

## COMMENTARIES

# Monocytes/Macrophages in Diagnosis and Immunopathogenesis

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This article summarizes studies of human monocytes/macrophages carried out in my laboratory over a period of more than three decades. Modern studies of mammalian phagocytes began with Metchnikoff in the late 19th century. The early history of this field is summarized in Table 1. In vivo and in vitro studies in animal systems led to the concept of the mononuclear-phagocyte system as a cell system involved in host defenses, phagocytosis, and antigen presentation and processing (11). Following Metchnikoff's development of phagocyte theory, Wright described opsonins as factors in serum that facilitated phagocytosis. Aschoff defined the reticuloendothelial system as a cellular system in which tissue macrophages and monocytes share important functional characteristics, namely, phagocytic ability and adhesiveness to glass. Subsequently, the histologic development of silver stains by Del Rio-Hortega defined a type of macrophage-related cell in the brain, the microglia. In the mid-1960s, the late Zanvil Cohn and his collaborators carried out seminal studies of mononuclear phagocytes leading to concepts of macrophage differentiation, activation, secretion, and the relationship of macrophages to antigen presentation and processing. My laboratory has had a long-standing commitment to the investigation of human monocytes/macrophages, their normal structure and function, their role in the immunopathogenesis of various disorders, and their functional abnormalities in diseases.

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### ISOLATION AND PREPARATION OF HUMAN PERIPHERAL BLOOD MONOCYTES AND MACROPHAGES

In our initial studies, we utilized the monocyte isolation method initially developed for horse blood by Bennett and Cohn which we modified for human peripheral blood mononuclear cells, using flotation on high concentrations (28%) of bovine serum albumin (24). The ability to carry out investigations with isolated human monocytes from peripheral blood was greatly facilitated by this development of a method for enrichment and purification of these cells to >98% purity using adherence to microexudate-coated surfaces and subsequent detachment using 5 mM EDTA (1). Subsequently, we further modified this cell isolation method and utilized adherence to gelatin-coated surfaces for a simple and relatively rapid purification of human peripheral blood monocytes (15, 16).

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The availability of this technology has made it possible to maintain monocytes in long-term culture (34). Using this cell culture system, we were able to establish and investigate the in vitro differentiation of monocytes into cells which resemble tissue macrophages in structure and immunologic, enzymatic, and functional profiles (34). Furthermore, we demonstrated the constitutive production of lysozyme and inducible production of acid phosphatase by these cells after several days in culture. Following 5 to 7 days in culture, the cells morphologically and biochemically assumed properties of tissue macrophages.

### DISORDERS OF MONONUCLEAR PHAGOCYTES

The ability to study mononuclear phagocytes derived from peripheral blood has made it possible to initiate studies of disorders of this important cell system. Disorders of mononuclear phagocytes can be broadly classified into the following eight categories: cell number, proliferation, secretory functions, surface receptors, motility, microbicidal-activity-oxidative, immunoregulatory, and developmental.

### MONONUCLEAR PHAGOCYTE RECEPTORS

An array of monocyte/macrophage receptors have now been described (11). These include Fc receptors, complement receptors, cytokine receptors, receptors for peptides and small molecules, hormone receptors, lipoprotein-lipid receptors, receptors for coagulants and anticoagulants, complex saccharide receptors, and others, in particular cholinergic and adrenergic agonist receptors. These receptors have been utilized as markers to study the origin, growth, differentiation, activation, motility, and function of the mononuclear phagocyte. More recently, clinical assays have been developed to detect these receptors on monocytes/macrophages from patients with various disorders.

We were the first to show the immunoglobulin G (IgG) subclass specificity of human monocyte receptor sites, namely, binding of IgG1 and IgG3 (26). We also demonstrated the human monocyte Fc receptor for complement using erythrocytes sensitized with cold autoantibodies (25).

