REVIEW ARTICLE

THE GRANULOMATOUS INFLAMMATORY RESPONSE

The Granulomatous Inflammatory Response

A Review

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GRANULOMATOUS INFLAMMATION was recognized as a distinct entity in the early nineteenth century and has been of continuing interest since.[1] Yet, the granuloma remains enigmatic. Over the past decade, the biology of granulomas has been extensively studied, and our understanding of mononuclear phagocytes, which constitute granulomas, has increased significantly. The purposes of this review are to consider these developments and to put them into a reasonable perspective.

To define a granuloma is difficult. Currently employed definitions range from inflammation characterized by the presence of lymphocytes, monocytes, and plasma cells to reactions identified by the presence of mononuclear phagocytes including epithelioid and giant cells.[2,3] A *granuloma* is defined here as a compact (organized) collection of mature mononuclear phagocytes, which is not necessarily accompanied by accessory features such as necrosis. The former lesions can be termed *pure granulomas* and the latter *complex granulomas*. Organization and activation of the mononuclear phagocytes separate granulomas from simple chronic inflammation; specialized mononuclear elements such as epithelioid cells need not be present.

Granulomatous inflammation can best be analyzed when viewed in the light of how granulomas develop. Conceptually, granulomas evolve in three stages: the development of an infiltrate of young mononuclear phagocytes, the maturation and aggregation of these cells into a mature granuloma, and the potential, further maturation of these cells into an epithelioid granuloma. The present review is organized about this conceptual framework, so that the host's system of mononuclear phagocytes and their biologic behavior are first considered. The development, morphology and metabolism, induction, accessory features, and functions of granulomatous inflammation are then treated. A hypothetical model of granulomatous inflammation is presented. The review emphasizes the areas in need of further study, particularly the fundamental nature of and the regulatory mechanisms controlling granulomatous inflammation.

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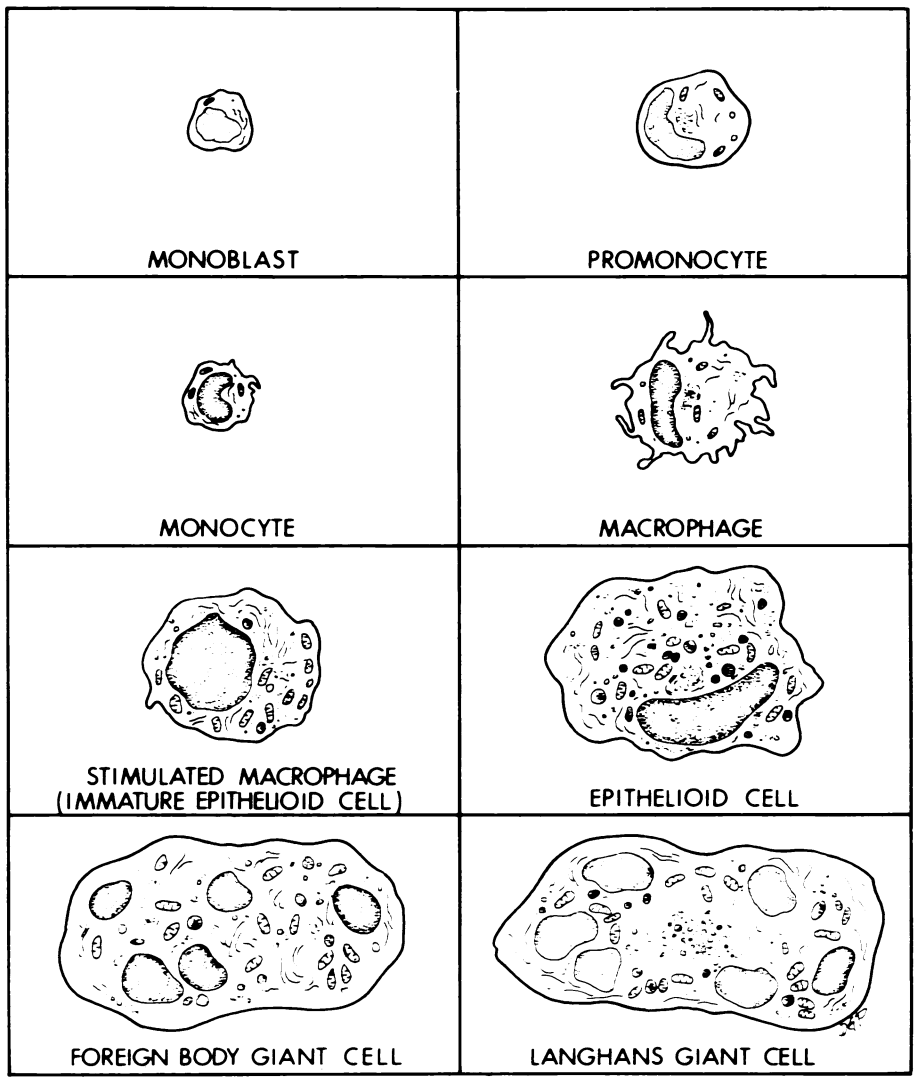
Supported in part by Grants 1-R01-CA-17684-01 and 1-R01-CA-14236-03 from the US Public Health Service and by Contract N01-CP-33313 from the National Cancer Institute.

Some of the terms as employed in this review can be briefly defined. *Monocytes* are circulating, relatively immature members of the mononuclear phagocyte system; *macrophages* are the mature form ordinarily found in the tissues (Text-figure 1). Upon stimulation, macrophages mature further into *immature epithelioid* cells and ultimately into *mature epithelioid* cells. *Mature granulomas* (foreign body granulomas) are dis-



TEXT-FIGURE 1—Schematic morphology of various elements of the mononuclear phagocyte system (*MPS*). Detailed definitions of the cells are found in Adams: Am J Pathol 76: 17–48, 1974.

tinct groups of compactly aggregated, interdigulated, mature macrophages, while *epithelioid granulomas* are similar lesions comprising epithelioid cells.

The Mononuclear Phagocyte System

These efficient, morphologically varied phagocytes constitute one widely distributed group of cells with a common origin, morphology, and function—the mononuclear phagocyte system (MPS)[4] (Table 1). Included are monocytes, macrophages, and specialized progeny such as epithelioid cells.[4] Cells of the system have the potential for avid pinocytosis and phagocytosis, strong adherence to glass, and the presence of receptors for activated $C\bar{3}$, and activated Fc, the N-terminal portion of immunoglobulin.[5,6] Mononuclear phagocytes are morphologically heterogenous because of their varied endocytic histories and degrees of maturation; but oval euchromatic nuclei, abundant cytoplasm and lysosomes, and prominent ruffles and pseudopods are common.[4,6]

Mononuclear phagocytes arise in the marrow and are released into the circulation as monocytes[7,8] (Table 1). After a brief sojourn there, they randomly leave the blood to become the tissue macrophages (histiocytes.)[7,8] The majority of mononuclear phagocytes in acute inflammatory foci have a similar origin, though a few are histiocytes.[9]

The morphology of the elements of the mononuclear phagocyte system has been extensively reviewed[10-14] (Text-figure 1). Their metabolism is also well understood. Mononuclear phagocytes are generally dependent upon glycolysis, though stimulated macrophages have marked increases in the hexosemonophosphate shunt.[15,16] These cells actively synthesize

Table 1—Cells of the Mononuclear Phagocyte System

Cell	Location	Half-life	Degree of maturity
Stem cell (Committed)	Marrow	?	0
Monoblast	Marrow	—	
Promonocyte	Marrow	1-2 days	±
Monocyte	Blood	1 day	+
Macrophages			
Normal tissue macrophage	Tissues	weeks	++
Inflammatory macrophage	Tissues	days to weeks	++
Hypermature macrophages			
Stimulated macrophage	Tissues	?	+++
Activated macrophage	Tissues	?	+++
Epithelioid cells	Tissues	weeks	++++
Giant cells	Tissues	days	++ to ++++

protein, much of which is packaged in the Golgi apparatus to accumulate in lysosomes.[6] Mononuclear phagocytes also manufacture a variety of proteins for extracellular release; these include: interferon, transferrin, endogenous pyrogen, complement components, lipids, lysozyme, acid hydrolases, neutral proteases, a cytotoxic factor, and several proteins that regulate the function and proliferation of other cells.[17,18] Cells of the macrophage family are actively motile and migrate by ameboid motion, engaging in both random motility and directed motility (chemotaxis).[19] They avidly ingest extracellular substances by pinocytosis and phagocytosis.[6] Once extracellular material is endocytosed, primary and secondary granules or lysosomes fuse with the endocytic vesicle to form secondary lysosomes.[6] Most complex molecules and organic particles are then attacked by the wide variety of lysosomal hydrolases and thoroughly degraded, e.g., to peptides in the case of proteins.[6] Various ingested microbes are readily killed in mononuclear phagocytes by mechanisms that are as yet incompletely established.[6] Finally, mononuclear phagocytes can injure or destroy allogeneic and neoplastic cells.[20-22]

Mononuclear phagocytes, unlike neutrophilic leukocytes, are not fully mature when released from the marrow.[23] Under the influence of yet unidentified stimuli, they mature into macrophages in the tissues.[23] This maturation has been extensively investigated *in vitro*, particularly in the laboratory of Cohn.[6,23,24] Small, immature mononuclear phagocytes develop greater size, large euchromatic nuclei, abundant cytoplasm, numerous Golgi profiles and lysosomes, stacks of smooth and rough endoplasmic reticulum, and many mitochondria.[24] These changes have biochemical counterparts: large increases in the content of protein, RNA, oxidative enzymes, and lysosomal hydrolases per cell.[24] Increases in acid hydrolases are particularly impressive, ranging up to fiftyfold.[24] Recently, maturation of mononuclear phagocytes has been directly demonstrated *in vivo*.[25]

