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THE MACROPHAGE—VERSATILE ELEMENT OF INFLAMMATION*

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DURING the course of an inflammatory reaction, the system of mononuclear phagocytes is involved in multiple and complex tasks—from the initial signaling of other cellular elements to the destruction of local tissues and ultimately to tissue remodeling and wound repair. In this context they are also significant components of the normal physiological process of cellular turnover and degradation as well as diverse pathological events prominent in the chronic arthritides and atherosclerosis.

To carry out these roles, bone marrow-derived cells must recognize, and emigrate to, the peripheral tissues. They must initiate the interiorization of environmental molecules of soluble and particulate nature and respond to other signals that turn on secretory cycles. In this discussion I review our current understanding of the mechanisms whereby this versatile element of inflammation carries out both beneficial and deleterious tasks in mammalian hosts.

I. Life History: Maintenance of the Tissue Compartment

The nature of the pools and compartments of mononuclear phagocytes represents information of relatively recent origin (van Furth and Cohn 1968; van Furth *et al.*, 1972) and leads to the unitarian view that heterogeneous tissue pools all arise from common progenitors in the marrow (Fig. 1). Although our knowledge stems largely from small laboratory rodents, the fragments of available information suggest that similar kinetics obtain in man. Three facts distinguish their life history

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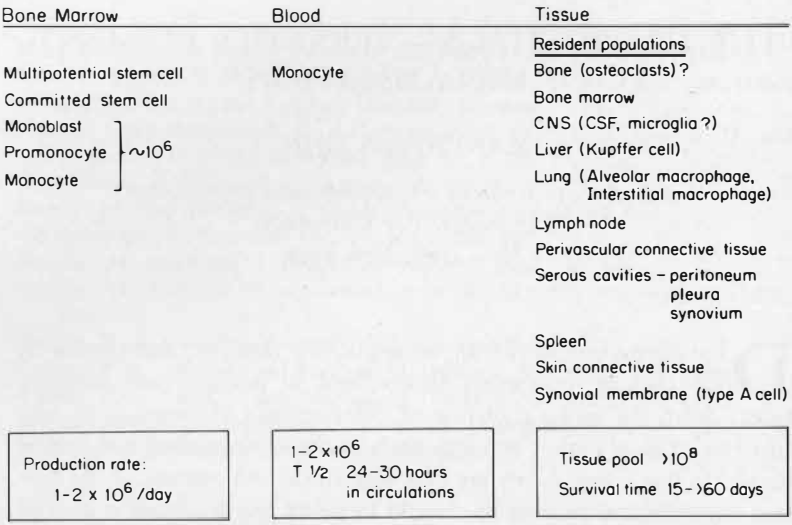

FIG. 1. Mononuclear phagocytes: pools and population (murine).

from that of other blood-borne phagocytes. First, the bone marrow pool is relatively small, without significant storage of mature members of the series. Second, the cell is released into the circulation in a relatively immature form and matures or differentiates into a larger and more active macrophage while exposed to the environmental stimuli of the tissues. Finally, the life-span in the tissues is long, as the cell resides in a G_o state and may undergo repeated cycles of endocytic and secretory activity. It is likely that the macrophages in the perivascular connective tissue outlining small blood vessels have half-lives much longer than alveolar or Kupffer cells, which are constantly exposed to particulate agents. What should be emphasized, however, is the large tissue pool some 500–1000 times greater than the bone marrow compartment. Parenchymal organs such as liver are particularly rich sources, and macrophages represent approximately 20–30% of the total cell number of this huge organ. Such long-lived macrophages of the tissue pool therefore represent a widely distributed *in situ* population poised, if you will, for an immediate response to perturbations by noxious stimuli—a population that is soon joined by emigrating monocytes and younger elements of the marrow compartment that swell local numbers, in part by

replication. Under steady state conditions, monocytes leave the circulation randomly, whereas they are selectively focused into areas of inflammation. In some instances, most of the daily production rate may enter an active lesion, leaving secondary sites relatively deficient in cells. This is also the result of the limited ability of the bone marrow (twofold) to increase its supply of cells by reducing the generation time and releasing more immature elements such as promonocytes.

