Cellular Hypersensitivity and Cellular Immunity in the Pathogenesis of Tuberculosis: Specificity, Systemic and Local Nature, and Associated Macrophage Enzymes

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INTRODUCTION

This review makes certain assumptions about cellular hypersensitivity, cellular immunity, and macrophage activation, which are consistent with current knowledge. These assumptions are then used to explain the known facts concerning the pathogenesis of tuberculosis. Finally, the relative importance of local and systemic aspects of cellular immunity is discussed.

Nature and Effects of Cellular Hypersensitivity

In tuberculosis, cellular hypersensitivity, the delayed type of allergy, may be defined as an immunological state in which lymphocytes and macrophages are directly or indirectly sensitive to tuberculin. As a result of this sensitivity, tuberculin in the appropriate low concentration will activate macrophages, i.e., cause the production of new lysosomes and other organelles as well...
as digestive enzymes and perhaps bactericidins. It will also cause macrophage proliferation. Cellular hypervisibility is a major factor in the development of cellular immunity (see next subsection).

Lymphocytes become sensitive to tuberculin (6b, 33, 110, 115, 123) and can passively transfer delayed hypervisibility to the normal host (12, 19, 166, 167). The question arises whether these lymphocytes, when exposed to tuberculin, merely produce a toxic or irritating product affecting macrophages, whether they sensitize macrophages to tuberculin by means of an antibody-like product, or whether they do both (6b, 11, 11a, 46; cf. 39a, 40). As far as the pathogenesis of tuberculosis is concerned, this question is somewhat academic. Lymphocytes and macrophages exist side by side in the tuberculous lesions of the host. The same result is attained whether tuberculin stimulates or injures macrophages directly, or only indirectly after it interacts with lymphocytes. For the purpose of this review, we have assumed that in the tuberculous host both macrophages and lymphocytes are specifically sensitive to tuberculin. However, the principles herein outlined would still be valid if the macrophages were affected only indirectly by this allergen.

Macrophages are activated by many types of irritant (26, 27, 36, 39). Tuberculin is such an irritant for sensitized cells, because it is toxic to them in high concentrations (3, 97, 134, 159). Thus, the assumption that the appropriate low concentration of tuberculin can activate "sensitive" macrophages seems completely justified even though direct proof is still lacking. Mackarness, in a recent editorial (108), summarizes the evidence in support of this assumption (see below, Systemic Infection and Systemic Immunity).

Macrophages will also proliferate in response to many types of irritant (93, 97, 146). Because tuberculin is an irritant for sensitized cells, it stimulates macrophage division (63, 83, 146, 163). In addition, by virtue of the inflammation produced, tuberculin causes macrophages to emigrate from the blood stream and accumulate in the local reaction.

Both macrophage activation and macrophage proliferation may be direct effects of tuberculin or indirect effects mediated by factors released from lymphocytes, and perhaps other cells. Such factors might include the migration inhibitory factor (6b, 6c, 11, 40-42), lymph node permeability factor (139, 169, 170a), and monocytogetic hormone (170).

Nature and Effects of Cellular Immunity

In tuberculosis, cellular immunity, i.e., acquired cellular resistance, may be defined as a state in which macrophages have been activated, have proliferated, and do possess an increased capacity to destroy tubercle bacilli. Activation has increased their digestive and microbicidal capabilities. Proliferation has increased their number. Both processes result in fewer intracellular bacilli in each macrophage. Once established, cellular immunity is mainly nonimmunological and nonspecific. However, as mentioned in the previous section, the development of cellular immunity, specifically the activation and proliferation of macrophages, can have both an immunological basis (namely, cellular hypervisibility) and a nonimmunological basis (namely, any irritation).

The cellular immunity of macrophages is mainly nonimmunological in nature, because it shows little if any specificity for the microorganism that caused its formation. Such "immune" macrophages have an increased capacity to destroy or inhibit many types of bacteria (51, 107, 108).

Macrophage activation is a general term applied rather loosely to an increase in many of the properties or activities of macrophages, including the size of the cell (7, 21), its processes (15a) and its Golgi apparatus (24), the number of lysosomes (15a, 21, 24) and mitochondria (7, 21), protein content (21), lysosomal enzymes (21, 37, 74) and mitochondrial enzymes (7, 36, 37), and in such physiological functions as phagocytosis (7, 13, 59, 71, 76, 77, 97, 144, 156). Some of these changes may contribute to the power of macrophages to destroy tubercle bacilli; others may not. There may also be factors like cidins, intracellular immune substances, and highly specific enzymes that are crucial to the cellular immunity of macrophages, but these factors have not yet been discovered. Nevertheless, the available evidence indicates that macrophages possessing increased ability to destroy intracellular bacteria show generalized cell activation and, conversely, that macrophages showing generalized activation have increased ability to destroy bacteria. (Data on the association of macrophage proliferation with cellular immunity are discussed in the section Macrophage Activation and Proliferation in Tuberculosis.)