The importance of mononuclear maturation lies in its direct relation to function. As mononuclear phagocytes mature, they attain increased functional capacity, which is so enhanced in three circumstances. First, macrophages maturing *in vitro* have more endocytic and degradative abilities than do monocytes.[15,26] The increased endocytosis encompasses both pinocytosis and phagocytosis. Second, mononuclear phagocytes, morphologically and biochemically similar to mature macrophages, are found at the site of microbial invasion in animals expressing cellular resistance.[27-29] These macrophages, termed activated macrophages, have enhanced microbicidal properties. Third, macrophages stimulated or elicited *in vivo* with a variety of agents such as peptone or BCG (the avirulent form of *Mycobacterium bovis*, strain Calmette-Guerin) have an increased capac-

ity to destroy neoplastic cells.[20-22] Mature, activated, and stimulated macrophages resemble one another in many ways and may all be examples of the same state of altered function. Yet, differences between them may exist.[30] The activated macrophages in salmonellosis have extremely potent bactericidal properties but a low content of acid hydrolases.[31] Stimulated macrophages, in one experimental system, have an increased phagocytic capacity towards starch granules and tubercle bacilli, while activated macrophages have increased phagocytic capacity only toward tubercle bacilli.[32] Although other factors such as availability of opsonins must be considered, the possibility that maturation, activation, and stimulation are separate physiologic states should be borne in mind; indeed, there may be different states of each. In this review, all instances of enhanced mononuclear function will be collectively termed *maturation*, with the realization that this may represent considerable simplification.

The regulation of mononuclear maturation as marked by the production and accumulation of acid hydrolases is incompletely understood (Table 2). Cohn's laboratory has shown that the interaction of macrophages with large anionic molecules, certain nucleotides, or antibodies directed against macrophage membranes stimulates maturation.[6,23] The phagocytosis of digestible particles produces maturation, although the phagocytosis of indigestible particles does not.[6,23] Soluble products of stimulated lymphocytes also produce maturation.[16,33,34] However, not all the hydrolases of mononuclear phagocytes respond similarly. Lysozyme is

Table 2—Representative Stimulants of Mononuclear Maturation *In Vitro*

Anionic molecules
Albumen
Fetuin
Polyglutamic acid
Heparin
Dextrose
Nucleotides
Adenosine
3',5'-Adenosinetriphosphate
Antibodies directed against macrophage membranes
Lymphokines
Digestible particles or substances
Erythrocytes
Carrageenan
Proteose peptone
Endotoxin
Lectins
Pathogens
Streptococci
Mycobacterium tuberculosis
Toxoplasma gondii

mostly secreted extracellularly, and its production is relatively independent of extracellular stimulants.[18] The production of plasminogen activator, a neutral serine esterase secreted extracellularly, is enhanced by stimulation but not in the same manner as are the acid hydrolases.[18]

Maturation of mononuclear phagocytes is relatively stable, requiring several days to occur.[24] In addition, mononuclear phagocytes may undergo short-lived and readily reversible changes in metabolism. Over a period of several hours, mononuclear phagocytes can become *excited* and develop increased oxygen consumption, phagocytic capacity, and motility.[32,35]

Mononuclear Phagocytes in Culture

The selective accumulation of leukocytes *in vivo* is incompletely understood but probably relates to the chemotaxis of these cells *in vitro* (see below).[36-37] Mononuclear phagocytes respond chemotactically to split fragments of complement, kallikrein, bacterial factors, basic peptides of neutrophils and a product of stimulated lymphocytes (chemotactic factor).[38-41] Ward and co-workers have found the latter two chemotactants to be specific for mononuclear phagocytes.[42,43] In comparison with neutrophilic leukocytes, mononuclear phagocytes have a slower rate of migration and do not respond to the activated fifth, sixth, and seventh components of complement ($C\overline{567}$ complex) that potently attracts neutrophils.[44] Interestingly, macrophages contain a proteinase capable of liberating chemotactic fragments from complement.[45]

The development of monocytes into epithelioid and giant cells can be easily reproduced *in vitro*. Over the past 50 years, numerous workers have observed that monocytes in culture develop into macrophages and ultimately into large epithelial-like cells or giant cells of both the Langhans's and foreign-body types.[46-48] Lewis, Willis, and Lewis demonstrated that the epithelial-like cells are histologically identical with epithelioid cells taken from tuberculous granulomas.[49] Sutton and Weis confirmed this observation ultrastructurally.[50] The development of monocytes into epithelioid cells is accompanied by the biochemical changes characteristic of maturation *in vitro*.[24] Epithelioid cells *in vitro* also have increased synthetic, phagocytic, bactericidal, and degradative capacities.[5] Thus, epithelioid cells *in vitro* are generally viewed as highly mature mononuclear phagocytes.[4,6,13,50] The development of epithelioid cells is stimulated by substances similar to those evoking maturation, such as mycobacteria, digestible particles, and surface active molecules.[25,26]

Giant cells are found under similar circumstances and arise by fusion of mature mononuclear phagocytes.[5] Employing Sendai virus, Gordon and

Cohn produced macrophage homokaryons (multinucleate giant cells).[23] Initially, the most mature macrophages fuse together and then undergo a random intermixing of cytoplasmic contents, followed by a colchicine-sensitive organization of the organelles.[23] The giant cells initially formed are of the foreign body type and those formed later are of the Langhans's type.[52] Giant cells seemingly arise by fusion of mature macrophages and take the foreign body or Langhans's form, depending on whether microtubule-mediated reorganization occurs.

Macrophages can also recapitulate the tissue-level organization of a granuloma in culture. As liquid cultures of mononuclear phagocytes mature, the macrophages grow apposite, touch, and develop complex interdigitations of cytoplasm.[46] If the cultures are in a semi-solid medium, the suspended cells cluster and form round aggregates.[53-55] Stimulated macrophages are more apt to aggregate than are nonstimulated macrophages.[55] The basis of this clustering is not known, but several explanations can be suggested. First, increased bulk could promote aggregation. Second, macrophages might secrete substances inducing aggregation. Among the numerous products secreted by macrophages is a factor which induces formation of hematopoietic colonies from stem cells.[56] Third, fundamental cell-to-cell recognition factors such as those operative in embryogenesis could be involved.[57-59] All three possibilities are consistent with the observation that maturation promotes aggregation. Production of colony-stimulating factor is greater by mature than by immature mononuclear phagocytes.[56] Likewise, morphogenesis may be regulated by surface antigens; [57-59] the specific surface antigens of macrophages are increased on mature forms.[60,61]

The division of mononuclear phagocytes is a frequent feature of granulomatous inflammation.[9] Replication of these cells is difficult to study *in vitro* because they are blocked in the G_0 phase and do not normally divide.[6] However, the application of a growth factor from conditioned medium plus a serum cofactor leads to their division.[62] Macrophages stimulated by thioglycolate are more susceptible to this induction than are nonstimulated cells.[63] The synthesis of DNA in cultured macrophages is also greater in mature than in immature mononuclear phagocytes.[64] Macrophages have recently been found to secrete substances capable of inducing other cells to divide,[65] so the possibility that they regulate their own growth should be considered.

Origin and Kinetics of Granulomatous Inflammation

The principal elements of a granuloma are mononuclear phagocytes.[5] Even granulomas induced by antigen in previously primed animals and

characterized by numerous eosinophils comprise over two-thirds mononuclear cells.[66] Granulomas induced by Freund's adjuvant comprise over 80% mononuclear phagocytes.[67] Most of the mononuclear phagocytes derive from monocytes, though a few are local histiocytes.[5,67] Ebert and Florey directly observed monocytes to immigrate into the tissues and there to develop into macrophages and epithelioid cells.[68] Spector and colleagues found the macrophages of granulomas derive principally from monocytes.[5,9,67] For example, the mononuclears of granulomas evoked by incomplete Freund's adjuvant resemble monocytes, not lymphocytes, in both their phagocytic capacity and pattern of DNA synthesis. Irradiation of these animals abolishes the granulomatous response; this suppression can be reversed by the infusion of marrow cells but not of thymic or lymph node cells. Epithelioid cells *in vivo* arise from macrophages.[9,69,70] Giant cells of the foreign body type arise by fusion of either macrophages or epithelioid cells;[5,52] Langhans's giant cells derive from foreign body giant cells.

The tempo at which granulomatous inflammation develops can vary considerably and depends upon both the inciting agent and the state of the host. In one series of experiments, the maturation of loose collections of monocytes into mature granulomas required 3, 9, and 10 days in lesions evoked by killed *M. tuberculosis*, living BCG vaccine, or barium sulfate, respectively.[25,71,72] The further development into epithelioid lesions required 4 and 22 days in the former two instances. The accumulation and number of epithelioid cells are accelerated in hosts displaying strong delayed hypersensitivity to the evoking agent.[73]

Once established, granulomas maintain themselves by various combinations of exudation and proliferation.[5] The kinetics of granulomas induced by many agents reveals two basic patterns: low turnover and high turnover. Low-turnover granulomas, induced by inert substances such as carbon or carrageenan, generally resemble mature (foreign body) granulomas and contain the irritant in most of the macrophages.[74,75] Immigration of exogenous monocytes and proliferation of endogenous macrophages are both low, and the lesions are mostly maintained by the long life-span of the constituent macrophages.[76] The small amount of replacement is mostly by proliferation.[75] High-turnover granulomas, induced by relatively toxic substances such as paraffin oil or mycobacteria, generally resemble epithelioid granulomas and contain the irritant in a small proportion of the macrophages.[74,75] Immigration and proliferation are both brisk, and the constituent macrophages have a relatively short life-span of several days. Maintenance of the lesions depends more on immigration. The natural history of high-turnover granulomas is to develop into low-turnover lesions as the inciting agent is degraded.[5,77]