II. The Migratory Event

The flight of monocytes from the circulation and their entry into the tissues occurs in a random fashion under steady state conditions and rather selectively during inflammation. It entails initial recognition of an endothelial cell surface, attachment, spreading, translational motility and directed movement or chemotaxis, basement membrane penetration, and fixation in the tissues. These multiple events are imperfectly understood and represent important regulatory steps in the inflammatory state. There are a number of products, however, that enhance the spreading of monocytes and macrophages (Bianco *et al.*, 1976, 1979; Götze *et al.*, 1979) and may play a role *in vivo*. One of the best studied is the large split product of factor B of the alternative pathway of complement fixation. Factor Bb is a diisoprophyl fluorophosphate (DFP)-inhibitable serine esterase that, at microgram levels, rapidly spreads mononuclear phagocytes, inhibiting motility and in this sense representing a defined macrophage inhibitory factor (MIF). It is of interest that factor B is synthesized by macrophages and both secreted and placed on the surface of the plasma membrane. Since many of the spreading factors are proteinases (Rabinovitch and De Stefano, 1973), the cleavage of factor B on the macrophage surface may be the common denominator that controls attachment spreading and tissue localization.

A second molecule that initiates the attachment of macrophage is fibronectin (Bevilacqua *et al.*, 1981). Although macrophages, like many other cells, synthesize fibronectin, they have on their surface a receptor that recognizes this molecule. With the exposure of collagens and the subsequent binding of plasma fibronectin during tissue injury, the fibronectin receptor could play a significant role in many pathological states leading to macrophage accumulation and perhaps granuloma formation.

III. The Macrophage as an Endocytic Cell—The Vacuolar Apparatus

The vacuolar apparatus of macrophages represents a particularly dynamic series of organelles comprising the plasma membrane, endosomes, and lysosomes. Considerable information has accrued over the years concerning the regulation and packaging of acid hydrolases (Cohn and Benson, 1965; Cohn *et al.*, 1966). This information and the related studies on the initiation and maintenance of pinocytosis and phagocytosis (reviewed in Silverstein *et al.*, 1977) left us with knowledge of lysosomal contents—enzymes, substrates, and hydrolysis—but few insights into the membrane of the organelles and its composition, fluxes, and turnover.

Figure 2 and the accompanying expanded legend outlines in a sim-

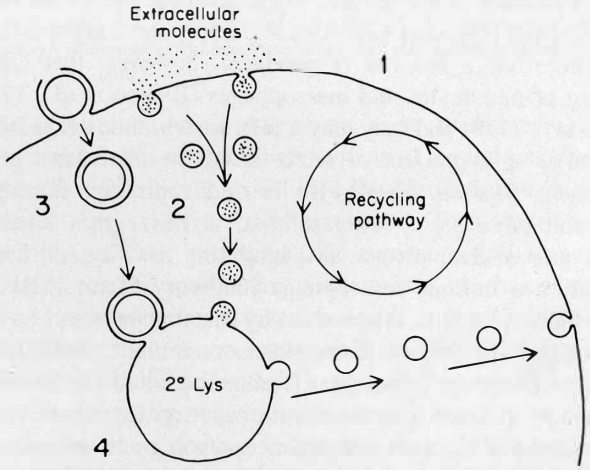

Fig. 2. Selected properties and dynamics of the vacuolar apparative of macrophages. 1. Plasma membrane: a. Surface area of resident, mouse peritoneal cell = 825 μm^2. b. Total volume of resident, mouse peritoneal cell = 395 μm^3. c. Fractional rate of internalization as pinocytic vesicles = 3.1% of total surface area per minute. d. Membrane in selectively labeled with soluble lactoperoxidase, ^{125}I, glucose oxidase, glucose, method. e. There are approximately 24 exteriorly disposed, iodinatable glycoproteins. f. Labeled membrane proteins turn over synchronously with a half-life of ± 25 hours. g. Monoclonal antibodies recognize six distinctive membrane antigens. h. Both iodinatable polypeptides and antigens (g) cycle from cell surface, through the vacuolar apparatus, and back to cell surface as a unit. i. Endocytic receptors include two distinct FcRs, $C_{3b}R$, and mannose R.

plified fashion the properties of the plasma membrane (1), pinocytic vesicles (2), phagocytic vacuoles (3), and secondary lysosomes (4). A more complete description can be found in a review (Cohn and Steinman, 1982). Figure 2 stresses our current information on the recycling and the reutilization of plasma membrane components as they flow from

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j. Membrane influx is inversely correlated with saturation of membrane phospholipid fatty acids. k. Arachidonic acid comprises 25% of total fatty acids. l. 5′-Nucleotidase and phosphodistinase I are useful ectoenzyme markers.