Local and Systemic Aspects of Cellular Immunity

The generalized nature of cellular hypervisibility has made many assume that cellular immunity in tuberculosis is also of a generalized nature. As a result, several investigators, myself included, have endeavored to find changes in the systemic macrophages of the immune host to which the cellular immunity of tuberculosis might be attributed. Apparently, such changes in systemic macrophages are slight compared with
changes in those macrophages actually participating in the local defense reaction (see below, Local Nature of Cellular Immunity in Tuberculosis). In other words, the major part of cellular immunity in tuberculosis seems to reside in macrophages that are locally activated in each lesion (37).

A minor part of this immunity, however, would be systemic in nature. If one assumes that the macrophages throughout the host are sensitive to tuberculin, they could be specifically stimulated by small amounts of this product released periodically into the general circulation from the local lesions. They could also be nonspecifically stimulated by other products of host or bacillary origin. The resulting activation and cellular immunity developed in these systemic macrophages seems to be of low magnitude compared to that developed by macrophages in situ (37), but it seems sufficient, especially with more generalized infections, to increase the resistance of the host against a variety of unrelated microorganisms (106–109).

General Aspects of Cellular Hypersensitivity and Cellular Immunity in the Pathogenesis of Tuberculosis

Bacillary Components Responsible for Each

Although cellular hypersensitivity and immunity are intimately related and develop almost simultaneously, they have been thought to be responses of the host to different components of the tubercle bacillus (3, 38, 96). Its proteins, i.e., tuberculin, are associated with delayed hypersensitivity (72, 121, 128, 141); its lipids or polysaccharides, or both, are associated with the development of immunity (18, 84, 119, 164).

The roles of these lipopolysaccharides in the development of immunity are not clearly understood. One of these roles is the nonspecific stimulation of macrophages in the local lesion (60, 61, 173). Another is the production of specific antibodies to the phospholipids and polysaccharides of the bacillus (18, 54, 112, 113, 122, 152, 172). However, the major role of these lipopolysaccharides is probably to act as a nonspecific adjuvant (107, 128, 168) for the establishment of delayed hypersensitivity to tuberculin protein.

Detrimental and Beneficial Effects of Cellular Hypersensitivity

The macrophage is the defense cell that determines the course of tuberculosis (15, 97, 98, 134). An inhaled bacillus is ingested by a pulmonary alveolar macrophage, which either destroys it or permits it to multiply intracellularly (cf. 13, 97). In the latter case, the macrophage also multiplies, and a primary lesion is established. About 2 weeks after the primary tubercle begins, cellular hypersensitivity and cellular immunity develop (97). At this time, because of this hypersensitivity, the bacillus and its tuberculin-like products become stimulatory (and in higher concentrations toxic) to the macrophage (see next section). Simultaneously, because of cellular immunity, the macrophage develops an increased ability to destroy the bacillus and, in doing so, differentiates into first an immature and then a mature epithelioid cell (91, 97, 104). An immature epithelioid cell is an activated macrophage, rich in lysosomes (cf. 21, 25, 52, 151a). A mature epithelioid cell is a macrophage containing many residual bodies, i.e., undigested, frequently lipid material in former phagolysosomes (digestive vacuoles) (173). Both immature and mature epithelioid cells arise in sequence following the ingestion (and digestion) of tubercle bacilli (91, 97, 104) as well as other substances (cf. 173).

The dose of allergen determines whether cellular hypersensitivity is detrimental or beneficial (3, 97, 159). If the tuberculin-like products of the bacilli are in high concentration, they cause caseous necrosis of the now “sensitized” macrophages and surrounding tissue. In addition, part of the tissue necrosis arises from factors accompanying the allergic inflammation, such as sluggish local circulation and blockage of small blood vessels with cellular infiltration and leukocyte thrombi (3), and perhaps toxic products released from dead and dying cells (159a).

Thus, delayed hypersensitivity is responsible for much of the tissue damage in tuberculosis (134). The elimination of such cellular allergy with the retention of immunity should eliminate the disease in man, but this does not seem possible.

On the other hand, if the tuberculin-like products of the bacilli are in low concentration, they cause the accumulation and multiplication of “sensitized” macrophages (see below, Macrophage Proliferation), so that more defense cells become locally available to combat the infection. In addition, low concentrations of tuberculin probably activate macrophages. Thus, delayed hypersensitivity contributes to the development of cellular immunity, although other bacillary products like lipids and polysaccharides must play some role, especially at the local level.

In tuberculosis, hypersensitivity produces the delayed type of inflammation at the local site. The lymph node permeability factor of Wollhoughby, Boughton, and Schild (139, 169, 170a), present in lymphocytes or macrophages, or in both, probably acts as one of the mediators of this type of inflammation. The increased blood supply brings more defense cells to the area,
as well as serological factors including oxygen and nutrients for these defense cells (96). If the inflammation is mild, there would be more drainage of bacilli into the local lymph nodes where they often can be destroyed (96–98). If the inflammation is severe, the fibrin formed tends to fix the bacilli locally (96, 97).