The proliferating cells of granulomas are predominantly macrophages, particularly immature forms.[5] The macrophages divide to form small, mononuclear phagocytes which resemble lymphocytes histologically.[78] Young epithelioid cells can also divide, apparently having a proliferative capacity similar to that of macrophages.[69] Fully mature epithelioid cells do not divide in dermal lesions induced by BCG, though those of pulmonary lesions do.[79,80] Giant cells also do not proliferate, though some of their nuclei have a limited capacity for division.[52]

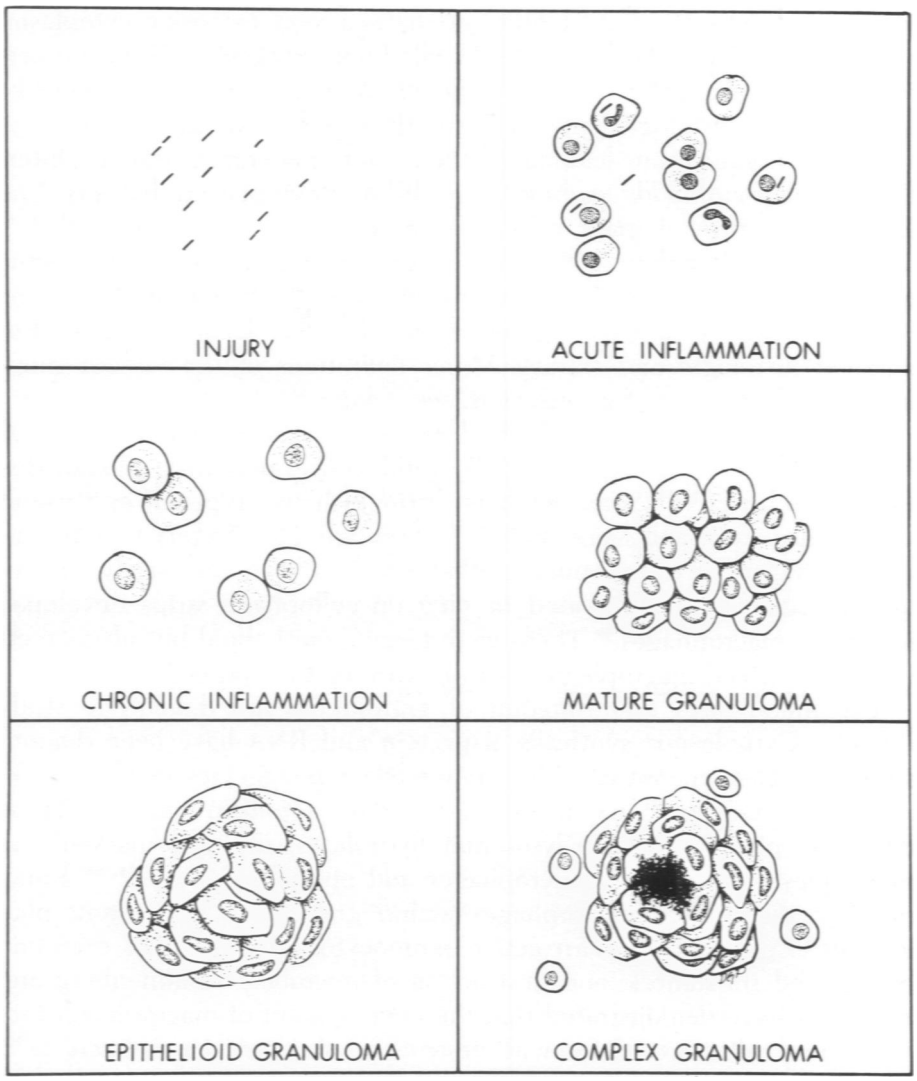
The life-span of the individual elements of a granuloma is varied. Macrophages of low-turnover lesions survive 4 to 8 weeks; those of high-turnover granulomas only a few days.[5] Epithelioid cells live 3 to 4 weeks, but giant cells survive only a few days.[52,69] The fate of these cells is not certain. The granuloma itself persists until the inciting agent is destroyed (see below). The constituent macrophages could have several possible ends: division, emigration, or death.[5,51] All of these have been shown to occur.[5,51,81,82] Recently, the population of mature elements such as epithelioid cells and macrophages has been shown to have an additional fate—reversion to less mature forms.[71] The relative contributions of these potential fates have not been ascertained.

Morphology and Function of the Granuloma

Although the histogenesis of a granuloma depends on both host and inciting agent, a composite picture can be drawn [25,83-85] (Text-figure 2). The initial event is acute inflammation. Within 24 hours, mononuclear phagocytes resembling promonocytes or monocytes dominate the leukocytic infiltrate and have phagocytosed most of the inciting agent. Over the next several days, the young mononuclears develop into loose sheets of immature macrophages.[25] By 3 to 7 days, the reaction comprises mostly mature macrophages plus a few foreign body giant cells.[25] The macrophages and giant cells are compactly aggregated into organized nests and sheets. These *mature granulomas* are often referred to as *foreign body granulomas,* although substances other than foreign bodies evoke this reaction.

Certain stimuli induce the further development of a mature granuloma.[25,83] The macrophages enlarge and become densely intertwined, so that previously crisp cytoplasmic borders are blurred. Their nuclei become eccentric and vesicular and the cytoplasm extensive and pale. These typical epithelioid cells—arranged in tightly packed nests and swirls, often around central Langhan's giant cells—form *epithelioid granulomas.*

Ultrastructural studies illuminate these observations. Scanning microscopy reveals young mononuclear phagocytes to be small, round cells having smooth surfaces. Mature macrophages are large, flattened cells



TEXT-FIGURE 2—Schematic diagram of the morphogenesis of a granuloma.

with a surface covered by numerous ridges, flanges, and villi.[86-89] Epithelioid cells are even larger and interlace with one another closely by intertwinings of the surface processes.[87] By transmission microscopy, young mononuclear phagocytes have heterochromatic nuclei and sparse, simple cytoplasm.[25] Mature macrophages have euchromatic nuclei, and an abundant cytoplasm filled with numerous organelles. Their pseudopods touch but do not interlock with those of adjoining macrophages.

Epithelioid cells are even larger and have a very extensive cytoplasm, complexly organized and completely filled with organelles. Their numerous pseudopods interlock with those of their neighbors in zipper-like arrays. Their histologic appearance is thus explained, the complex cytoplasmic organization leading to their pale appearance and the intertwining of pseudopods leading to the blurred cytoplasmic borders. The general structure of giant cells resembles that of epithelioid cells.[90,91] Those of the Langhans's type have extensively organized cytoplasmic organelles and peripherally located nuclei; those of the foreign-body type have randomly dispersed organelles and diffusely located nuclei. (For detailed histologic and ultrastructural definitions of the various mononuclear phagocytes of granulomas, see Adams.[25])

The fine structure of mononuclear phagocytes is generally similar whether observed in experimentally produced granulomas, human disease, or culture.[92-102] Some workers distinguish two types of epithelioid cells—phagocytic and secretory [92,94,100]—though the secretory type resembles immature or developing epithelioid cells.[25] In one study, mononuclear phagocytes cultivated *in vivo* on cellophane strips developed into large macrophages.[99] These were termed epithelioid but ultrastructurally resembled macrophages or immature epithelioid cells.[25]

The metabolism and function of granulomas are difficult to study directly. Cytoplasmic synthesis of protein and RNA have been demonstrated.[103] Histochemically, the mononuclear phagocytes contain respiratory and lysosomal enzymes similar to those found in culture.[51] These enzymes, particularly the lysosomal hydrolases, increase markedly as monocytes develop into macrophages and epithelioid cells.[104,105] Functionally, the mature macrophages within granulomas are potent phagocytes.[5] Epithelioid cells are active in pinocytosis, degradation, excretion of ingested substances, and destruction of microbes.[77] Dannenberg and associates have demonstrated that the development of macrophages into epithelioid cells is associated with destruction of ingested mycobacteria.[106] However, the phagocytic capacity of epithelioid cells may be less than that of macrophages. Although epithelioid cells do frequently contain recently formed phagosomes,[25] Spector's laboratory has found that epithelioid cells phagocytose fewer latex beads and bacteria than do macrophages.[77] This might relate to the complex interdigitations of the cell's pseudopods.[107]

The nature of epithelioid cells is clarified by observations of developing granulomas, indicating that young mononuclear phagocytes develop into mature macrophages and ultimately into epithelioid cells. Morphologically, metabolically, and funtionally, this development *in vivo* closely

resembles maturation of these cells *in vitro*. Most authors accordingly view epithelioid cells *in vivo* as mature mononuclear phagocytes.[24, 25, 50, 51] Spector has emphasized that epithelioid cells may have important secretory functions.[5] Certainly, mature mononuclear phagocytes secrete a wide variety of products and epithelioid cells are well-equipped morphologically and metabolically for such a role. Epithelioid cells are active in endocytosis, degradation, and microbial destruction and may have important secretory functions as well.

Induction of Granulomatous Inflammation

Certain foreign substances, especially particulate or oily foreign compounds and chronic intracellular parasites, evoke the granulomatous inflammatory response [108,109](Table 3). These substances presumably have properties in common that produce this response. To analyze these properties, the development of a granuloma can be divided into three steps: a) the development of a monocytic infiltrate; b) the aggregation, maturation, and organization of these cells into a mature granuloma; and c) the further evolution of this granuloma into an epithelioid one.

