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NEW APPROACHES TO MACROPHAGE FUNCTION

Barry R. BLOOM*

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This presentation will attempt to review some of the basic characteristics and functions of macrophages and indicate newer approaches to understand these mechanisms in molecular terms.

1. Origins. Macrophages derive initially from a pluripotential stem cell in the bone marrow, transforms into a monoblast, which, in the presence of colony stimulating factor differentiates into a promonocyte which then leaves the bone marrow, appears in the blood as the monocyte and has the potential of localizing in the tissues. There are approximately 0.6×10^5 mouse monocytes produced per hour, and the turnover time of promonocytes is approximately 32 hours. Inflammatory reactions increase their production 1.5 ×. While monocytes exist in the circulation for only 36 to 104 hours, they can live for very long periods of time in the tissues, as evidenced by tatoos.

2. Characteristics of monocytes.

a) Functions. Macrophages are highly motile, highly endocytic cells, which ingest large particles by phagocytosis and smaller particles or liquid phase materials by pinocytosis through a variety of specific and non-specific mechanisms.

b) Ten to 20% of macrophages appear to possess Ia antigens and are required for antigen presentation to some T-cell subsets.

c) Macrophages have the capability of carrying out intracellular and extracellular killing of parasites and tumor cells, digestion of intracellular materials and release of products which may be involved in tissue damage.

d) Receptors and markers. Macrophages are known to possess receptors for immunoglobulins, and through the use of monoclonal antibodies produced by hybridomas it has been possible unambiguously to define them as being receptors for IgG_1, IgG_{2a} and IgG_3 in the mouse. In addition they have receptors for $C3_b$ and for a variety of hormones, including insulin.

e) Enzymes. The enzyme constituitively secreted by macrophages and no other blood cells is lysozyme, which becomes an important marker for identifying macrophages. In addition, they have lysosomal hydrolases which include esterases, proteases, lipases, nucleases, glycocylases, amidases, hydrases, phosphoamidases, all of which work best at the acid pH found in the lysosome, pH 3.0～4.0.

3. Endocytosis. There are a number of pathways by which extracellular materials are constantly being taken up by the macrophage including fluid phase pinocytosis, adsorptive pinocytosis, and phagocytosis of larger particles. This is followed by fusion of the phagocytic or pinocytic vesicle with primary lysosomes containing proteolytic enzymes, killing and/or digestion of material in those secondary lysosomes, release of degraded material and recycling of the membrane. As an example of the normal degradative power of the macrophage, an adult individual has 5×10^{13} red blood cells of which 1/120 or 5×10^{11} are removed each day by splenic macrophages. In one year, splenic mononuclear phagocytize, eat and digest 2.7 kg of hemoglobin.

a) Energy requirements. While pinocytosis is linear from 2°–38° phagocytosis fails to occur at temperatures less than 18°C. Phagocytosis is inhibited by glycolytic but not respiratory inhibitors, e.g. NaF, but not DNP. The energy for phagocytosis comes from ATP and creatine phosphate. The energy for pinocytosis requires both glycolysis and respiration, and can be inhibited by NaCN.

* From the Professor and Chairman, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Yeshiva University, U. S. A.

Endocytosis results in a major change in the surface of the cell. When a macrophage ingests 1.1 micron latex particles, after one hour 31% of the total plasma membrane area is interiorized in the phagocytic vesicle. In pinocytosis, 3.1% of the surface area is interiorized each minute, or 25% of the cell volume. In one hour 186% of the cell surface is interiorized, yet the lysosomal conpartment remains constant at 3% of the cell volume. Since synthesis of membrane lipids is a slow process, it has clearly been demonstrated that a large part of the membrane recycles from the lysosome back to the plasma membrane. Adsorption of soluble molecules to Fc receptors increases the rate of pinocytosis 4,000-fold greater than just fluid phase pinocytosis.

b) Contractile elements. The contractile elements of all cells include microtubules and microfilaments. Colchicine, which disrupts microtubules, has no effect on phagocytosis or pinocytosis. Cytocholasin B, which blocks microfilaments, abolishes phagocytosis. Microfilaments consist of actin and myosin which when bound neither gel nor contract. In the presence of an actin-binding protein, there is gelation but not contraction. Finally in the presence of ATP and a cofactor, possibly a protein kinase, there is contraction. Contracted actin myosin filaments are depolymerized by a calcium dependent regulatory protein.

