those who ask the gods for immortality should not forget to include eternal youth.[38] Although Metchnikoff may not himself have achieved longevity, his legacy remains immortal.

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