We were the first to demonstrate the dynamic turnover of

TABLE 1. Early history of research on mononuclear phagocytes

Yr	Researcher	Area
1883	Elie I. Metchnikoff	Phagocyte theory: microphages (neutrophils), macrophages
1903	Almoth E. Wright	Opsonins
1924	L. Aschoff	Reticuloendothelial system
1932	P. Del Rio-Hortega	Microglia, silver stains
1965	Zanvil A. Cohn	Mononuclear-phagocyte differentiation

the human monocyte IgG receptor. In a unique experiment, Schmidt and I added latex particles to adherent monocytes. Following phagocytosis of latex, the IgG Fc receptor was no longer detectable with antibody-coated erythrocytes. This plasma membrane receptor reappeared over a 6- to 8-h period *in vitro* (32). These experiments were among the first showing the dynamics of plasma membrane receptors in cell biology.

We have also utilized ferritin-labeled antibody to demonstrate at the electron-microscopic level the Fc receptor binding sites on monocytes as delineated by the Rh antibody bound to erythrocytes (5). Using the freeze fracture technique, I demonstrated aggregation of intramembranous particle distribution during interaction of erythrocyte-bound ligands with immunoprotein receptors, in particular Fc and complement receptors (9). These receptors are also readily demonstrable by flow cytometry methods. Using both isotopic and morphological methods, we studied monocyte Fc and complement receptors in clinical disorders.

### CLINICAL DISORDERS OF MONOCYTE RECEPTORS

We were able to demonstrate, using quantitative dilutions of Forssman antibody that monocyte Fc receptor activity is enhanced in patients with pulmonary sarcoidosis (6). We further showed increased turnover of Fc receptors, as well as enhanced monocyte Fc receptors in patients with Crohn's disease and with sarcoidosis (7, 8, 33). We studied monocyte receptors on monocytes derived from patients with immune hemolytic anemias. In these studies, we demonstrated enhanced uptake of autologous coated cells indicating a unique association between the patient's autoantibody and the monocyte Fc receptor (27).

### MICROBICIDAL DISORDERS OF MONOCYTES

Using the albumin flotation technique, we were the first to show a microbicidal defect in circulating monocytes in cells from children with chronic granulomatous disease (4). This study was directly related to the pathophysiology of the disease, namely, the occurrence of lipid-laden macrophages in granulomas and tissue parenchyma. Our studies of monocytes have also included studies on protein calorie malnutrition, a situation in which subtle microbicidal defects occur (10, 14).

### MONOCYTES AND HIV INFECTION

We established a system to study monocyte infectivity of human immunodeficiency virus (HIV) using our *in vitro* culture system (2, 3, 18, 23). We demonstrated that cord blood monocytes/macrophages are more readily infected than monocytes/macrophages derived from adults (22). This important difference may be significant in determining HIV infectibility of the neonate. We have utilized microglia as a system for studying the brain-derived macrophage (17, 23).

### NEUROPEPTIDES AND MONOCYTES/MACROPHAGES

We have recently shown that the addition of the neuropeptide substance P (SP) to cultured monocytes/macrophages derived from healthy controls and individuals infected with HIV enhanced HIV expression (19). SP augments interleukin-10 and tumor necrosis factor production by human monocytes and macrophages (20, 30). We have recently demonstrated SP gene expression in human monocytes and macrophages (21). HIV infection of monocytes/macrophages up-regulates the synthesis of SP gene-encoding mRNAs, including the beta, gamma, and delta isoforms of SP (28, 29). This exciting finding is

important in understanding the defects of SP and its role on the monocyte/macrophage. Thus, HIV infection of monocytes/macrophages through alteration of SP may be directly relevant to the immunopathogenesis of HIV. We have also developed a chromatographic assay for SP and plasma and demonstrated changes in SP in acute anxiety or stress (patients undergoing sigmoidoscopy [12, 13]), acute crisis in sickle-cell anemia (31), and physiological stress.

In conclusion, the mononuclear phagocyte affords a unique system for studying clinical and diagnostic aspects of disorders related to receptors, secretory functions, and motility in the monocyte phagocyte system.

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