JEM Journal of Experimental Medicine

PEOPLE & IDEAS

Siamon Gordon: A half-century fascination with macrophages

Stephanie Houston

Siamon Gordon is a Glaxo Wellcome Professor Emeritus of Cellular Pathology at the University of Oxford and a fellow of the Royal Society. Throughout his career, Siamon has focused on macrophages, and his work led to the identification of the pan-macrophage marker F4/80 and the description of a role for Dectin-1 in the innate recognition of β-glucans. I caught up with Siamon to discuss his career path and his thoughts on macrophages.

Background

My parents immigrated to South Africa from Lithuania in 1930. Due to the Depression, my father, trained as a rabbi, had to move to the small village of Darling, 50 miles from Cape Town, to serve a scattered Jewish community on the West Coast. I spoke Afrikaans and went to a local school until the age of 15 before moving to an English language school in Cape Town for the final 2 yr of high school. I studied medicine at the University of Cape Town, graduating in 1961, completed my residency at Groote Schuur Hospital, and left for further research experience abroad in 1964. After a year in the laboratory of Rodney Porter at the Wright-Fleming Institute in London and a further year with Alexander Bearn at the Rockefeller University in New York, I joined the doctoral program at Rockefeller in 1966.

When did your interest in macrophages begin?

I have been devoted to macrophages for over 50 yr, as a student, group leader, and mentor. After early experiments at Rockefeller with macrophages as a fusion partner for somatic cell genetics, I chose to study their role in inflammation for its own sake, as phagocytes and secretory cells. At Oxford, my group developed mAb to study their distribution and function as the mononuclear phagocyte system, representing a dispersed organ in all tissues of the body, throughout health and disease. Through plasma membrane receptors and responsiveness to change in their environment, they support specific organ functions and promote homeostasis beyond immunity and host defense.

Where and with whom have you studied (undergraduate, graduate, postdoc)?

In 1965, I was fortunate to meet my doctoral supervisor, Zanvil (Zan) Cohn, founder of cellular immunology at the Rockefeller University, mentor of a generation of macrophage investigators, and editor of the *Journal of Experimental Medicine*. He encouraged me to use primary mouse macrophages as a fusion partner for Sendai virus–induced hybridization with a malignant melanoma cell line. This experimental approach to studying somatic cell proliferation and differentiation had been pioneered by Henry Harris at Oxford (Harris, 1966). I could not foresee that I would move to the Sir William Dunn School of Pathology in 1976 for the rest of my career, focusing on macrophages for their own sake, on occasion reverting to cell fusion as a tool and phenomenon. A portrait of Elie Metchnikoff, the grandfather of macrophage immunobiology, hung in the office of James (Jim) Hirsch, codirector of the laboratory, and cast a benign presence over the group. This was a time of rapid progress in cell biology at Rockefeller, initiated by Palade, de Duve, Siekevitz, Allfrey, and, later, Blobel. Zan and Jim, protégés of microbiologist and environmentalist René Dubos, were influential in the field of leukocyte biology and helped to motivate the change in nomenclature from "reticuloendothelial" to "mononuclear phagocyte system" (van Furth et al., 1972).

Siamon Gordon

Why are macrophages such important cells to study?

Macrophages constitute a dispersed organ of cells distributed throughout different body compartments from embryonic development to adult life, adapted to respond to diverse tissue environments, internal

shouston@rockefeller.edu.

© 2020 Houston. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).

Group reunion in 2013, 5 yr after closing the laboratory.

changes, and exogenous stimuli. As migrating and sessile cells, they interact with neighboring and remote cells through a variety of recognition receptors and secretory responses, by direct contact and via soluble signals, to tailor tissue-specific gene and protein expression. Macrophage trophic, phagocytic, and digestive functions contribute to organ development, growth, cellular injury, and death, shaping tissue modeling and repair, as well as host defense against infection. By sensing and reacting to changes in their immediate and systemic environment, macrophages contribute to physiological homeostasis as well as many disease processes beyond innate and adaptive immunity.

Mouse peritoneal macrophages were a wonder to behold by phase-contrast microscopy, especially after inflammatory stimulation in vivo and isolation by their tenacious adhesion to tissue culture plastic in vitro. Their pseudopodia and dynamic membrane ruffles, combined with a busy cytoplasm of endocytic and secretory vesicles, captured my imagination. Macrophage-specific markers such as the phagocytic Fc receptors were extinguished in heterokaryons by fusion with melanoma cells, but were, however, retained in immortalized hybrids by fusing primary macrophages with a macrophage tumor cell line. The discovery that macrophage primary cells and hybrids constitutively released lysozyme led to an appreciation that macrophages are active secretory cells as well as professional phagocytes. Collaborative studies revealed that macrophages acquired the capacity to secrete plasminogen activator, generating the fibrinolytic enzyme plasmin, as well as other potent neutral proteinases, collagenase and elastase. These were early indications that macrophages are heterogeneous in phenotype, depending on their inflammatory status.

