MEETING REPORT

The macrophage – a cell for all seasons

Naomi Morrissette, Elizabeth Gold and Alan Aderem

Macrophages are extraordinarily versatile cells. They are found in some guise in practically every tissue in the body, where they participate in an overwhelming array of biological processes, ranging from development, to bone remodelling and wound healing. However, it is as sentinels of the immune system that macrophages exploit their full functional repertoire; they detect, ingest and destroy infectious agents; they initiate T-cell responses by antigen presentation, and they act as effector cells for both humoral and cell-mediated immune responses. Because of their powerful and diverse effects, macrophage function is tightly regulated. Sometimes, these controls go awry, and macrophages become the etiological agents of a wide array of inflammatory diseases. Much of our current understanding of macrophage biology is based on the pioneering work of Jim Hirsch and Zan Cohn. Zan, Jim and Ralph van Furth established a series of meetings in Leiden that facilitated an interdisciplinary discourse that allowed effective analysis of the wide array of macrophage functions. The Keystone meeting on Macrophage Biology* continued this tradition. Ralph van Furth (Leiden, The Netherlands) opened the symposium with a review of the Leiden Conferences.

Phagocytosis, phagocytic receptors and inflammation

One of the defining features of macrophages is their phagocytic capacity (Fig. 1). It is becoming apparent that phagocytosis is a highly heterogeneous phenomenon whose mechanism and biological outcome depends on the nature of the receptors engaged, particles internalized and activation state of the macrophage. For example, phagocytosis mediated through Fc receptors leads to pro-inflammatory outcomes, whereas complement-mediated uptake does not initiate the same responses. A number of presentations focused on regulation of the actin cytoskeleton during phagocytosis and emphasized the role of the Rho family of small GTPases [Steven Greenberg (New York, USA), Alan Hall (London, UK) and Alan Aderem (Washington, USA)]. The Rho family consists of three homologous subgroups – Rho, Rac and Cdc42 – all of which regulate the actin cytoskeleton. There was consensus regarding the importance of these molecules in phagocytosis, but there was disagreement about their precise role. Hall reported that Rac and Cdc42 are necessary for Fc receptor-mediated phagocytosis, whereas Rho regulates complement receptor-mediated uptake. However, work from the Aderem laboratory suggested that each of these three members participates in both types of phagocytosis.

Macrophages ingest vast numbers of apoptotic cells, sculpt the interdigital spaces during development, clear senescent red blood cells and remove apoptotic thymocytes during positive and negative selection of T cells. Unlike the uptake of infectious agents that cause pro-inflammatory responses, phagocytosis of apoptotic cells results in anti-inflammatory responses (Peter Henson, Denver, USA). Indeed, macrophages that have ingested apoptotic cells actively suppress the release of inflammatory cytokines triggered by bacterial lipopolysaccharide, by a mechanism involving transforming growth factor β, prostaglandin E_2 and platelet-activating factor. Multiple macrophage receptors, including the scavenger receptor type A (SRA), participate in the uptake of apoptotic cells (Nicholas Platt, Oxford, UK). Interestingly, SRA-null mice are more susceptible to endotoxic shock, suggesting that this receptor can downmodulate