2. Pinocytosis and pinocytic vesicles: a. Both fluid-phase and adsorptive pinocytosis occur. b. Adsorptive vesicles exhibit clathrin coats. c. Average vesicle diameter = 0.202 μm. d. Average vesicle surface area = 0.196 μm^2. e. Average vesicle volume = 0.0143 μm^3. f. Percentage of total cell volume = 2.5%. g. Percentage of total surface area = 12.5%. h. Steady state number per cell = 250–1000. i. Activation energy for generation = 18 kcal/mol. j. Selectively labeling follows the fluid-phase uptake of an iodinating system. k. Polypeptide composition is indistinguishable from that of plasma membrane. l. Fluid phase vesicles are generated at a continuous and constant rate. m. Vesicle fusion with lysosomes is selectively inhibited with tetravalent concanavalin A. n. Rate of generation is linear between 6 and 38°C. o. Increased saturation of fatty acids inhibits total vesicle formation without modifying activation energy. p. Pinocytosis is not inhibited with cytochalasin. q. It is focused into perinuclear zone by a saltatory flow mechanism.

3. Phagocytosis and phagocytic vacuoles: a. Phagocytosis is a discontinuous and localized process. b. It is a receptor-mediated event—F_c, C_{3b}, mannose. c. Vacuoles conform to size and shape of particles < 1 to > 10 μm. d. Internalization requires the circumferential flow of membrane, utilizing receptor–ligand interactions; "zipper mechanism." e. Activation energy is 45 kcal/mol. f. Phagocytosis consumes high energy stores of creatine phosphate and ATP. g. Vacuoles are generated at temperatures only above 18 ± 2°C. h. Vacuoles are transiently associated with clathrin. i. Vacuole generation is inhibited with cytochalasin. j. Vacuoles fail to fuse with secondary lysosomes at temperatures below 18 ± 2°C. k. Increased saturation of membrane phosphatide fatty acids decreases total particle internalization *and* elevates the activation energy for particle uptake. l. Polystyrene latex (1.1 μm) phagocytic vacuoles contain a representative sample of plasma membrane polypeptides, antigens, and ectoenzymes.

4. Phagolysosomes (secondary lysosomes): a. Secondary lysosomes (2° Lys.) may represent from 15 to 55% of the total cell surface area. b. Phagolysosomes are a fusion product of primary and secondary lysosomes with endosomes. c. Size depends upon both input of extracellular solutes and the rate of hydrolysis. d. Intravacuolar pH = 4.7. e. Contain ± 30 hydrolytic enzymes with acid pH optima. f. Membranes are permeable to amino acids and selected dipeptides. g. Luminal surface of membrane is selectively labeled with lactoperoxidase–latex bead iodinating systems. h. Labeled polypeptides (iodinated) are indistinguishable from those of plasma membrane, phagocytic vacuole, and pinocytic vesicle. i. Centrifugal flow of polypeptides of plasma membrane occurs within 5 minutes of "recycling." j. Rapid and continuous contact with extracellular medium is via pinocytic vesicles. k. Storage of macroanions leads to a selective block in fusion with phagosomes but not pinosomes.

the cell surface through the cytosol and then return to the surface after delivering their contents to the digestive bodies or secondary lysosomes. The basis for this scheme can be found in a number of papers, which encompass stereological approaches, selective labeling of the vacuolar organelles, and turnover of intrinsic membrane glycoproteins.

The initial information was derived from studies on the rate at which fluid phase and adsorbed solutes were interiorized within pinocytic vesicles (Steinman and Cohn, 1972a,b). These studies, at the biochemical and ultrastructural level, emphasized the rapidity of both solute interiorization and the rate of intralysosomal degradation. It was clear that molecules adsorbed to the plasma membrane Fc receptor were captured at rates 4×10^2 to 10^3-fold greater than the fluid phase reactants. Fluid phase uptake was a constant, constitutive nonsaturable event, whereas adsorptive endocytosis exhibited saturation kinetics and was discontinuous. It was not, however, until Steinman's careful stereological analysis that we were aware of both the actual dimensions of the vacuolar system and the large quantity of membrane that was continually flowing into the cell (Steinman *et al.*, 1976). During each 60 seconds, 3.1% of the total surface area of the macrophage (830 μm^2) was entering the cytoplasm as vesicles or a *total* surface area each 33 minutes. Since the individual plasma membrane polypeptides had half-lives of 24–30 hours (Kaplan *et al.*, 1979), one could discount any extensive digestion and synthesis to explain the results. A more likely possibility was that vesicle membrane was being reutilized and cycled back to the cell surface. We were assuming in some of these speculations that a representative sample of polypeptides was present in the vesicles and were cycling in unison. This assumption was by no means clear and in fact had been challenged by a number of authors.