The differential accumulation of leukocytes in inflammatory foci may be an active rather than a passive process.[37] Possible explanations include: cell-specific chemotaxis, differential persistence of chemotactants, inhibitors of specific chemotactants, differences in migratory speed of various leukocytes, and differential persistence of leukocytes. Separate forces do seem to attract neutrophils and monocytes into granulomas.[37] For example, the number of monocytes entering granulomas produced by

Table 3—Representative Evokers of Mononuclear Inflammation

Chronic inflammation
Simple delayed hypersensitivity
Carbon
Fibrinogen
Salmonella typhi
Carmine
Mature granulomas
Inert particles
Lipids
High molecular-weight polymers
Delayed hypersensitivity to particle-bound antigens
Brucella abortus
Histoplasma capsulatum
Epithelioid granulomas
Mycobacterium tuberculosis
Granulomatous hypersensitivity
Blastomyces dermatitidis
Treponema pallidum
Chlamydia trachomatis

Freund's adjuvant is low but constant, while the number of entering neutrophils is initially high but soon falls to almost zero.[110] Leukocyte-specific chemotaxis may be operative. The potency of the $C\overline{567}$ complex in attracting neutrophils suggests inflammatory foci characterized by activated complement will be initially dominated by neutrophils. Several experimental studies do confirm this point.[111,112] For example, antigen–antibody complexes formed in antigen excess may evoke neutrophilic responses and those in extensive antibody excess evoke granulomas.[113] Differential persistence of chemotactants may also be operative. In peritoneal exudates induced by glycogen or mycobacteria, neutrophils and neutrophilic chemotactants decline concomitantly, while monocytes increase, and monocytic chemotactants remain constant.[114,115] Berenberg and Ward have recently described inhibitors of both neutrophilic and monocytic chemotactic factors.[116] The inflammatory accumulation of neutrophils and of monocytes is blocked by different metabolic inhibitors.[37] Finally, neutrophils are reported to migrate faster and to survive for less time than do monocytes.[19,44] The relative contribution of these possible mechanisms remains to be established.

Once a loose collection of mononuclear phagocytes has formed, the cells may disperse, maintain that composition, or develop into a granuloma. The development into a granuloma is characterized by the transition of a loose infiltrate of monocytes into an organized aggregate of mature macrophages. The maturation also marks the onset of extensive proliferation by the macrophages.[82] The ability of a foreign substance to evoke a granuloma might correlate with its content of substances capable of inducing maturation of macrophages. Products of streptococcal cell walls evoke modest maturation of macrophages *in vitro* and mature granulomas *in vivo*.[117] More to the point, the colloidal form of carrageenan evokes both, while the calcium form can evoke neither.[118] However, the ability to stimulate maturation cannot alone evoke a granuloma. Endotoxin potently stimulates macrophages in culture but does not evoke granulomas.[117,118] One additional property needed for granuloma development is the persistence of the evoking agent.[9] Microorganisms which can be completely degraded by macrophages in culture evoke only transient, acute inflammatory responses *in vivo*; only those organisms resistant to degradation produce granulomas.[119] Granulomas persist only so long as does the inciting agent.[71,78] Agents which possess these properties are generally complex substances, such as incomplete Freund's adjuvant or paraffin oil, rather than simple molecules, such as carbon or fibrinogen.[78] A further property promoting granuloma development may be particulate form. Some hypersensitivity-mediated granulomas require the agent to be

in particulate form (see below). Finally, a high local concentration of a given agent is more likely to evoke a granuloma.[78] In summary: Agents which evoke granulomas are likely to be persistent, particulate substances that are capable of producing modest maturation of macrophages.

Under appropriate circumstances, mature granulomas can evolve into epithelioid lesions, a change representing intensification of maturation.[25] The two processes are similar metabolically and morphologically and both are reversible after removal of the stimulant.[71] This step in the development of a granuloma relates to the character of the evoking agent. For example, particles of both oak and pine produce mature granulomas but only pine pollen, which has a high content of lipid, evokes epithelioid lesions.[120] Those substances evoking epithelioid lesions *in vivo* should thus produce extensive maturation *in vitro*. The methanol extraction residue (MER) of tubercle bacilli has been so tested and does have such a twofold effect.[121] Epithelioid granulomas seemingly arise when substances capable of evoking mature granulomas also stimulate mononuclear phagocytes to mature fully.

The factors which induce mononuclear maturation *in vivo* are not well characterized. The various lipid and wax fractions of the tubercle bacillus have long been viewed as responsible for producing epithelioid cells in some circumstances.[122-124] These studies have been critically considered.[125,126] In summary: Large amounts of lipid are required to produce lesions, and many question whether the lesions contain true epithelioid cells.[125,126] Another suggestion has been that colloidal substances evoke epithelioid cells.[127] Again, it may be questioned whether true epithelioid cells are produced. Finally, the presence of delayed hypersensitivity can also evoke epithelioid cells (see below). Attempts need be made to characterize precisely the substances evoking mononuclear maturation *in vivo*.

Delayed Hypersensitivity and Formation of the Granuloma

The role of delayed hypersensitivity in inducing granulomatous inflammation has been extensively investigated, particularly in the laboratories of von Lichtenberg and of Warren.[128,129] Delayed hypersensitivity to the substances evoking a granuloma is not necessary for development of the lesion.[128] Inert particles such as polystyrene beads or barium which evoke no hypersensitivity can induce granulomas.[127,128] Even in the experiments described below, small but definite granulomas are found in bead-treated control groups, where either antigen or sensitization is absent. On the other hand, delayed sensitivity alone does not evoke a granuloma. Skin-test reactions are certainly not granulomas. In sensitized hosts, intact mycobacteria evoke large granulomas but finely disrupted organisms pro-

duce only transient mononuclear responses resembling skin-test reactions.[130]

Delayed hypersensitivity can, however, greatly augment and accelerate the development of mature granulomas.[129,131] Experimental animals, sensitized to a given antigen and subsequently challenged with that antigen bound to inert particles, develop granulomas where the particles lodge.[128,129] The granulomas form faster and reach a larger size than in nonsensitized animals challenged with particle-bound antigen or in sensitized animals challenged with particle alone. The accelerated and augmented granulomas contain epithelioid and giant cells plus eosinophils, while the control lesions resemble typical mature granulomas.[128,129,131] Accelerated granuloma development is immunologically specific, dependent upon carrier rather than hapten recognition, transferred by T lymphocytes but not by serum, and is suppressed by thymectomy or the administration of antilymphocyte globulin.[129,132] Lymphokines, soluble products of stimulated lymphocytes, could mediate this response. *In vitro*, lymphokines attract and immobilize macrophages and stimulate them to mature.[133] Lymphokines have been implicated in inflammatory responses *in vivo*,[134-136] and antigen-induced granulomas can be stimulated *in vitro* to produce substances capable of inhibiting macrophage migration.[137]

The relationship of delayed sensitivity to the development of epithelioid granulomas is similarly complex. It is not obligate. Although some authors suggest that all epithelioid granulomas are hypersensitivity reactions,[131] cellophane strips, antigen–antibody complexes, and the methanol extraction residue of tubercle bacilli evoke epithelioid granulomas.[5,121] Furthermore, epithelioid granulomas can be evoked in thymectomized animals, which have significantly prolonged survival of allogeneic skin grafted across a strong transplantation barrier.[138] On the other hand, the presence of delayed sensitivity does accelerate and augment the development of epithelioid cells. In granulomas induced by BCG, the rate of development and the number of epithelioid cells are greatest when delayed sensitivity is maximal and bacillary products are still present.[79] This phenomenon is well demonstrated in leprosy, where the formation of epithelioid cells and the strength of cell-mediated immunity are directly correlated.[139]

Granulomatous hypersensitivity encompasses a group of diseases, characterized by the indolent development of epithelioid granulomas after the injection of specific antigen into a sensitized host.[140] These diseases are typified by deodorant-induced or zirconium granulomatosis. In these patients, the injection of elemental zirconium specifically produces epithelioid granulomas 6 to 8 weeks later. Injection of zirconium into nonsensitized patients produces only chronic inflammation or mature

granulomas.[140] Granulomatous berylliosis is a similar disorder. Peripheral lymphocytes from these latter patients undergo specific blastogenesis when challenged with beryllium.[141,142] Recently, an experimental model of beryllium sensitivity has been described in which the animals express delayed sensitivity to beryllium.[143] Lymphocytes of these animals undergo blastic transformation when specifically challenged with beryllium and can transfer the sensitive state to naive animals. It is tempting to group sarcoidosis with these disorders. The injection of Kveim antigen does evoke epithelioid granulomas within 6 to 8 weeks. The reaction of peripheral leukocytes from patients who have sarcoidosis to Kveim antigen is currently unsettled; various laboratories have reported both positive and negative responses.[144,145]

Accessory Features of Granulomatous Inflammation

Emphasis has been placed on the accumulation and maturation of mononuclear phagocytes in granulomas. Other histologic characteristics which may be present (such as subacute inflammation or necrosis) are viewed as being superimposed. Conceptually, this view is useful because it links otherwise disparate disorders such as actinomycosis and sarcoidosis.[108] Factually, it can be defended because pure granulomas can easily be transformed into complex ones by subtle alterations in host or agent. For example, the onset of delayed hypersensitivity generally marks the development of caseous necrosis in tuberculosis.[51,125] In coccidioidomycosis, the release of endospheres from the spherules converts mature granulomas into subacute inflammatory responses.[146]

Necrosis accompanies many granulomas. In many cases, the development of delayed hypersensitivity to the intruder produces necrosis. For example, the onset of sensitivity and caseous necrosis are coincident in tuberculosis;[51,125] tubercle bacilli do not generally evoke necrosis in unsensitized hosts.[125] The effector mechanism producing the necrosis is not known. *In vitro*, stimulated lymphocytes release factors which are nonspecifically toxic for many cells.[133] Vascular spasm and subsequent, central ischemia have been observed in hypersensitivity reactions.[147] Finally, macrophages die much more rapidly in sensitized than in nonsensitized hosts.[73] Factors other than hypersensitivity also produce necrosis. Foreign substances such as silica are directly toxic to macrophages and cause leakage of lysosomal enzymes.[117] Substances such as endotoxin, certain bacteria, and antigen–antibody complexes are not toxic but do cause macrophages to release large quantities of acid hydrolases into the extracellular compartment.[117] Lastly, the potent neutral proteases, elastases, and collagenases of macrophages are extensively secreted ex-

tracellularly after a complex triggering by ingested particles.[148,149] The enzymes of macrophages remain active after the macrophages die.[150] Although granulomas are not always accompanied by necrosis, their constituent macrophages do contain potent lytic enzymes capable of extensive tissue destruction when released extracellularly by a variety of mechanisms.