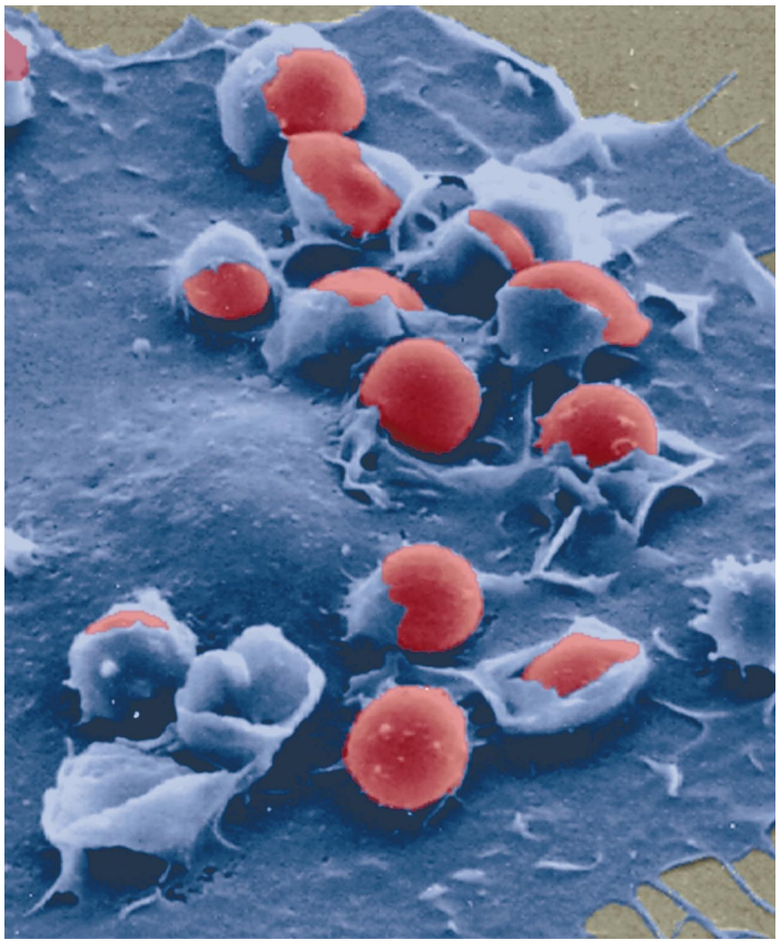

FIGURE 1

Bone-marrow-derived macrophages phagocytosing IgG-coated red blood cells. (Scanning electron micrograph kindly provided by Joel Swanson and M. Diakonova.)

*Macrophage Biology, Keystone, Colorado, USA; 22–28 January 1999. Organized by Samuel C. Silverstein, Siamon Gordon and Ralph Steinman.

The authors are in the Dept of Immunology (N. M., A. A.) and Division of Cardiology (E. G.), University of Washington School of Medicine, Seattle, WA 98195-7650, USA.
E-mail: aaderem@u.washington.edu

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inflammation by sequestering bacterial lipopolysaccharide (Siamon Gordon, Oxford, UK). The role of SRA in internalizing oxidized lipoproteins was illustrated by the observation that the hypercholesterolemic ApoE-null mouse develops substantially less atherosclerosis when crossed with SRA-null animals (Siamon Gordon). The progression of atherosclerotic lesions is dependent on the interaction between macrophage receptors and modified extracellular matrix. A model system demonstrated the binding interaction of SRA to glycated collagen that contained oxidized LDL. Adherent macrophages produce inflammatory mediators in response to CD36 ligation by oxidized LDL (Samuel Silverstein, New York, USA).

The theme of phagocytic receptors and the regulation of inflammation was extended by the analysis of Fc-receptor-null mice (Jeffery Ravetch, New York, USA). There are two types of Fc receptors: activating receptors bearing ITAM motifs, and inhibitory receptors containing ITIM motifs. The balance between these receptors modulates the response of the effector cell; this often reflects the differentiation state of the macrophage. Unexpectedly, Fc-receptor-null mice have defects in type I, type II and type III inflammation, pointing to a central role for these receptors in such pathways.

Innate recognition of pathogen products

The Fc receptors of macrophages permit recognition of pathogens detected by the adaptive immune response (antibodies). However, in their capacity as innate immune cells, macrophages detect infectious agents by expression of pattern-recognition molecules. These molecules serve as both opsonins (mannose-binding protein) and cell-surface-bound receptors (mannose receptor) to recognize a broad range of pathogens through terminal sugars. This family includes croquemort, a *Drosophila* homologue of CD36, which acts as a phagocytic receptor for apoptotic cells (Alan Ezekowitz, Boston, USA). The Toll receptors, initially characterized in *Drosophila*, represent another evolutionarily conserved family of receptors important in innate immune system signalling. The mammalian homologues of the Toll receptors participate in CD14-dependent responses to bacterial lipopolysaccharide (LPS). The GPI-linked CD14 binds to LPS but requires interaction with Toll receptors to transduce responses through activation of NFκB (Carsten Kirschning, San Francisco, USA). In another model of LPS signalling in macrophages, LPS binding protein and CD14 serve as lipid-transfer molecules to catalyse insertion of LPS into the plasma membrane. This is followed by rapid reverse transport of LPS to the Golgi stacks. LPS antagonists also insert into the plasma membrane but do not traffic to the Golgi or activate downstream events. Thus, the behaviour of distinct types of LPS in the plasma membrane is crucial for both reverse transport and signalling (Samuel Wright, Rahway, USA).