A more definitive technique was required to follow the circuitry of a large group of plasma membrane polypeptides. This was accomplished utilizing the basic lactoperoxidase (LPO) iodination method developed by Hubbard and Cohn (1972). For this purpose, however, LPO was covalently coupled to carboxylated polystyrene latex beads via a water-soluble carbodiimide, yielding a particle that could be rapidly established within the secondary lysosome (Muller *et al.*, 1980a,b). In the presence of glucose oxidase-generated hydrogen peroxide and iodide isotopes, rapid labeling occurred on the luminal surface of secondary lysosomes with only trace labeling of contents. This was carried out at

4°C, minimizing membrane movement and resulting in the lysosomal localization of radioactive grains on electron microscopic autoradiography. As the temperature was raised to 37°C, a rapid flow of grains took place to the cell periphery, and at 30 minutes they were randomized on both the superior and inferior surfaces.

Similar experiments were then carried out to label specifically either the exteriorly disposed surface of the plasma membrane or the newly formed pinocytic vesicles (Mellman *et al.*, 1980). The polypeptide composition of each of the members of the vacuolar system were compared, i.e., plasma membrane, pinosome, and secondary lysosome. On single-dimension, gradient SDS gels, approximately 24 iodinated polypeptides were easily discernible on each of the three organelles, and these were virtually indistinguishable. More recently Muller *et al.* (1983) have compared the labeled plasma membrane and secondary lysosome on two-dimensional isoelectric focusing–SDS systems. Again no distinctions between the two membranes are apparent. Finally, examination of more "minor" membrane proteins detected with monoclonal antibodies indicates that the pinosome and secondary lysosome membrane are representative samples of the plasma membrane. In Fig. 1, therefore, the flow or cycling process represents a membrane continuum connected by means of extensive membrane fusions.

The above synthesis of these results does not, however, preclude that receptor-mediated events could not lead to higher concentration of given polypeptides in the system. One such situation occurs with the Fc_{II} receptor of murine cells, which recognizes immune complexes. This receptor has been purified by Mellman and Unkeless (1980), and monoclonal and polyclonal antibodies were prepared against various epitopes expressed on both the exterior and cytoplasmic face of this transmembrane molecule. One antibody in particular recognizes the receptor on the macrophage surface even after its active site has been occupied (Mellman *et al.*, 1983). This has allowed us to follow the internalization, cycling, and degradation of the receptor in the presence and in the absence of ligands of various valences. Turnover of the receptor was compared to four other exteriorly expressed unrelated antigens for which we had monoclonal probes. In the absence of an Fc ligand, each of the plasma membrane components had similar half-lives and expression in the vacuolar apparatus. However, in the presence of heavily opsonized erythrocyte ghosts about 50% of the Fc receptors

were occupied internalized, and cleared from the surface. Subsequently, both the receptor and ligand were rapidly digested—the receptor demonstrating a half-life of only 2 hours under these conditions. In contrast, other membrane antigens retained their normal expression and turnover, which was more than 20 hours. Reexpression of the Fc receptor was slow and in keeping with new synthesis.

The focal ablation of a receptor from a cycling membrane system requires rather selective mechanisms. We believe that this does not take place at the plasma membrane by the generation of a coated vesicle of restricted composition. Certainly, the receptor may be clustered and enriched in this structure, but on a basal level of other membrane polypeptides. We think it more likely that selective degradation occurs within the secondary lysosomal compartment. Two possible mechanisms are suggested in Fig. 3. Both are based upon the movement and clustering of the receptor in the plane of the membrane—a process that occurs with ease, as demonstrated by Silverstein and his colleagues. On the left, receptor aggregation leads to its local accumulation similar to the capping phenomenon in B lymphocytes. This in turn initiates the formation of an autophagic vacuole and a multivesicular body, both of which are commonly encountered in macrophage cytoplasm (Hirsch *et al.*, 1968). The other mechanism would be shedding of the clustered