4. Continuous Macrophage-like Cell Lines. One approach to understanding the molecular mechanism of these processes is to use continuous macrophage-like cell lines as models, and to produce mutations in the various individual steps underlying the complicated process of the macrophage, such as endocytosis. By analyzing the defect in each of the mutants, it may be possible to understand the steps involved. We have developed a strategy for obtaining mutants unable to phagocytize opsonized red cells based on a "Trojan horse" strategy in which a poison, tubercidin, is contained within the red cells. Cells able to phagocytize through Fc receptors ingest the poisoned rbc and are killed, non-phagocytic cells are spared, and can be isolated by cloning. A large variety of non-phagocytic clones have been obtained. Phagocytosis of Ab-coated rbc in some clones can be restored to normal levels by addition of cAMP, the first demonstration that the AMP may be involved in the normal process of phagocytosis. Insulin inhibits phagocytosis, and the insulin effect can be overcome by cAMP in normal macrophage cells. At least one mechanism responsible for the inability of a mutant to phagocytize has been identified, in which it has been found that one set of non-phagocytic variants express less than one-third the receptors for IgG_{2a} immunoglobulin. When cAMP is added, the IgG_{2a} receptors return to normal levels and phagocytosis proceeds. Thus cAMP may be responsible for receptor expression. There are other mutants, with normal levels of receptors, whose phagocytosis is also facilitated by cAMP, and there, protein kinases, responsible for phosphorylating unknown proteins, possibly those of the contractile system, are important.

5. Macrophage activation. Macrophages can be activated by products of activated T-cells, e.g. MIF or MAF, to show a variety of altered properties including: i) increased spreading; ii) metabolic changes including a shift to oxidation by way of the hexose monophosphate shunt; iii) phagocytosis through the C3 receptor; iv) secretion of neutral proteases, including plasminogen activator, collagenase and elastase; v) production of superoxide (0_2^-) and hydrogen peroxide (H_2O_2); and vi) enhanced microbicidal and tumoricidal activity.

The secreted enzymes from activated macrophages may be involved in tissue damage. As an example, it has been found that MIF causes the production of plasminogen activator by normal macrophages in culture. Plasminogen activator in the presence of serum converts plasminogen to plasmin, a trypsin-like enzyme which has the ability to degrade normal body constituents such as the basic protein in myelin. Release of macrophage neutral proteases may be responsible for nerve damage in such diseases as multiple sclerosis and tuberculoid leprosy. Development of experimental allergic encephalomyelitis can be inhibited in experimental animals by treatment with protease inhibitors known to block plasminogen activator.

5. Cytocidal mechanisms.

There are a variety of specialized mechanisms which permit macrophages to kill ingested microorganisms including: i) low pH; ii) lysozyme, which attacks certain bacterial cell walls; iii) proteases; iv) cationic proteins; and v) oxygen radicals. Within 15～30 seconds after phagocytosis there is a 10～15 × increase in consumption of oxygen, which is insensitive to cyanide and hence not the usual mitochondrial oxidation. This results ultimately in the oxidation of glucose-$^{1\sim14}$C to CO_2 by way of the hexose monophosphate shunt. NADH and NADPH are produced by the hexose monophosphate shunt and in the process of glucose oxidation, oxygen radicals including O_2^-, H_2O_2, the

hydroxyl radical (OH·) and singlet oxygen ($^1O_2$) are produced. In the presence of appropriate halides, H_2O_2 will be acted on by myeloperoxidase to produce free radical halide or OX·. All of these radicals are short-lived, strong oxidizing agents which are highly bactericidal and tumorcidal.

Patients with chronic granulomatous disease are known to have a genetic deficiency in the oxidative cytocidal mechanism of polymorphs and macrophages and are highly susceptible to bacterial infection. Their cells fail to produce O_2^- or H_2O_2. It is unclear at present what the nature of the enzyme in which is responsible for the initial reduction of molecular oxygen to produce the oxygen radicals, and which of the radicals is responsible for killing individual organisms. In my laboratory we have developed a series of mutants in oxidative metabolism in a continuous macrophage- like cell line using a standard clinical test, the NBT test, to select against cells capable of producing oxygen radicals. In the case of one intracellular parasite tested, *T. cruzi*, it is clear that the parental cells (clone 16) which have oxidative metabolism are cytocidal to phagocytized trypanosomes. Mutants lacking the oxidative mechanism (clone C_3C) are unable to kill the parasites.This system provides a unique model for studying the mechanism by which oxygen radicals mediate killing, because it may be possible to reconstitute the mutant with simple enzyme systems capable of generating either O_2^-, H_2O_2, or both, and permit the identification of the radicals involved in killing particular bacteria. In this regard, it is not without interest that many intracellular parasites, including *Mycobacterium tuberculosis* and *lepramurium* are known to contain high levels of superoxide dismutase and catalase, enzymes which presumably evolved to break down the oxidative killing radicals O_2^- and H_2O_2 respectively. By understanding the role of oxidative cytocidal mechanisms, it may be possible to design new therapeutic approaches based on the ability to inhibit the enzymes of microorganisms which naturally destroy the oxidative killing radicals, and permit natural host defense to operate effectively against intracellular parasites.