Macrophage–environment interactions

Macrophages from different tissues exhibit a wide range of phenotypes with regard to both their morphology and function; the local environment has a profound effect on macrophage differentiation. For example, microglia are refractory to a spectrum of inflammatory signals, which serves to protect the surrounding neurons. This non-inflammatory phenotype seems to be mediated, in part, by microglial adhesion to neuronal proteins. Inflammatory stimuli, such as direct injection of BCG into the brain, are not sufficient, under normal circumstances, to permit these cells to present antigen. However, microglia can present antigen after activation by peripherally presensitized T cells (Hugh Perry, Oxford, UK).

Macrophages also interact with the neuroendocrine system. For example, alveolar macrophages increase IL-1β and decrease NO production in response to stress. This occurs through catecholamine-mediated release of IL-6 by type II pneumocytes (George Kraal, Amsterdam, The Netherlands). Fertility studies on the CSF-1-deficient *op*/*op* mouse illustrate that macrophages are also important trophic cells for establishing the hypothalamic–pituitary–gonadal axis (Jeffrey Pollard, Albert Einstein, USA).

Bone morphogenesis and remodelling reflects a balance between osteoblasts, which synthesize bone, and specialized macrophages called osteoclasts, which resorb bone. Osteoprotegerin ligand (OPGL) is an osteoclast differentiation factor that can both activate mature osteoclasts and promote osteoclastogenesis when combined with CSF-1. OPGL-null mice exhibit severe osteopetrosis, stunted growth and a defect in tooth eruption. These mice contain haematopoietic precursors that can develop into mature osteoclasts *in vitro* when presented with recombinant OPGL and CSF-1. Thus, OPGL is essential for osteoclast development (William Boyle, Thousand Oaks, USA). Bone remodelling and osteoclast recruitment are also crucially dependent on the matrix metalloprotease gelatinase B (Zena Werb, San Francisco, USA).

Dendritic cells: highly differentiated antigen-presenting cells

While macrophages perform a wide array of functions, the related dendritic cells have evolved as specialized antigen-presenting cells. Dendritic cells derive from bone marrow precursors and can establish themselves in an immature form in tissues. These immature cells, for example Langerhans cells in the skin, are highly phagocytic but are unable to present antigen. Upon encountering an infectious agent, they migrate to lymph nodes, where they mature their antigen-presenting capacity and activate T cells.

Dendritic cell maturation is crucially dependent on the extracellular environment, a fact that prevents them from presenting antigen at inappropriate times and places. Monocytes can differentiate into dendritic cells when they encounter the correct signals (William Muller, New York, USA). Monocytes that migrate through the endothelium and remain within the matrix acquire the phenotype of mature tissue macrophages. Surprisingly, monocytes that reverse transmigrate emerge with the phenotype of immature dendritic cells. When reverse transmigration occurs in the presence of inflammatory stimuli, the cells that emerge are mature dendritic cells. Dendritic cell maturation is accompanied by an alteration in their expression of chemokine receptors, leading to developmentally regulated localization (Christophe Caux, Dardilly, France). Immature dendritic cells express the chemokine receptor CCR6 and respond consequently to the chemokine MIP3α, whereas mature dendritic cells express CCR7 and respond to MIP3β. In areas of tissue injury, MIP3α is expressed, recruiting immature dendritic cells, which are phagocytic and capable of

loading antigen. As these cells mature, they downregulate CCR6, allowing them to leave the local environment. The subsequent upregulation of CCR7 causes maturing dendritic cells to home to T-cell areas in the tonsil, where MIP3β is expressed.