Leukocytes other than mononuclear phagocytes may accompany granulomas. Small mononuclear cells having small dark nuclei and histologically resembling lymphocytes are common. Most of these are very young mononuclear phagocytes, because they have the DNA-synthesis and the phagocytic characteristics of monocytes not lymphocytes.[5] Lymphocytes are present in some chronic inflammatory responses. Contact-sensitivity sites and granulomas induced by *M. tuberculosis* in sensitized animals both contain short-lived, replicating, steroid-sensitive T-cells.[151,152] B-cells have been identified in chronic inflammatory responses.[153-156] Lymphocytes are generally present in granulomas when delayed hypersensitivity is operative.[67] Eosinophils accumulate around antigen–antibody complexes *in vivo*, if thymic function is intact. Lymphocytes secrete a chemotactant precursor, which attracts eosinophils after it has been activated by antigen–antibody complexes.[134] The appearance of eosinophils may then indicate the presence of antigen–antibody complexes plus thymus-dependent lymphocytes.[157]

Granulomatous Inflammation in the Host's Economy

One of the basic functions of the granuloma is to rid the host of unwanted substances.[158-160] Most foreign substances and toxins are eventually phagocytosed by macrophages and therein detoxified and degraded as much as possible. Macrophages are also important in the destruction of autochthonous tissue debris, effete cells, and metabolites.[12,158] If these exogenous and endogenous substrates cannot be degraded, they are contained within macrophages and thus segregated from the host's *milieu interieur*.[12]

Granulomatous inflammation is highly effective in destroying pathogens.[159] Pathogens not destroyed by the acute inflammatory response, generally facultative or obligate intracellular parasites, become contained in macrophages, which may coalesce to form mature granulomas.[161,162] This sequence of events can be seen in chronic granulomatous disease, where organisms that normally evoke suppurative inflammation produce granulomas instead.[161] The resistant organisms may be destroyed within the macrophages. If they persist, delayed hypersensitivity often develops. Epithelioid granulomas then develop, and the organisms are destroyed.

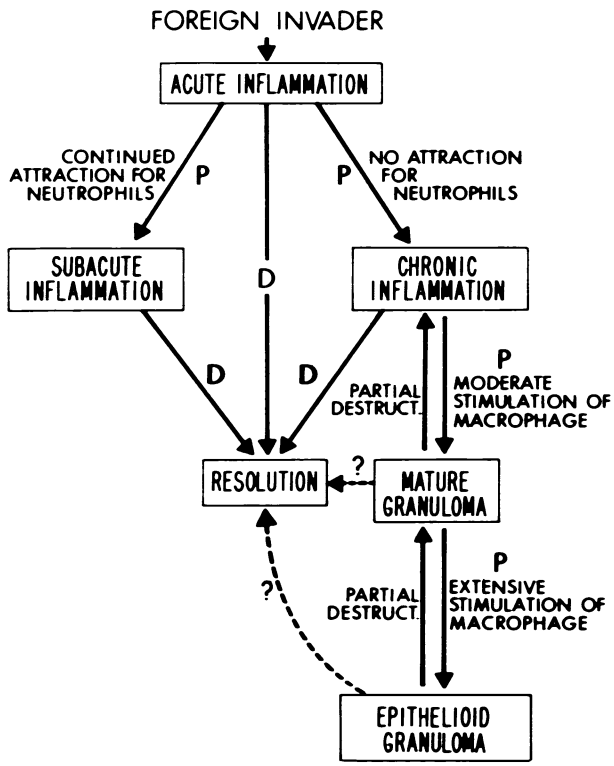
Thus, the development of a granuloma frequently heralds destruction of persisting, resistant, intracellular parasites.

Granulomas may also be operative in inducing immunity. Since macrophages are important in the induction and regulation of the immune response,[164,165] the injection of an antigen into areas of granulomatous inflammation increases the immune response to that antigen.[166] This adjuvant effect involves stimulation of macrophages as well as of T and B lymphocytes.[166,167] The presence of a granuloma suggests that cellular and humoral immunity to antigens contained within the granuloma will be augmented. Living, intracellular parasites are particularly likely to stimulate delayed sensitivity.[28] It is interesting that those invaders, which are not directly destroyed by macrophages, have the capacity to stimulate T cells and thus ultimately focus the activities of macrophages upon destroying the invaders.[28]

Finally, granulomas may take part in the destruction of neoplasms. Macrophages are important in the host's general resistance to tumors and their cytotoxic effectiveness *in vitro* is increased when they are stimulated.[20] An example of this augmentation *in vivo* is found in the regression of localized nodules of tumor injected with BCG.[168] Hanna, Zbar, Rapp, and associates studied intradermally transplanted hepatomas in guinea pigs and found that intratumoral injection of BCG produces complete regression of the local tumor and of nodal metastases.[169] The regressing tumors, local and distant, are characterized by the presence of granulomas. Similar observation have been made in humans and in other experimental systems.[168]

Coda

On the basis of current information, a hypothetical model of granulomatous information can be constructed (Text-figure 3). The introduction of a foreign substance or parasite into the host evokes acute inflammation, including exudation of neutrophilic and mononuclear phagocytes. These leukocytes engulf the invader and attempt to destroy it. If their attempt is successful, the inflammatory response subsides. If not, the invader comes to lie within mononuclear phagocytes. The continued presence of the invader, especially in a particulate form and in a high concentration, may stimulate mononuclear phagocytes to mature, coalesce, and divide. A mature granuloma thus results. If the intruder and its components are particularly stimulatory or if delayed sensitivity develops, the macrophages mature even further into epithelioid cells. The resultant epithelioid granuloma persists until the foreign intruder is destroyed. Then, the lesions slowly resolve as the mononuclears die, revert to less



TEXT-FIGURE 3—Conceptual model of granulomatous inflammation. Persistence of the foreign invader (*P*) and its destruction (*D*) are denoted.

mature forms, or wander off. Resolving epithelioid granulomas first take the form of mature granulomas, which in turn further develop into simple chronic inflammation. Alterations in either the intruder or the host can change this picture by superimposing additional features such as necrosis or influx of other leukocytes.

The definition and classification of granulomatous inflammation are to some extent arbitrary. Several definitions of a granuloma are possible: a) a focal collection of mononuclear cells including lymphocytes, mononuclear phagocytes, and plasma cells; b) a focal collection of mononuclear phagocytes alone which has maintained that appearance since its inception; c) a focal, organized collection of mononuclear phagocytes; or d) a focal organized collection of mononuclear phagocytes containing epithelioid cells. The first two definitions do not distinguish granulomatous from chronic inflammation. Because the third permits histologic distinction of a granuloma, it is employed here. However, it does exclude disorders such

as typhoid and anthracosis, which have been classed as granulomas.[159] The fourth is unnecessarily restrictive, excluding most reactions to foreign substances. Grouped here as mature and epithelioid types, granulomas might also be classified on the basis of turn-over (high or low) or origin (immunologic or nonimmunologic).[107] The latter two classifications cannot be applied histologically and could be confusing, in that histologically similar lesions might be classified differently.

What is the fundamental significance of granulomatous inflammation? The development of a granuloma may represent a specific, inflammatory response evoked by certain foreign invaders.[108] Alternatively, its presence may merely indicate that a foreign substance is being contained and degraded by the mononuclear phagocyte system and that this is occurring under certain conditions of macrophage number and maturation such that the histologic form, which we recognize as a granuloma, is produced. To some extent, the issue is philosophic and not capable of resolution. The presence of a granuloma does signify that a foreign substance has resisted destruction by the acute inflammatory response and is being sequestered and destroyed by mature elements of the mononuclear phagocyte system. From a phylogenetic point of view, phagocytosis by mononuclear phagocytes is one of the host's most primitive defense systems.[170] The primitive phagocytes of molluscs, for example, surround foreign objects and then form morulae about the invader.[160] The granuloma will undoubtedly remain important as a diagnostic reaction to the surgical pathologist, but its position as a specific inflammatory response needs to be reassessed critically.

Recent advances in our understanding of mononuclear phagocytes plus extensive studies of mononuclear inflammation have provided a large body of data on the granuloma. A conceptual framework has been constructed and employed to assess this data. Viewed in this fashion, many aspects of granulomatous inflammation can be explained, though many others need further study. What factors regulate the aggregation, division and maturation of mononuclear phagocytes *in vivo*? What is the fate of the cells of a granuloma? The function and metabolism of epithelioid cells need more investigation, particularly in regard to secretory activity. What factors are responsible for the differential accumulation of leukocytes, for the development of granulomas, and for the production of epithelioid cells? What are the mechanisms by which delayed hypersensitivity alters granulomas? What is the immunologic basis of granulomatous hypersensitivity, as well as the cause of necrosis in granulomas? The analytical framework and developmental model presented here may have to be modified or discarded in the light of such studies, but hopefully it will first fulfill a heuristic function.