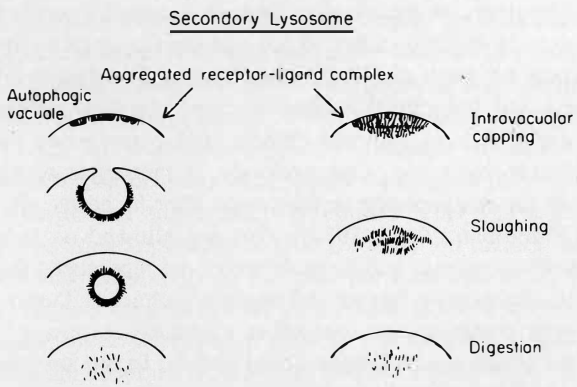

FIG. 3. Mechanisms for the selective intracellular degradation of a receptor–ligand complex.

receptor into the lumen and digestion. In both instances the receptor would be selectively removed from the recycling pool of membrane.

IV. The Macrophage as a Major Secretory Cell

Macrophages that enter the tissues or find themselves attached to the wall of sinusoids are long lived and maintain significant synthetic properties. Within the last decade our attention has been focused on the wide range of potentially important macromolecules that these cells release or secrete into their environment (Table I). Almost all these experiments have dealt with cultured cells, which are either exposed to a variety of stimuli or in different states of activation.

One is immediately struck by the wide range and diversity of this list of products that may be employed in tissue injury, wound healing, and tissue remodeling (Nathan *et al.*, 1980a,b). Some of my colleagues now refer to the macrophage as the "circulating hepatocyte." This analogy although somewhat facetious, nevertheless focuses on the importance of an emigrating, mobile population with this potential.

Our initial studies on macrophage secretion dealt with what has turned out to be one of the two or three bulk secretory products, i.e., lysozyme (Gordon *et al.*, 1974). This enzyme is produced constitutively by most populations of mononuclear phagocytes. Many of the products are solely produced, or have their rate of secretion enhanced, under conditions of macrophage activation. This process, which is brought about both by nonspecific inflammatory environments as well as specific lymphoid products, has been reviewed (Cohn, 1978). Although our original concepts were biased toward enhancement of function, we now know, as outlined in Table II, that important molecules are lost or modulated. In particular, the striking depression in the levels of arachidonate metabolites, certain ectoenzymes, and plasma membrane antigens are noteworthy.

Many questions remain concerning the secretion of these products. What is the species variation in both qualitative and quantitative expression? What is their temporal sequence? Are there selective triggers, and can a cell return to a resting state to be stimulated in the future? Certainly these and other related queries are of concern in understanding the complexities of inflammation.

TABLE I

SECRETORY PRODUCTS OF MONONUCLEAR PHAGOCYTES THAT MODULATE THE INFLAMMATORY AND IMMUNE RESPONSES

Complement components
C1
C4
C2
C3
C5
Factor B
Factor D
Properdin
C3b inactivator
β1H

Coagulation factors
X
IX
VII
V
Thromboplastin
Prothrombin
Prothrombinase

Other enzymes
Lysozyme[a]
Neutral proteases
  Plasminogen activator
  Collagenase
  Elastase
  Angiotensin-convertase
Acid hydrolases
  Proteases
  Lipases
  Ribonucleases (deoxy)
  Phosphatases
  Glycosidases
  Sulfatases
Arginase
Lipoprotein lipase

Enzyme Inhibitors
Plasmin inhibitors
α_2-Macroglobulin

Binding Proteins
Transferrin
Transcobalamin II
Fibronectin
Apolipoprotein E[a]

Oligopeptides
Glutathione

Bioactive lipids
Arachidonate metabolites
  Prostaglandin E_2
  Prostaglandin $F_{2\alpha}$
  6-Keto-prostaglandin $F_{1\alpha}$ (from prostacyclin)
  Thromboxane A_2
  Leukotrienes B, C, D, E (including SRS-A)
  Monohydroxy-eicosatraenoic acids (5-; 12-; 15-)
  Dihydroxyeicosatra-enoic acids
Platelet-activating factors

Nucleosides and metabolites
Thymidine
Uracil
Uric acid

Reactive metabolites of oxygen
Superoxide anion
Hydrogen peroxide
Hydroxyl radical
Singlet oxygen (?)