Macrophage heterogeneity is a well-established concept. What are your insights?

Upon setting up my own laboratory at Oxford, it was natural to use hybridoma technology to generate new tools to characterize macrophage heterogeneity in situ. Hybridization of immunized splenic B lymphocytes and myeloma cells yielded cell-specific mAb. The pan-macrophage F4/80 mAb (Austyn and Gordon, 1981), directed against the EMR1 antigen, made it possible to identify mouse macrophages in embryonic and adult tissues in the steady-state and a range of disease models. Functional screens were subsequently used to develop a panel of further mAb directed against macrophage surface receptors involved in adhesion, migration and phagocytosis (Taylor et al., 2005).

Fast forward to the past decade, during which a paradigm shift has been brought about by lineage-tracing methods, that macrophages in tissues originate from yolk sac and fetal liver progenitors which seed developing tissues of the fetus, then turn over slowly as resident populations in all organs throughout adult life (Yona et al., 2013). Hematopoietic stem cell–derived blood monocytes replenish macrophages in tissues with high turnover such as gut and are recruited in response to increased demands during inflammation and infection. Adult tissues therefore contain mosaic macrophage populations of embryonic and bone marrow origin.

Immunocytochemical analysis of antigen markers established that macrophages can be distinguished from other cell types in situ; particularly striking was the discovery that resident macrophages in different organs expressed tissue-specific signatures as well as common antigen markers. Recent gene expression analysis by single-cell RNA sequencing has begun to generate a wealth of information regarding cellular composition and diversity in different tissues, including macrophage populations. Although further validation of protein expression is needed, this information has already greatly extended knowledge of organ-specific functions; atlases of tissue and blood cell composition and gene and protein expression document extensive cellular heterogeneity, novel cell types, and subpopulations of macrophages as well as other lineages (https://www.proteinatlas.org/humanproteome/tissue).

Mackaness and colleagues initially described macrophage activation by acquired immunity after Bacille-Calmette-Guérin (BCG) infection; enhanced anti-mycobacterial activity depended on prior stimulation of T lymphocytes but was antigen nonspecific, since priming by BCG also promoted resistance to challenge with *Listeria monocytogenes*, an unrelated pathogen (Mackaness, 1964). Subsequent studies by Steinman and Cohn revealed the role of dendritic cells in the activation of naive CD4 lymphocytes (Moberg, 2011), and others identified interferon gamma as the macrophage-activating cytokine. Many studies of resident and monocyte-recruited tissue macrophages in inflammation and infection revealed striking differences in macrophage phenotype associated with innate and adaptive, humoral, and cellular immunity; prominent changes were observed in MHC and costimulatory antigen expression, generation of reactive oxygen and nitrogen metabolites, and secretion of pro- and anti-inflammatory cytokines, enzymes, and inhibitors (Mosser and Edwards, 2008). Characteristic gene expression markers of differential cell polarization can be induced in cell culture by treatment of monocyte/macrophages with TH1- and TH2-type cytokines

and interferon gamma and IL4/13, respectively, termed classical and alternative activation. Subsequent studies have identified more complex signatures of altered gene expression by these and other prototypic immunoregulatory stimuli (Martinez and Gordon, 2014). Phagocytosis of microorganisms provides further activation after priming by BCG, whereas uptake of apoptotic cells after IL-4 stimulation enhances the anti-inflammatory response. In vivo studies have shown that phagocytosis of apoptotic cells can override tissue-specific gene expression. Netea et al. (2019) have demonstrated that priming of macrophages and their bone marrow progenitors by BCG and yeast particles such as zymosan induces innate "memory" in macrophages, mediated by altered metabolic and epigenetic mechanisms. After 50 yr of studying macrophage "activation," I have learned to appreciate the remarkable plasticity of macrophage populations in their ability to respond to their environment by enhanced trophic, host-protective responses, as well as by inflicting lethal injury.

How do macrophages maintain tissue diversity?

