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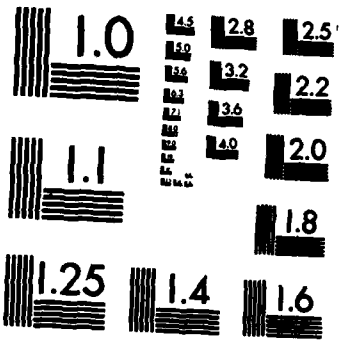

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WORKSHOP ON MACROPHAGE ACTIVATION

Final Report

D.O. Adams, M.D.

June 1982

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Duke University Medical Center
Durham, NC 27710

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A workshop on activation of mononuclear phagocytes was held. Topics included cell surface of the macrophages, macrophage receptors, induction of macrophage activation, regulation of macrophage activation, mechanisms of tumoricidal lysis, and mechanisms of bactericidal destruction.

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"SUMMARY ON MACROPHAGE WORKSHOP"

D.O. Adams

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On the 101st anniversary of Metchnikoff's observation of phagocytosis by wandering coelomocytes of starfish, the beach seemed a particularly appropriate setting for a conference on mononuclear phagocytes[1]. Over the past two decades, ample evidence has demonstrated that mononuclear phagocytes can be altered to express vastly enhanced microbicidal and tumoricidal capacities (i.e., activated). The induction and control of activation are complexly regulated; and macrophages, once activated, are characterized by numerous alterations in metabolism and structure. Two overriding questions are thus to define the induction and expression of macrophage activation in cellular and molecular terms and to relate these pleiotypic alterations, to the inductive process on the one hand and to the bases of microbicidal and tumoricidal functions on the other.

The exterior surface of macrophages proved a key focus. Although the plasma membrane of macrophages is continually and rapidly interiorized -- shuttling between several interior compartments and the exterior, many proteins are similarly distributed in the interior and exterior. The interaction of receptors with ligands not only demonstrates lateral mobility of membrane receptors but points up two conspicuous contradictions to the general pattern of even distribution of proteins in cellular membranes: 1) endocytosis of receptors leads to selective diminution of receptor proteins at the surface and 2) selective clustering of receptors about an attached particle leads to topologic heterogeneity. The turnover of membrane proteins is fairly rapid (half-life of approximately 7 hours), even in the absence of receptor engagement, and can differ quantitatively between macrophages in various stages of activation. The ectoenzyme 5'nucleotidase, for example, is greatly diminished in the latter 3 stages of activation (vide infra). This diminution is hypothesized to be a downregulation necessary for full activation of cAMP and/or of methylation reactions. On the other hand, IA molecules, necessary for effective Mϕ-T-cell interactions and hence for generation of lymphokines, are increased in the latter two stages of activation. Other alterations observed in the latter stages of activation are decreases in the mannose-fucose receptor, the FcII receptor, and the F4/80

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1 Workshop on Macrophage Activation; Hilton Head, South Carolina, U.S.A.; May 23-26, 1982.

antigen and increases in the FcI receptor and the binding site for tumor cells; all these alterations can be induced by lymphokines. Other surface constituents, such as the Mac-I antigen and the receptor for acetylated-maleylated proteins, are not apparently altered during activation.

Monoclonal antibodies, in addition to providing powerful probes for addressing such questions, are proving very helpful in studying macrophage heterogeneity. Already established is considerable heterogeneity in expression of a given epitope between populations of macrophages which have had different histories or which have been taken from different anatomic sites, plus molecular heterogeneity presumably due to differences in glycosylation. Convincing evidence of distinct macrophage subpopulations, akin to the known subsets of T lymphocytes, has yet to emerge. Of particular interest, surface proteins defined by monoclonal antibodies are now being assigned specific functions. The well-known Mac-I antigen appears to be part of the CR_3 receptor (receptor for C3bi), and to share its beta subunit with LFA-1 (Lymphocyte Function-Associated Antigen), which is important to the interaction between lytic T-cells and targets.

Receptors on mononuclear phagocytes received particular attention. Over 15 distinct receptors recognizing globulins, complement components, hormones, and various other macromolecules (e.g., lactoferrin or acetylated proteins) plus several binding sites have now been defined on macrophages. As would be expected, most of the receptors are quite mobile (e.g. as elegantly demonstrated by the zipper model of phagocytosis) and clear rapidly after specific engagement with ligand (t 1/2 for clearance is $\sim$ 2-5 minutes). The number of activities on mononuclear phagocytes controlled by receptors is almost staggering, because the various receptors both regulate macrophage development and activation (e.g. colony stimulating factor interacts via a receptor to induce differentiation of immature Mϕ) and effect, induce, or control many macrophage functions (e.g., facilitated pinocytosis, phagocytosis, chemotaxis, and secretion of numerous substances).

Control of macrophage function thus appears to rest in good part upon regulation of surface receptors. In addition to alterations in receptor number as described above, control can also be exerted by changes in ligand affinity or lateral mobility; lymphokines can induce these 2 alterations as well. A new and exciting fourth possibility is change of a receptor's function. The receptor for chemotactic peptides, when in the low affinity state, induces secretion of lysosomal enzymes and superoxide anions, but engagement of the receptor in the high affinity state induces chemotaxis.