References

1. Long ER: A History of Pathology. New York, Dover Publications, Inc., 1965
2. Ward PA: Inflammation. Principles of Pathobiology. Second edition. Edited by MF LaVia, RB Hill Jr. New York, Oxford University Press, 1975, pp 97–140
3. Sell S: Immunology, Immunopathology, and Immunity. New York, Harper and Row, Publishers, 1972
4. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL: The mononuclear phagocyte system: A new classification of macrophages, monocytes, and their precursor cells. Bull WHO 46:845–852, 1972
5. Spector WG: The macrophage: Its origins and role in pathology. Pathobiol Annu 4:33–64, 1974
6. Steinman RM, Cohn ZA: The metabolism and physiology of the mononuclear phagocytes. The Inflammatory Process, Vol I, Second edition. Edited by BW Zweifach, L Grant, RT McCluskey. New York, Academic Press, Inc., 1974, pp 449–510
7. Volkman A: The origin and fate of the monocyte. Ser Haematol 3:62–92, 1970
8. van Furth R: Origin and kinetics of monocytes and macrophages. Semin Hematol 7:125–141, 1970
9. Spector WG: The granulomatous inflammatory exudate. Int Rev Exp Pathol 8:1–55, 1969
10. Fedorko ME, Hirsch JG: Structure of monocytes and macrophages. Semin Hematol 7:109–124, 1970
11. Carr I: The Macrophage. New York, Academic Press, Inc., 1973
12. Vernon-Roberts B: The Macrophage. Cambridge, Cambridge University Press, 1972
13. Nelson DS: Macrophages and Immunity. New York, North Holland Publishing Co.–Wiley Interscience, 1969
14. Nichols BA, Bainton DF: Ultrastructure and cytochemistry of mononuclear phagocytes. Mononuclear Phagocytes in Immunity, Infection, and Pathology. Edited by R van Furth, Oxford, Blackwell Scientific Publications, 1975, pp 17–56
15. Axline SG: Functional biochemistry of the macrophage. Semin Hematol 7:142–160, 1970
16. David JR: A brief review of macrophage activation by lymphocyte mediators. The Phagocytic Cell in Host Resistance. Edited by JA Bellanti, DH Dayton. New York, Raven Press, 1975, pp 143–149
17. Adams DO: The mononuclear phagocyte system: Human disorders and their evaluation. Annu Rev Allergy, 1975, In Press
18. Gordon S, Unkeless JC, Cohn ZA: The macrophage as secretory cell. Immune Recognition. Edited by AS Rosenthal. New York, Academic Press, Inc., 1975, pp 589–614
19. Stossel TP: Phagocytosis. New Engl J Med 290:717–723, 774–780, 833–839, 1971
20. Levy MH, Wheelock EF: The role of macrophages in defense against neoplastic disease. Adv Cancer Res 20:131–163, 1974
21. Nelson DS: Macrophages as effectors of cell-mediated immunity. Macrophages and Cellular Immunity. Edited by AI Laskin, H Lechevalier. Cleveland, CRC Press, 1972, pp 45–76
22. Cerottini J-C, Brunner KT: Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. Adv Immunol 18:67–132, 1974
23. Gordon S, Cohn ZA: The macrophage. Int Rev Cytol 36:171–214, 1973
24. Cohn ZA: The structure and function of monocytes and macrophages. Adv Immunol 9:163–214, 1968
25. Adams DO: The structure of mononuclear phagocytes differentiating *in vivo*. I. Sequential fine and histologic studies of the effect of bacillus Calmette-Guerin (BCG). Am J Pathol 76:17–48, 1974

26. Blanden RV: Modification of macrophage function. J Reticuloendothel Soc 5:179–202, 1968
27. WHO Scientific Group: Cell-mediated immunity and resistance to infection. WHO Techn Rep Ser 519, 1973, pp 1–64
28. Mackaness GB: Lymphocyte-macrophage interaction. Inflammation: Mechanisms and Control. Edited by IH Lepow, PA Ward. New York, Academic Press, 1972, pp 163–176
29. North RJ: Cell mediated immunity and the response to infection. Mechanisms of Cell-Mediated Immunity. Edited by RT McCluskey, S Cohen. New York, John Wiley and Sons, 1974, pp 185–219
30. Allison AC, Davies P: Mononuclear phagocyte activation in some pathological processes. Activation of Macrophages. Edited by WH Wagner, H Hahn. Amsterdam, Excerpta Medica, 1974, pp 141–154
31. Mackaness GB: Discussion. Immunobiology of Tumor Host Relationship. Edited by RT Smith, M Landy. New York, Academic Press, Inc., 1975, p 138
32. Karnovsky ML: Biochemical aspects of the functions of polymorphonuclear and mononuclear leukocytes. The Phagocytic Cell in Host Resistance. Edited by JA Bellanti, DH Dayton. New York, Raven Press, 1975, pp 25–39
33. Adams DO, Biesecker JL, Koss LG: The activation of mononuclear phagocytes *in vitro*: Immunologically mediated enhancement. J Reticuloendothel Soc 14:550–570, 1973
34. Simon HB, Sheagren JN: Cellular immunity *in vitro*. I. Immunologically mediated enhancement of macrophage bactericidal capacity. J Exp Med 133:1377–1389, 1971
35. Dannenberg AM, Jr, Walter PC, Kapral FA: A histochemical study of phagocytic and enzymatic functions of rabbit mononuclear and polymorphonuclear exudate cells and alveolar macrophages. II. The effect of particle ingestion on enzyme activity: two phases of *in vitro* activation. J Immunol 90:444–465, 1963
36. Keller HU, Sorkin E: Chemotaxis of leukocytes. Experientia 24:641–652, 1968
37. Perper RJ, Sandar M, Stecher VJ, Oronsky AL: Physiological and pharmacological alterations of rat leukocyte chemotaxis *in vivo*. Ann NY Acad Sci 256:19–209, 1975
38. Wilkinson PC: Chemotaxis and Inflammation. Edinburgh, Churchill-Livingston, 1970
39. Snyderman R, Mergenhagen SC: Chemotaxis of macrophages. Immunobiology of the Macrophage. Edited by DS Nelson. New York, Academic Press, Inc., (In press)
40. Ward PA: Leukotaxis and leukotactic disorders. Am J Pathol 77:520–538, 1974
41. Keller HU, Hess MW, Cottier H: Physiology of chemotaxis and random motility. Sem Hematol 12:47–57, 1975
42. Ward PA, Remold HG, David JR: A leukotactic factor produced by sensitized lymphocytes. Science 163:1079–1081, 1969
43. Ward PA: Chemotaxis of mononuclear cells. J Exp Med 128:1201–1221, 1968
44. Ward PA: Comparisons and contrasts in the chemotactic behavior of mononuclear cells and neutrophils. Second International Congress on Immunology. Edited by S Cohen, G Cudkowicz, RT McCluskey, Basel, S. Karger, 1971, pp 131–196
45. Snyderman R, Shin HS, Dannenberg AM Jr: Macrophage proteinase in inflammation: The production of chemotactic activity from the fifth component of complement by macrophage proteinase. J Immunol 109:896–898, 1972
46. Jacoby F: Macrophages. Cells and Tissues in Culture, Vol II. Edited by EN Willmer. New York, Academic Press, 1965, pp 1–93
47. Lewis MR, Lewis WH: Transformation of mononuclear blood-cells into macrophages, epithelioid cells and giant cells in hanging-drop blood cultures from lower vertebrates. Contrib Embryol 18:95–120, 1926
48. Bloom W: Fibroblasts and macrophages. Handbook of Hematology, Vol II. Edited by H Downey. New York, Paul B. Hoeber, 1938, pp 1336–1373

49. Lewis MR, Willis HS, Lewis WH: The epithelioid cells of tuberculous lesions. Bull J Hopkins Hosp 36:175–184, 1925
50. Sutton JS, Weiss L: Transformation of monocytes in tissue culture into macrophages, epithelioid cells and multinucleated giant cells: An electron microscope study. J Cell Biol 28:303–332, 1966
51. Dannenberg AM Jr: Cellular hypersensitivity and cellular immunity in the pathogenesis of tuberculosis: Specificity, systemic, and local nature and associated macrophage enzymes. Bacteriol Rev 32:85–102, 1968
52. Mariano M, Spector WG: The formation and properties of macrophage polykaryons (inflammatory giant cells). J Pathol 113:1–19, 1974
53. Lin H-S, Stewart CC: Colony formation by mouse peritoneal exudate cells in vitro. Nature [New Biol] 243:176–177, 1973
54. von Heyden HW, von Heyden D: Characteristics of macrophages *in vitro* derived from peripheral blood cells. Blut 29:37–42, 1974
55. Stewart CC, Lin H-S, Adles C: Proliferation and colony-forming ability of peritoneal exudate cells in liquid culture. J Exp Med 141:1114–1132, 1975
56. Golde DW, Finley TN, Cline MJ: Production of colony-stimulating factor by human macrophages. Lancet 2:1397–1399, 1972
57. Bennett D, Boyse EA, Old LJ: Cell surface immunogenetics in the study of morphogenesis. Proceedings of the Third Lepetit Colloquium. Edited by LG Silvestri. Amsterdam, North Holland Publishing Co, 1972, pp 247–263
58. Roth S: A molecular model for cell interactions. Q Rev Biol 48:541–563, 1973
59. Moscona AA: Surface specification of embryonic cells: Lectin receptors, cell recognition and specific cell ligands. The Cell Surface in Development. Edited by AA Moscona. New York, John Wiley and Sons, 1974, pp 67–99
60. Feldman JD, Tubergen DG, Pollock EM, Unanue ER: Distribution of a macrophage-specific antigen. Cell Immunol 5:325–337, 1972
61. Virolainen M, Lahti A, Häyry P: Differentiation of macrophage antigen(s) in mouse bone marrow and buffy coat cultures. The Reticuloendothelial System and Immune Phenomena. Edited by NR DiLuzio, K Flemming. New York, Plenum Press, 1971, pp 47–51
62. Virolainen M, Defendi V: Dependence of macrophage growth *in vitro* upon interaction with other cell types. Growth Regulating Substances for Animal Cells in Cultures. Wistar Institute Symposium Monograph No. VII. Philadelphia, Wistar Institute, 1967, pp 67–83
63. Mauel J, Defendi V: Regulation of DNA synthesis in mouse macrophages. II. Studies on mechanisms of action of the macrophage growth factor. Exp Cell Res 65:377–385, 1971
64. Wiener E: DNA: Synthesis in peritoneal mononuclear leukocytes. Exp Cell Res 45:450–459, 1967
65. Calderon J, Kiely J-M, Lefko JL, Unanue ER: The modulation of lymphocyte functions by molecules secreted by macrophages. J Exp Med 142:151–164, 1975
66. Speirs RS, Speirs EE, Ponzio NM: Eosinophils in humoral and cell-mediated responses. Developments in Lymphoid Cell Biology. Edited by AA Gottlieb. Cleveland, CRC Press, 1974, pp 51–75
67. Spector WG: Chronic inflammation. The Inflammatory Process, Vol III, Second edition. Edited by BW Zweifach, L Grant, RT McCluskey, New York, Academic Press, 1974, pp 277–290
68. Ebert RH, Florey HW: The extravascular development of the monocyte observed *in vivo*. Br J Exp Pathol 20:342–356, 1939
69. Papadimitriou JM, Spector WG: The origin, properties, and fate of epithelioid cells. J Pathol 105:187–203, 1971
70. Epstein WL, Kransnobrod H: The origin of epithelioid cells in experimental granulomas of man. Lab Invest 18:190–195, 1968