Macrophages are terminally differentiated, relatively long lived, biosynthetically and metabolically active phagocytes and endocytic cells. Their receptor repertoire is broad, including ligand proteins, peptides, amino acids, carbohydrates, lipids, and nucleic acids. They express activatory and inhibitory, opsonic and nonopsonic, Toll-like, lectin-like, plasma membrane, vacuolar, and cytoplasmic receptors. They bind extracellular matrix through integrins and leukocyte adhesion GPCR, such as EMR2, related to EMR1, and contribute to local tissue interactions. In hematopoietic organs, they express CD163 for uptake of haptoglobin–hemoglobin complexes and heme metabolism; as osteoclasts, they express vitronectin receptors for adsorption and vacuolar ATPase to resorb bone (Gordon and Plüddemann, 2017). Although it is widely assumed that the local microenvironment, including the microbiome, together with chromatin conformation and transcription determine macrophage tissue-specific phenotype, the mechanisms of diversification remain obscure. Recent studies (Bonnardel et al., 2019) have tracked the sequence of events when monocytes replace dying Kupffer cells in the liver macrophage niche. They also mapped the interactions by which stellate cells, hepatocytes, and endothelial cells imprint Kupffer cell identity. A companion study by Sakai et al. (2019) provided evidence for a two-step model in which liver-derived signals sequentially reprogram myeloid enhancers to initiate and maintain Kupffer cell identity. Together with extensive metabolic plasticity, it remains a formidable challenge to account for the remarkable diversity of the macrophage phenotype in vivo.

What are some interesting areas of ongoing research in the field?

The placenta contains fetal and maternal macrophages in close proximity to two different circulations in which transfer of genetically distinct monocytes and other precursors in both directions is conceivable. The F4/80 EMR1 antigen has been implicated in peripheral tolerance. If systemic tolerance persists in both circulations, these cells may enter every organ in the mother and/or fetus, yielding a mosaic population capable of long-term engraftment in each recipient. In the brain, this could give rise to a unique biological intimacy beyond birth. Appropriate genetic and cellular markers are available to test this speculation.

A further example of maternal–neonatal transfer of macrophages (Darby et al., 2019) demonstrated that preconception helminth infection can transfer cellular immunity to offspring via nursing through T cells rather than IgA. Since milk is a rich source of macrophages, it is plausible that macrophages surviving the acid environment of the stomach can enter the infant body through an immature or leaky intestine.

We are now in a position to decipher the functions of macrophages in every tissue, their regulation in situ, and possible manipulation for therapeutic purposes, converting a tumor-promoting trophic macrophage phenotype to a cytocidal one, or the converse, in immunodeficiency. Organ repair by monocyte adoptive therapy, without fibrosis, remains a challenge. We have come a long way from Metchnikoff, but my own reading of macrophage history is that we have climbed another step of a spiral stair, returning to the pioneers who described the fundamental features of the mononuclear phagocyte system, but hopefully at a higher level.

What has been the biggest challenge in your career?

Although I had received an excellent clinical training in South Africa, my limited background in advanced biophysics, chemistry, and mathematics was a handicap I never fully overcame. There was no formal teaching at Rockefeller and I remember well the admonition of a senior tutor to try and visualize molecular structures better, and in three dimensions.

What is the best advice you have been given?

The nature of scientific research is very similar to that of literary biography, as practiced by my wife Lyndall Gordon. She provided advice relevant to my writing in immunobiology and its history: decide early what story you want to tell in order to maintain narrative momentum. My mentor Zanvil Cohn added to this: be positive and acknowledge your predecessors, but do not spend time rebutting the perceived deficiencies of others.

Acknowledgments

I dedicate this essay to the memory of Zanvil A. Cohn, mentor and prince among men.

I thank Annette Pluddemann for indispensable editorial assistance.

References

Austyn, J.M., and S. Gordon. 1981. *Eur. J. Immunol.* https://doi.org/10.1002/eji.1830111013
Bonnardel, J., et al. 2019. *Immunity*. https://doi.org/10.1016/j.immuni.2019.08.017
Darby, M.G., et al. 2019. *Sci. Adv*. https://doi.org/10.1126/sciadv.aav3058
Gordon, S., and A. Plüddemann. 2017. *BMC Biol*. https://doi.org/10.1186/s12915-017-0392-4
Harris, H., et al. 1966. *J. Cell Sci.*
Mackaness, G.B.. 1964. *J. Exp. Med*. https://doi.org/10.1084/jem.120.1.105
Martinez, F.O., and S. Gordon. 2014. *F1000Prime Rep*. https://doi.org/10.12703/P6-13
Moberg, C.L.. 2011. *J. Exp. Med*. https://doi.org/10.1084/jem.20112294
Mosser, D.M., and J.P. Edwards. 2008. *Nat. Rev. Immunol*. https://doi.org/10.1038/nri2448
Netea, M.G., et al. 2019. *Cell Host Microbe*. https://doi.org/10.1016/j.chom.2018.12.006
Sakai, M., et al. 2019. *Immunity*. https://doi.org/10.1016/j.immuni.2019.09.002
Taylor, P.R., et al. 2005. *Annu. Rev. Immunol*. https://doi.org/10.1146/annurev.immunol.23.021704.115816
van Furth, R., et al. 1972. *Bull. World Health Organ.*
Yona, S., et al. 2013. *Immunity*. https://doi.org/10.1016/j.immuni.2012.12.001