Activation of murine peritoneal macrophages procedes via a sequential interaction between multiple signals and macrophages in several, short-lived stages of development. Young mononuclear phagocytes from sites of sterile inflammation (i.e., responsive macrophages), though not resident macrophages unless cultured for several days, interact with a second signal to gain responsiveness to yet a third signal (i.e., they become primed macrophages). The interaction of primed macrophages with the appropriate third signal leads to development of cytolytic competence (i.e., development of the fully activated macrophage). Events leading to enhanced responsiveness remain ill defined. Lymphokines, usually crude supernatants of stimulated lymphocytes, are a potent second signal but gamma interferon may be able to serve as well. Third signals appear to be quite diverse and include endotoxin, acetylated/maleylated proteins, and high concentrations of lymphokine. Precise analysis is hampered by the current state of impurity of most of the signals. Lymphokines, for example, are heterogeneous at the macromolecular level, heterogeneous with respect to both targets and target effects, and micro-heterogeneous due to differences in glycosylation. Nevertheless, good separation of several lymphokines by classic molecular sieving has been achieved. Two new approaches, utilization of supernatants of T-cell hybridomas and affinity chromatography with isolated receptors for lymphokine, appear to have great promise - particularly in cooperation with subsequent gel filtration. Once activated, macrophages can also be shut off; prostaglandin E (PGE) and α-2-macroglobulin-protease complexes, for example, can

do so quite effectively. Finally, the activation potential and path of human monocytes appears to be quite distinct from that of murine macrophages.

The obverse of stimulants from the inflammatory milieu regulating macrophages is macrophages regulating the inflammatory response. Among the 60 plus substances secreted by macrophages are components of the complement cascade, proteases, anti-proteases, procoagulants, prostaglandins, leukotrienes including Leukotriene C which has SRS-like activity, oxygen radicals, interferons, interleukin(s), apolipoprotein E, and numerous factors that regulate other cells (e.g. induce vascular proliferation or fibroblast growth or regulate lymphocyte function). This plethora of molecules can obviously induce, promote and control both acute and chronic inflammatory responses and help to shut off such responses as well - in part by destroying humoral regulators of these responses via proteases and oxygen radicals. Of particular importance, compounds from macrophages which regulate the activation of macrophages (e.g. prostaglandins, protease-antiprotease complexes, interferon, and complement components) could provide positive and negative feedback controls for macrophage activation. For example, macrophages during phagocytosis secrete a 20,000 Mr protein which increases monocytopoeisis, while quiesient macrophages appear to release a substance of Mr 50,000 that shuts off monocytopoeisis.

One of the outcomes of activation, the capacity for destruction of tumor cells, is seen in 2 distinct circumstances - a relatively slow spontaneous lysis and a rapid, antibody-dependent lysis. Both forms of cytolysis are hypothesized to occur in two steps: 1) target attachment to the macrophages, and 2) secretion of lytic substances from the macrophages. Attachment is via a surface binding site for structures contained in the plasma membrane of tumor cells in the spontaneous form of lysis and via the Fc-II receptor in the antibody-dependent. Of the multiple substances macrophages secrete now proven to be capable of damaging tumor cells, emphasis is currently placed upon a cytolytic protease and H_2O_2. A particularly important and new observation is cytolytic

synergy between two or more toxic compounds (e.g., between the cytolytic protease and H_2O_2 or between eosinophil peroxidase and H_2O_2). Target alterations observed in the spontaneous form of lysis include mitochondrial injury, cytostasis, and ultimately cytolysis. The relationship between these target alterations and the various toxic mediators remain unclear, since certain populations of macrophages are cytolytic, some cytostatic, and some both. One model relates activation and target destruction, in suggesting the signals needed to induce activation are the sum of those signals required to induce augmented capacity for attachment plus those required to induce augmented capacity for secretion of lytic mediators.

The relationship between macrophages and tumor cells is by no means a one-way street. Tumor cells can powerfully influence interactions between the two cells, by blocking the accumulation of macrophages in vivo, inhibiting the development of macrophage activation, stifling the release of lytic substances from macrophages, or inhibiting the injurious effects of lytic mediators once they have been released.

The activation of macrophages for microbicidal destruction, resembles but is clearly not synomynous with activation for lysis of tumor cells. The sequential development of bactericidal function by murine macrophages, while requiring lymphokines plus an additional signal, does not seem to require responsive macrophage to initiate the cascade. Furthermore, two separate lymphokines induce anti-Leishmanial and anti-Rickettsial kill, while a third distinct lymphokine induces these two activities plus antitumor and anti-Schistisomal kill. Though not addressed in detail, the mechanisms macrophages use to recognize microbes encompass both extracellular attachment and intracellular uptake leading to ultimate dispersal into several different intracellular compartments (e.g., phagosomes, phago-lysosomes, and cellular cytoplasm) via multiple recognition mechanisms including Fc receptors, C3 receptors, and binding sites for various glycoproteins. One critical question, as in destruction of tumor cells, is identification of those toxic substances actually involved in microbicidal destruction. Without question,

oxygen radicals, alone and in the presence of halides, are extremely deleterious to most microbes. Abundant evidence in several model systems, including kill of Candida, toxoplasma, trypanosomes, and Leishmania, indicate that oxygen radicals are major effectors. The role of oxygen-independent mediators, including lysozyme, fatty acids, cationic peptides and two novel cationic proteins from macrophage is being intensively pursued.

Macrophage activation thus clearly represents a complex and intricately coordinated pattern of alterations in macrophage metabolism, morphology, constitutive proteins, and functions, that culminate in the ability to kill microbes and tumor cells with great efficiency. Numerous changes in the plasma membrane and its constituents, particularly its receptors, in response to lymphokines appear to be a key part of these alterations. As these signals and alterations become clearly defined in molecular terms, the celular biology and physiology of activation should emerge more clearly. These, in turn, should permit employment of precisely defined probes and hence the use of genetic technology to address the fundamental question of whether macrophage activation represents the modulation or differentiation of mononuclear phagocytes.

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