Immature dendritic cells have a high phagocytic capacity, but the bulk of their MHC class II molecules are intracellular, rendering them incapable of antigen presentation. Maturation, induced by factors such as LPS or TNFα, causes a change in the phenotype of the cells – they lose their phagocytic ability and shift MHC class II from internal stores to the plasma membrane. The targeting of class II molecules to the cell surface is regulated by the balance between cathepsin S and its specific inhibitor, calpstatin. In the ER, nascent class II molecules complex with a protein, the invariant chain (Ii), which both blocks the peptide-binding groove and targets the MHC complex to the endosomal compartment. Proteolytic processing of Ii by cathepsin S results in the targeting of class II to the MIIC compartment, where peptide is loaded, following which the complex traffics to the plasma membrane. Dendritic cell maturation is associated with decreased levels of calpstatin, resulting in increased cathepsin S activity and transport of class II to the cell surface [Ira Mellman (New Haven, USA), Ralph Steinman (New York, USA)].

Immature dendritic cells can internalize both necrotic and apoptotic cells. Mature dendritic cells can present these internalized antigens in the context of class II. This process might have significant implications for the maintenance of peripheral tolerance (Ralph Steinman). Interestingly, dendritic cells can also present antigens derived from apoptotic cells in the context of MHC class I. Such cross-priming, which drives a CTL response, might be important in immunity to viral infection, tumour immunity and also in autoimmune diseases. Macrophages phagocytose apoptotic cells more efficiently than dendritic cells but cannot cross-prime for a $CD8^+$ response (Nina Bhardwaj, New York, USA).

Concluding remarks

In the spirit of the Leiden Conferences, the first Keystone Meeting on Macrophage Biology brought together scientists from a wide range of disciplines. Precisely because macrophages perform such diverse functions, this meeting had an extraordinarily broad scope. While this was exhilarating to experience, it is impossible to report in full. We have highlighted, therefore, only a few themes and have had to omit many outstanding presentations that fell outside of these boundaries.

Automated analysis of patterns in fluorescence-microscope images

Michael V. Boland and Robert F. Murphy

The widespread proliferation of automated fluorescence-microscope systems has made the acquisition of digital images commonplace in cell-biology research. This has created a need for computer applications that automate the analysis of these images and an opportunity to develop new approaches to classical problems. Most software developed for analysing fluorescence images has focused on problems such as the quantitation of total cellular DNA content[1], determination of intracellular ion concentrations[2] or analysis of fluorescence *in situ* hybridization (FISH) experiments[3]. These applications of quantitative methods, in which the goal is measurement, can be distinguished from potential applications of pattern-analysis methods, in which the goal is the interpretation of images. There is an extensive literature describing the application of pattern recognition, pattern analysis and machine vision to images from many other disciplines, but little work has been done on applying pattern-analysis methods to fluorescence-microscope images. We describe here some recent work[4,5] aimed at making pattern-analysis tools available to cell biologists.

Selection of representative images

Investigators in many disciplines, but especially in cell biology, regularly need to choose a single image from a collection for presentation or publication. The unspoken implication of this choice is that the selected image is representative of the set. However, it is difficult to describe the biases, both conscious and unconscious, of the investigator making the selection. Furthermore, without making the entire set of images, and a set of explicit selection criteria, available to the research community, the degree to which the choice is representative cannot be verified by other investigators. We have developed, therefore, a system for automating the choice of representative microscope images and demonstrated that results consistent with biological knowledge can be obtained using it[5].

The starting point for the system is a set of digital images, all of which nominally contain the pattern of interest (for example, a set of images obtained via indirect immunofluorescence using a monoclonal antibody against a new protein). Ideally, the images will have been corrected for any artifacts or nonlinearities occurring during the image-acquisition process (i.e. out-of-focus fluorescence or nonlinear illumination), although such correction is not necessary. The system describes each image using a set of numerical 'features' and then uses these features to rank the images in order of their 'typicality'. The system has been implemented as a Web server (TypIC; for: 'typical image chooser'; see Box 1) so that biologists anywhere can upload a set of images and receive the rankings via e-mail.

The authors are affiliated with the Biomedical Engineering Program, Center for Light Microscope Imaging and Biotechnology and Dept of Biological Sciences, Carnegie Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213, USA. E-mail: murphy@cmu.edu

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