Chemotactic factors
For neutrophils
For fibroblasts

Factors regulating synthesis of proteins by other cells
Hepatocytes
  Serum amyloid A
  Haptoglobin
Synovial-lining cells
  Collagenase
  Prostaglandins
  Plasminogen activator
Adipocytes
  Lipoprotein lipase

Factors promoting growth of:
Lymphocytes (T and B cells)
Myeloid precursors (colony-stimulating factors, factor inducting monocytopoiesis)
Erythroid precursors
Fibroblasts
Capillaries (angiogenesis factor)

Factors inhibiting growth of:
Lymphocytes
Myeloid precursors
Tumor cells
Viruses (interferon, α, β)
Listeria monocytogenes

Other hormone-like factors
Endogenous pyrogens (two M_r species)
Insulin-like activity
Thymosin B_4

[a] Major bulk products.

TABLE II

PROPERTIES OF ACTIVATED MACROPHAGES

Structural	Metabolic	Cell surface determinants	Endocytosis
	Elevated		
Spreading	Glucose cons.	Ia antigen	C3b mediated
Ruffled membranes	O_2 cons.	Alkaline PDE I	Fluid pinocytosis
Polarization	HMP shunt		EA ingestion
Secondary lysosomes	O_2^-		
	OH^-		
	H_2O_2		
	Secretion of proteinases, etc.		
	Depressed		
	Cyclooxygenase	5′-Nucleotidase	
	PGE_2	Mannose receptor	
	Prostacyclin	Antigen F4/80	
	Lipoxygenase		
	Leukotrienes		
	Mono HETEs		

[a] HMP, hexose monophosphate; PDE, phosphodiesterase; PGE, prostaglandin E; HETE, 12L-hydroxy-5,1,10,14-eicosatetranoic acid.

V. The Macrophage as an Effector Cell

A. The Intracellular Milieu

It was apparent from the earlier work of Rich, Lurie, and Danenberg, Suter and Mackaness and North, that the activation of macrophages during infection with intracellular microbial pathogens lead to an enhanced microbicidal state in the animal. This was followed by the elegant demonstrations of the important link between sensitized T cells, the production of lymphokines, and the activation of macrophages by Mackaness, North, David, and Bloom. Only more recently have we begun to understand the underlying mechanisms that lead to intracytoplasmic killing and extracellular cytotoxic reactions.

Our progress in this area was related to the availability of large, easily

scored targets that replicated only within living host cells. In addition, our ability to manipulate relatively homogeneous populations of resident, elicited, and activated cells under the defined conditions of an *in vitro* environment allowed us to approach biochemical distinctions. These methodological advances were aided by the extensive knowledge of neutrophils, oxidases, the generation of oxygen intermediates and myeloperoxidase. This system, which lead to microbial halogenation or the "Klebanoff pathway," set the groundwork for examining the macrophage. It soon became evident that macrophages in the resting state failed to generate appreciable quantities of either superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2) (Johnston *et al.*, 1981; Nathan and Root, 1977). However, when harvested from animals undergoing infection with *Listeria* or bacillus Calmette-Guérin they produced large amounts of both products. To examine this modulation in more detail, we chose the *in vitro* environment and the host–parasite interaction of *Trypanosoma cruzi* tryposmastigotes with resident murine peritoneal macrophages (Nogueira and Cohn, 1978). In the presence of usual media these organisms replicated free in the cytoplasm of the macrophage, having lysed the endocytic vacuole shortly after ingestion (Nogueira and Cohn, 1976). After 72 hours the resulting amastigotes ruptured their hosts and initiated another cycle of infection.

The addition of supernatants from specific antigen-triggered sensitized T cells (lymphokines) lead to a dramatic increase in the ability of the macrophage to destroy the trypomastigotes (Nogueira and Cohn, 1978). At concentrations as low as 2% and treatment from 24 hours *in vitro,* resident macrophages took on the structural properties of the activated mode and sterilized the intracellular milieu. Organisms initially free in the cytoplasm at the onset of lymphokine therapy were seen in various stages of degeneration within newly formed autophagic vacuoles. Subsequently, we extended this analysis to *Toxoplasma* and *Leishmania* with basically similar results (Murray *et al.*, 1979).

One could now correlate the formation of toxic oxygen intermediates with the microbicidal process. A clear-cut association between the formation of H_2O_2 and intracytoplasmic killing was established in activated cells of both the intact mouse and macrophages exposed to lymphokines *in vitro* (Nathan *et al.*, 1979a,b,c). In addition, parasites cultured in the presence of variable fluxes of glucose oxidase-generated H_2O_2 were appropriately sensitive to this reagent. Monocytes rapidly

lose their endogenous supply of azurophil granule localized myeloperoxidase during maturation to the macrophage. Therefore, the microbicidal process of activated macrophages appears to depend largely on reactive oxygen intermediates of which H_2O_2 is only one example. This is not to exclude other factors such as proteases, which may in fact operate through an oxygen-dependent mechanism (Johnston *et al.*, 1981).