71. Adams DO: The structure of mononuclear phagocytes differentiating *in vivo*. II. The effect of *Mycobacterium tuberculosis*. Am J Pathol 80:101–116, 1975
72. Goldner RW, Adams DO: The structure of mononuclear phagocytes differentiating *in vivo*. III. The effect of barium sulfate, talc and Thorotrast. (In preparation)
73. Dannenberg AM Jr, Ando M, Shima K: Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. III. The turnover of macrophages and its relation to their activation and antimicrobial immunity in primary BCG lesions and those of reinfection. J Immunol 109:1109–1121, 1972
74. Papadimitriou JM and Spector WG: The ultrastructure of high- and low-turnover inflammatory granulomata. J Pathol 106:37–43, 1972
75. Ryan GB, Spector WG: Macrophage turnover in inflamed connective tissue. Proc R Soc B 175:269–292, 1970
76. Ryan GB, Spector WG: Natural selection of long-lived macrophages in experimental granulomata. J Pathol 99:139–151, 1969
77. Spector WG, Heesom N, Stevens JE: Factors influencing chronicity in inflammation of rat skin. J Pathol 96:203–213, 1968
78. Spector WG, Mariano M: Macrophage behavior in experimental granulomas.[14] pp 927–942
79. Ando M, Dannenberg AM Jr, Shima K: Macrophage accumulation, division, maturation and digestive and microbicidal capacities in tuberculous lesions. II. Rate at which mononuclear cells enter and divide in primary BCG lesions and those of reinfection. J Immunol 109:8–19, 1972
80. Shima K, Dannenberg AM Jr, Ando M, Chandrasekhan S, Seluzicki J, Fabrikant JI: Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. I. Studies involving their incorporation of tritiated thymidine and their content of lysomal enzymes and bacilli. Am J Pathol 67:159–180, 1972
81. Ginsberg I, Mitranis S, Ne'eeman N, Lahav M: Granulomata in streptococcal inflammation: Mechanisms of localization, transport, and degradation of streptococci in inflammatory sites.[14] pp 981–1014
82. Smith JB, McIntosh GH, Morris B: The migration of cells through chronically inflamed tissues. J Pathol 100:21–29, 1970
83. Spector WG, Lykke AWJ: The cellular evolution of inflammatory granulomata. J Pathol Bacteriol 92:163–177, 1966
84. Chapman JS: A review of early events in tubercule formation. Acta Pathol Microbiol Scand [A] 80:(Suppl 233) 189–194, 1972
85. Berry CL: The development of the granuloma of histoplasmosis. J Pathol 97:1–10, 1969
86. Carr I, Clarke JA, Salsburg AJ: The surface structure of mouse peritoneal cells: A study with the scanning electron microscope. J Microsc 89:105–111, 1969
87. Papadimitriou JM, Finlay-Jones J-M, Walters MN-I: Surface characteristics of macrophages, epithelioid and giant cells using scanning electron microscopy. Exp Cell Res 76:353–362, 1973
88. Parakkal P, Pinto J, Hanifin JM: Surface morphology of human mononuclear phagocytes during maturation and phagocytosis. J Ultrastruct Res 48:216–226, 1974
89. Albrecht RM, Hinsdill RD, Sandok PL, MacKenzie AP, Sachs IB: A comparative study of the surface morphology of stimulated and unstimulated macrophages prepared without chemical fixation for scanning EM. Exp Cell Res 70:230–232, 1972
90. Black MM, Epstein WL: Formation of multinucleate giant cells in organized epithelioid cell granulomas. Am J Pathol 74:263–274, 1974

91. Papadimitriou JM, Archer M: The morphology of murine foreign body multinuclear giant cells. J Ultrastructural Res 49:372–386, 1974
92. Williams WJ, James EMV, Erasmus DA, Davies T: The fine structure of sarcoid and tuberculous granulomas. Postgrad Med J 46:496–500, 1970
93. Williams WJ, Fry E. James EMV: The fine structure of beryllium granulomas. Acta Pathol Microbiol Scand [A] 233:195–202, 1972
94. Wanstrup J, Christensen HE: Sarcoidosis. Ultrastructural investigations on epithelioid cell granulomas. Acta Pathol Microbiol Scand 66:169–185, 1966
95. Williams WJ, Erasmus DA, Jenkins D, James EMV, Davies T: A comparative study of the ultrastructure and histochemistry of sarcoid and tuberculous granulomas. Fifth International Conference on Sarcoidosis, 1969. Prague, Universita Karlova Praha, 1971, pp 115–120
96. James EMV, Williams WJ: Fine structure and histochemistry of epithelioid cells in sarcoidosis. Thorax 29:115–120, 1974
97. Elias PM, Epstein WL: Ultrastructural observations on experimentally induced foreign-body and organized epithelioid-cell granulomas in man. Am J Pathol 52:1207–1223, 1968
98. Dumont A, Sheldon H: Changes in the fine structure of macrophages in experimentally produced tuberculous granulomas in hamsters. Lab Invest 14:2034–2055, 1965
99. Galindo B, Imaeda T: Cellular response to Freund's adjuvant in the rabbit lung. An electron microscope study. Lab Invest 15:1659–1681, 1966
100. Gusek W: Histologie und elektronenmikroskopische komparative Zytologie tuberkulöser und epitheloidzelliger Granulome. Bibl Tuberc 14:97–156, 1965
101. Hirsch JG, Fedorko ME, Dwyer CM: The ultrastructure of epithelioid and giant cells in positive Kveim test sites and sarcoid granulomata. La Sarcoidose: Rapports De La IV^{e} Conference Internationale. Paris, Masson et Cie, 1967, pp 59–70
102. Williams D, Williams WJ, Williams JE: Enzyme histochemistry of epithelioid cells in sarcoidosis and sarcoid-like granulomas. J Pathol 97:705–709, 1969
103. McDougal JS, Azar HA: Tritiated proline in macrophages: In vivo and in vitro uptake by foreign-body granulomas. Arch Pathol 93:13–17, 1972
104. Papadimitriou JM, Wyche PA: An examination of murine foreign body giant cells using cytochemical techniques and thin layer chromatography. J Pathol 114:75–83, 1974
105. Dannenberg AM Jr, Meyer OT, Esterly JR, Kambara T: The local nature of immunity in tuberculosis, illustrated histochemically in dermal BCG lesions. J Immunol 100:931–941, 1968
106. Ando M, Dannenberg AM, Sugimoto M, Tepper BS: Histochemical studies relating the activation of macrophages with the subsequent destruction of tubercle bacilli. In press
107. Schaberg A, Editor: Discussion of Paper 60.[14] pp 938–942
108. Forbus WD: Granulomatous Inflammation. Springfield, Ill., Charles C Thomas Publishers, 1949
109. Refvem O: The pathogenesis of Boeck's disease (sarcoidosis). Acta Med Scand, Suppl 294:1–168, 1954
110. Spector WG, Walters MN-I, Willoughby DA: The origin of the mononuclear cells in inflammatory exudates induced by fibrinogen. J Pathol Bacteriol 90:181–192, 1965
111. Slauson DO, Dahlstrom MA: The pulmonary inflammatory response: cellular events in experimental pulmonary arterial hypersensitivity disease. Am J Pathol 79:119–130, 1975
112. Ward PA: Complement-dependent phlogistic factors in rheumatoid synovial fluids. Ann NY Acad Sci 256:169–176, 1975