B. The Extracellular Milieu

Much of the hydrogen peroxide generated by activated macrophages is liberated into the extracellular milieu. Here depending upon local concentrations it can destroy sensitive eukaryotic targets including tumor cells (Nathan *et al.*, 1979a,b). As in the case of intracellular targets, toxic oxygen intermediates are responsible for much of the activity of macrophages against neoplastic cells. A number of studies have demonstrated the oxygen dependence of the process and its correlation with the formation of H_2O_2 (Nathan *et al.*, 1980a,b). It seems likely that H_2O_2 secretion may be vectorial and limited to the membrane zone exposed to the inducing stimulus. Secretion may then occur within lacunae, separated from the general environment and reminiscent of the bone absorbing events carried out by osteoclasts.

Both tumor cells and intracellular microbes demonstrate considerable variation in their sensitivity to oxygen intermediates (Nathan *et al.*, 1980a,b). With murine tumor cells this is best correlated with their content of scavengers of oxygen radicals and in particular with cellular levels of gluthathione (Arrick *et al.*, 1982). Figure 4 illustrates some of the pathways implicated in the maintenance of reduced glutathione (GSH). Factors including oxygen per se and oxidizable substrates (glucose) influence GSH levels. Figure 4 includes more specific agents that inhibit synthesis (buthionine sulfoximine), GSH peroxidase (selenium deficiency), GSSG reductase (BCNU) and shows electrophiles that complex GSH (CDNB + SLs). Each to a greater or lesser extent can sensitize tumor cells for an oxidative attack and may shift the dose-response titration with H_2O_2 by as much as 50- to 100-fold (Arrick *et al.*, 1982). One may also kill tumor cells in the peritoneal cavity by generating high local concentrations of H_2O_2 via a glucose oxidase–latex bead (Nathan and Cohn, 1981). It is of interest that the rate-

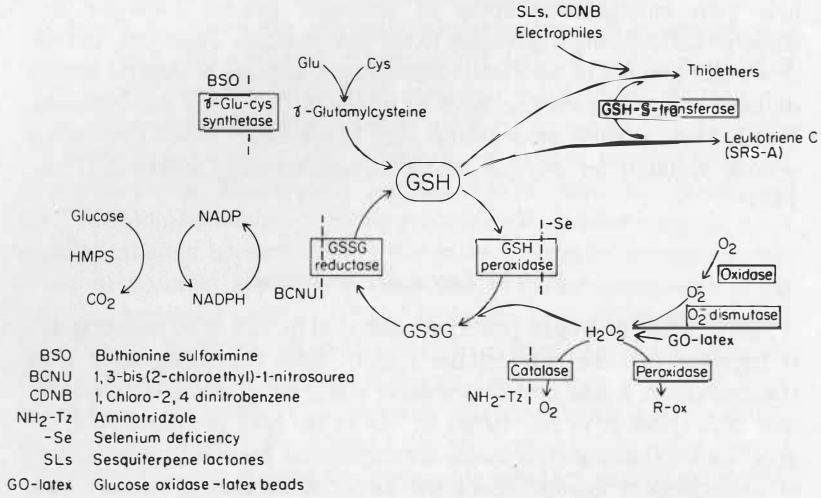

FIG. 4. Pathways implicated in the maintenance of reduced glutathione (GSM).

limiting reactant under these conditions is molecular oxygen, and tumor-killing can be enhanced by exposing animals to a high pO_2. Both the generation of local concentrations of toxic oxygen intermediates and the inhibition of tumor scavenging systems may well have a place in the therapeutic armamentarium of the future.

VI. The Macrophage as a Mediator of Inflammation

The more classic view has considered the macrophage only in terms of chronic, granulomatous forms of inflammation. There is now rather compelling evidence that suggests important roles of macrophages in immediate forms of allergy as well. These include the presence of large, constant tissue pools and the production of potent metabolites that can influence the microvasculature and smooth muscle-containing structures. One of the more interesting concerns the metabolites of arachidonic acid (20:4), an unsaturated fatty acid present in macrophage membrane phospholipids at the high level of 25 mol%. Exposure of macrophages to particulate stimuli leads to the release of as much as 60–70% of the total arachidonate as metabolites of both the cycloox-

ygenase and lipoxygenase pathways (Scott *et al.*, 1980). The products of mouse populations is outlined in Table III (Rouzer *et al.*, 1980a, 1982). It is apparent that large amounts are formed in response to immune complexes of IgG and IgE. Phagocytosis per se is not required; merely the interaction of a multivalent ligand with the Fc receptor on the cell surface is sufficient to trigger the secretory event (Rouzer *et al.*, 1980b). This is a new role for the Fc receptor in addition to its better

TABLE III

THE ARACHIDONATE METABOLITES OF MURINE MACROPHAGE POPULATIONS

Macrophage source	Stimulus	Product	Amount (pmol/10^7 cells)
Resident peritoneal	Zymosan	PGE_2	14,000
		6-Keto-$PGF_{1\alpha}$	6,000
		HETEs	3,300
		Leukotriene C	4,500
	IgG immune complexes	PGE_2	5,400
		6-Keto-$PGF_{1\alpha}$	13,000
		Leukotriene C	2,000
	IgE immune complexes	PGE_2	5,400
		6-Keto-$PGF_{1\alpha}$	13,000
		Leukotriene C	2,000
	IgE immune complexes	PGE_2	4,800
		6-Keto-$PGF_{1\alpha}$	8,200
		Leukotriene C	1,100
C. parvum-elicited peritoneal	Zymosan	PGE_2	3,000
		6-Keto-$PGF_{1\alpha}$	150
		TXB_2	650
		HETEs	850
		Leukotriene C	95
Interstitial pulmonary	Zymosan	PGE_2	2,300
		6-Keto-$PGF_{1\alpha}$	1,100
		TXB_2	1,500
		HETEs	5,600
		Leukotriene C	3,800
		Unidentified	3,000

[a] PGE, $PGF_{1\alpha}$, prostaglandins, E_1 and $F_{1\alpha}$; IgG, immunoglobulin G; HETE, 12 L-hydroxy-5,8,10,14-eicosatetranoic acid; TXB, thromboxane B.

known activity for initiating phagocytosis. A second point is that the process of activation leads to a marked depression in the production of arachidonate metabolites (Scott *et al.*, 1982b). At present the mechanisms that modulate the phospholipases—and therefore the release of membrane 20:4 as well as the metabolic enzymes responsible for the production of the active metabolites—are unclear. One can consider that such regulating events might well influence the overall inflammation process.

Although specific membrane stimuli are required for the production of cyclooxygenase and lipoxygenase products from the esterified 20:4 of phospholipids, free 20:4 can be extensively metabolized without a stimulus (Scott *et al.*, 1982a,b). Large quantities of products are continually formed from the free fatty acid via what has been called the *exogenous pathway.*

VII. Macrophage and Cell-Mediated Immunity

There is little doubt that macrophages are important effector cells in immune responses characterized by delayed forms of hypersensitivity, nor that their secretory products such as prostaglandin E_2 may suppress the function of lymphoid cells (Nussenzweig *et al.*, 1980). There is even some unanimity of opinion that they may on occasion support the growth of lymphoid cells in the artificial test tube environment. What is less clear is their role as ''processors'' or ''presenters'' of antigen for the stimulation of T cells. We suspect that in fact macrophages play rather minor roles in this process, their preeminence being usurped by an adherent cell type that for years has been hiding among the forest of macrophage ruffles. This is the *dendritic cell,* a novel character in immune responses, which has been so elegantly isolated and characterized by Steinman and his colleagues (Steinman and Nussenzweig, 1980). The dendritic cell constitutively expresses large amounts of Ia antigen on its surface and serves as the most potent stimulator of the allogeneic and syngeneic mixed lymphocyte reaction (MLR) and other forms of reactions that lead to T-cell replication and the formation of cytotoxic T cells. Monoclonal antibodies specific for dendritic cells and C′ can destroy the cell in mixed spleen populations and subsequently ablate the MLR, whereas similar studies employing anti-monocyte monoclonals do not alter the allogeneic MLR.

Studied initially in the mouse, an analogous cell type with similar

properties is present in the rat and in man as well. In the human species it is present in small numbers in the circulation and has been purified by Van Voorhis *et al.* (1982). It is clear that the dendritic cell, serving as a passenger leukocyte in transplant studies, will have a major impact on transplantation and in the control of graft rejection. As the reagents for its study become more readily available, immunologists will cease their practice of referring to "adherent" populations and begin to distinguish between members of the mononuclear phagocytes and the dendritic cells.

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