113. Spector WG, Heesom N: The production of granulomata by antigen-antibody complexes. J Pathol 98:31–39, 1969
114. Wilkinson PC, O'Neill GJ, McInroy RJ, Cater JC, Roberts JA: Chemotaxis of macrophages: The role of a macrophage-specific cytotoxin from anaerobic corynebacteria and its relation to immunopotentiation *in vivo*. Immunopotentiation. Edited by GEW Wolstenholme, J Knight. Amsterdam, Associated Scientific Publishers, 1973, pp 121–140
115. White RG: Granuloma formation and its relation to chemotaxis: A discussion of the role of two groups of micro-organisms: the mycobacteria and the anaerobic coryneforms. Antibiot Chemother 19:382–408, 1974
116. Berenberg JL, Ward PA: Chemotactic factor inactivator in normal human serum. J Clin Invest 52:1200–1206, 1973
117. Davies P, Allison AC: Secretion of macrophage enzymes in relation to the pathogenesis of chronic inflammation. Immunobiology of the Macrophage. Edited by DS Nelson. New York, Academic Press, Inc., 1975, pp 428–463
118. Page RC, Davies P, Allison AC: Participation of mononuclear phagocytes in chronic inflammatory diseases. J Reticuloendothel Soc 15:413–438, 1974
119. Spector WG, Reichhold N, Ryan GB: Degradation of granuloma-inducing microorganisms by macrophages. J Pathol 101:339–354, 1970
120. Adams DO: Experimental pine pollen granulomatous pneumonia in the rat. Am J Pathol 49:153–165, 1966
121. Weiss DW: Nonspecific stimulation and modulation of the immune response and of states of resistance by the methanol-extraction residue fraction of tubercule bacilli. Natl Cancer Inst Monogr 35:157–171, 1972
122. Canetti G: The Tubercule Bacillus in the Pulmonary Lesion of Man. New York, Springer Publishing Co, 1955
123. Anderson RJ: The chemistry of lipids of the tubercule bacilli. Physiol Rev 12:166–189, 1932
124. Sabin FR: Cellular reactions to fractions isolated from tubercule bacilli. Physiol Rev 12:141–165, 1932
125. Rich AR: The Pathogenesis of Tuberculosis, Second edition. Springfield, Ill., Charles C Thomas Publishers, 1951
126. Ungar J, Muggleton PW: The production of experimental granulomatous lesions induced by injections of fatty acids and fractions of tubercule bacilli. Am Rev Resp Dis 84(5) Pt 2:76–80, 1961
127. Shelley WB, Hurley HJ Jr: Experimental sarcoid reactions in human skin. Am Rev Resp Dis 84(5) Pt 2:45–48, 1961
128. von Lichtenberg F, Smith TM, Lucia HL, Doughty BL: New model for schistosome granuloma formation using a soluble egg antigen and bentonite particles. Nature 229:199–200, 1971
129. Warren KS: Granulomatous inflammation. Inflammation, Mechanisms and Control.[28] pp 203–217
130. Reid JD, Mackay JB: The role of delayed hypersensitivity in granulomatous reactions to mycobacteria. II. Reactions to intradermal injections of intact and disintegrated organisms. Tubercule 48:109–113, 1967
131. Epstein WL: Granulomatous hypersensitivity. Prog Allergy 11:36–88, 1967
132. Unanue ER, Benacerraf B: Immunologic events in experimental hypersensitivity granulomas. Am J Pathol 71:349–364, 1973
133. Bloom BR: *In vitro* Approaches to the mechanism of cell-mediated immune reactions. Adv Immunol 13:101–208, 1971
134. Yoshida T, Cohen S: In vivo manifestations of lymphokine and lymphokine-like activity. Mechanisms of Cell-Mediated Immunity.[29] pp 43–60
135. Galindo B, Myrvik QN, Love SH: A macrophage agglutinating factor produced in

a pulmonary delayed hypersensitivity reaction. J Reticuloendothel Soc 18:295–304, 1975
136. James SL, Colley DG: Eosinophils in immune mechanisms: Production of the lymphokine eosinophil stimulation promoter (ESP) *in vitro* by isolated granulomas. J Reticuloendothel Soc 18:283–294, 1975
137. Boros DL, Warren KS, Pelley RP: The secretion of migration inhibitory factor by intact schistosome egg granulomas maintained in vitro. Nature 246:224–226, 1973
138. Rothwell TLW, Spector WG: The effect of neonatal and adult thymectomy on the inflammatory response. J Pathol 108:15–21, 1972
139. Turk JL, Bryceson ADM: Immunological phenomena in leprosy and related diseases. Adv Immunol 13:209–266, 1971
140. Epstein WL: Metal-induced granulomatous hypersensitivity in man. Immunology and the Skin, Vol 11, Advances in Biology of the Skin. Edited by W Montagna, RE Billingham. New York, Appleton-Century-Crofts, 1969, pp 313–335
141. Henderson WR, Fukuyama K, Epstein WL, Spitler LE: *In vitro* demonstration of delayed hypersensitivity in patients with berylliosis. J Invest Dermatol 58:5–8, 1972
142. Hanfin JM, Epstein WL, Cline MJ: *In vitro* studies of granulomatous hypersensitivity to beryllium. J Invest Dermatol 55:284–288, 1970
143. Marx JJ, Burrel R: Delayed hypersensitivity to beryllium compounds. J Immunol 111:590–598, 1973
144. Rocklin RE: Clinical applications of *in vitro* lymphocyte tests. Progr Clin Immunol 2:21–67, 1974
145. James DG, Neville E, Walker A: Immunology of sarcoidosis. Am J Med 59:388–394, 1975
146. Forbus WD, Bestebreurtje AM: Coccidioidomycosis: Study of 95 cases of the disseminated type with special reference to the pathogenesis of the disease. Milit Surg 99:653–719, 1946
147. Waksman BH: Tissue Damage in the "delayed" (cellular) type of hypersensitivity. Mechanisms of Cell and Tissue Damage Produced by Immune Reactions, Second International Symposium on Immunopathology. Edited by P Grabar, P Miescher. New York, Grune and Stratton Inc, 1962, pp 146–160
148. Werb Z, Gordon S: Secretion of a specific collagenase by stimulated macrophages. J Exp Med 142:346–360, 1975
149. Werb Z, Gordon S: Elastase secretion by stimulated macrophages. J Exp Med 142:361–377, 1975
150. Tsuda T, Dannenberg AM, Ando M, Rojas-Espinosa O: Enzymes in tuberculous lesions hydrolyzing protein, hyaluronic acid, and chondrotin sulfate: A study of isolated macrophages and developing and healing rabbit BCG lesions with substrate film techniques; the shift of enzyme pH optima towards neutrality in "intact" cells and tissues. J Reticuloendothel Soc 16:220–231, 1974
151. McGregor DD: Cytokinetics and fate of sensitized lymphocytes. J Reticuloendothel Soc 17:126–132, 1975
152. Asherson GL: The role of T-cells in inflammation: Activated T-cells which move to sites of inflammation and suppresor T-cells which depress contact sensitivity. Future Trends in Inflammation. Edited by GP Velo, DA Willoughby, PG Giroud. Padua, Piccin Medical Books, 1973, pp 449–480
153. Allison AC, Davies P: Mechanisms underlying chronic inflammation.[152] pp 449–480
154. McCluskey RT, Leber PD: Cell-mediated reactions *in vivo*.[29] pp 1–25
155. Loewi G, Papamichail M: Studies of mononuclear cells from inflammatory joint effusions. Int Arch Allergy 45:285–289, 1973
156. Chused TM, Hardin JA, Frank MM, Green I: Identification of cells infiltrating the

minor salivary glands in patients with Sjogren's syndrome. J Immunol 112:641–648, 1974
157. Cohen S, Ward PA, Bigazzi PE: Cell cooperation in cell-mediated immunity.[29] pp 331–358
158. Pearsall NN, Weiser RS: The Macrophage. Philadelphia, Lea and Febiger, 1970
159. Forbus WD: Reaction to Injury, Vol I. Baltimore, The Williams and Wilkins Co, 1943
160. Stuart AE: The Reticuloendothelial System. Edinburgh, E & S Livingstone, Ltd., 1970
161. Suter E, Ramseir H: Cellular reactions in infection. Adv Immunol 4:117–173, 1964
162. Suter W: Interaction between phagocytes and pathogenic microorganisms. Bacteriol Rev 20:94–132, 1956
163. Quie PG: Pathology of bactericidal power of neutrophils. Semin Hematol 12:143–160, 1975
164. Unanue ER: The regulatory role of macrophages in antigenic stimulation. Adv Immunol 15:95–165, 1972
165. Argyris BF: Role of macrophages in immune response. Developments in Lymphoid Cell Biology. Edited by AA Gottlieb. Cleveland, CRC Press, 1970, pp 28–50
166. Myrvik QN: Adjuvants. Ann NY Acad Sci 221:324–330, 1974
167. Dresser DW, Phillips JM: The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants. Immunopotentiation. Edited by GEW Wolstenholme, J Knight. Amsterdam, Associated Scientific Publishers, 1973, pp 3–28
168. Bast RC, Zbar B, Borsos T, Rapp HJ: BCG and Cancer. N Engl J Med 290:1413–1420, 1458–1469, 1974
169. Hanna MG Jr: Immunologic aspects of BCG-mediated regression of established tumors and metastases in guinea pigs. Semin Oncol 1:319–335, 1974
170. Cooper EL, ed: Invertebrate Immunology. Contemporary Topics in Immunobiology, Vol 4. New York, Plenum Press, 1974
171. van Furth R, Langvoort HL, Schaberg A: Mononuclear phagocytes in human pathology: Proposal for an approach to improved classification.[14] pp 1–15
172. Goud ThJLM, Schotte C, van Furth R: Identification of the monoblast in mononuclear phagocyte colonies grown in vitro.[14] pp 189–203

Acknowledgments

I particularly thank my colleagues, Arthur Dannenberg, Ralph Snyderman, and Stephen Vogel, for their critical and helpful comments in reviewing this manuscript.

Data in Table 1 are taken primarily from van Furth *et al.*,[4] Spector,[5] Adams,[25] van Furth *et al.*,[171] and Goud *et al.*[172] Data in Table 2 are taken primarily from Gordon and Cohn [23] and Cohn;[24] data in Table 3 are taken primarily from Forbus,[160] Refven,[160] and Forbus.[159]

Data shown in Text-figure 1 are primarily taken from Fedorko and Hirsch,[10] Carr,[11] Vernon-Roberts,[12] Nelson,[13] Nichols and Bainton,[14] Adams,[25,71] Mariano and Spector,[52] Black and Epstein,[60] and Goud *et al.*[172] Data shown in Text-figure 2 are taken primarily from Adams.[25,71]

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