**Cellular Hypersensitivity and Resistance to Attack by Tubercle Bacilli**

The question frequently arises whether hypersensitivity increases resistance to attack by airborne tubercle bacilli (98) by hastening the development of immunity, or whether it actually lowers resistance by making alveolar macrophages more susceptible to injury by these bacilli. The answer is unequivocally the former. Inhaled bacilli reach the alveolar spaces only in small units of one to three bacilli (97, 99). This number of bacilli does not contain or release enough tuberculin to be toxic to the macrophages. It could only activate them, stimulate their proliferation, and recall their immunity. Cellular hypersensitivity, therefore, would be entirely beneficial as far as the prevention of exogenous pulmonary tuberculosis is concerned. It would also be beneficial in preventing secondary foci of infection via the hematogenous route, since only a very few bacilli would lodge in any one site at any one time.

**Liquefaction**

The softening of caseous tuberculous foci, which leads to cavity formation in the lung, is at least partly associated with delayed hypersensitivity (37, 88, 171). It is one of the most harmful processes in human tuberculosis, for, on the surface of the open cavity, bacilli multiply tremendously with little inhibition from the host (15). Among these bacilli, antibiotic-resistant mutants can arise. The large numbers of bacilli spread through the bronchial tree, causing disease in other parts of the lung and in other individuals. Except for cavity formation, pulmonary lesions would fibrose, inspissate (or calcify), and heal (15, 97, 134).

**Duration of Cellular Hypersensitivity and Immunity: Persistence of Bacilli**

In man, hypersensitivity to tuberculin may last for many years after the clinical healing of the primary infection, and it may persist for a lifetime. There seem to be two reasons for its long duration. The first is its immunological nature. Lymphocytes, and possibly macrophages, have "memory" for the antigen (or allergen) to which they previously reacted. The second reason is that the bacilli or their antigens may persist (111, 132, 149) in the caseous or liquefied centers of "healed" lesions and perhaps elsewhere. The occasional escape of bacilli or their products from encapsulated foci of infection should bolster and maintain hypersensitivity to tuberculin throughout the years. Only a minute dose of antigen would be required for this effect because of the immunological nature and "memory" involved.

In tuberculosis, systemic immunity associated with generalized macrophage activation is of relatively short duration and seems to disappear after the primary focus heals (87, 107, 108). Possibly systemic immunity requires for its maintenance the release into the circulation of much more tuberculin than is required for systemic hypersensitivity. The recall of systemic immunity upon reinfection will, however, occur more rapidly because the host retains or quickly regains hypersensitivity (87, 107, 108). Local immunity is probably of longer duration because of the persistence of bacilli at the local site. This local immunity would aid the host in preventing endogenous reinfection, but would be of little or no help in preventing exogenous reinfection.

In addition to sometimes remaining viable in "healed" caseous and liquefied foci, it is possible that typical bacilli, or protoplasts, weak-walled forms or L forms, may persist intracellularly in macrophages. (In leprosy, mycobacterial L forms have been recovered from lesions (171.) The incomplete elimination of the tubercle bacillus is responsible for the endogenous reinfections commonly occurring in man.

**Specificity of Cellular Hypersensitivity and Cellular Immunity in Tuberculosis**

**Immunological Factors**

*Cellular hypersensitivity* is highly specific for the infection involved (3, 97, 134). A positive tuberculin test is indicative of past or present exposure to *Mycobacteria* and no other microorganism.

*Cellular immunity*, on the other hand, is largely nonspecific; i.e., macrophages with acquired resistance to tuberculosis are also better able to handle other infections to which the host may be exposed (3, 45, 51, 79, 80, 97, 106–109, 114). There must be some degree of specificity to the antibacterial action of macrophages, but the responsible intra- or extracellular factors have yet to be identified. The various species of bacteria that grow inside macrophages differ in their cell wall composition as well as in their metabolic requirements. In response to such dif-
ferences, the types and amounts of macrophage enzymes and cins should vary to a certain degree. (References 81, 117, 130, 131, and 150 provide evidence that macrophages contain cins.) In addition, serological factors (49, 55, 64, 79, 113, 136) should contribute some specificity by combining with the surface components of the bacillus prior to its ingestion.

Youmans (176) proposed a “multiple response” theory of immunity in tuberculosis: (i) a nonspecific activation of the reticuloendothelial system by heat-stable (nontoxic) endotoxin-like components of the bacilli, (ii) a more “specific” immune mechanism perhaps stimulated by heat-labile components of viable bacilli and possibly mediated by an antibody, and (iii) a granulomatous response involving the rapid accumulation, proliferation, and activation of macrophages (perhaps produced directly or indirectly by hypersensitivity to tuberculin and also by the adjuvant effect of the lipid components of mycobacteria [168]). Each of these responses contributes to the total immunity of the host; yet each may be stimulated by a different component of the bacillus (176).

Hydrolytic Enzymes

In an effort to delineate some of the specific and nonspecific factors involved in the intracellular destruction of tubercle bacilli, we have spent several years studying the hydrolytic enzymes of macrophages, in particular, their proteases, esterases, acid phosphatase, β-galactosidase, β-glucuronidase, lipase, lysozyme, deoxyribonuclease, and ribonuclease (16, 34–36, 39, 116, 174; Meyer, Dannenberg, and Mizunoe, in preparation). These enzymes are not specific for tubercle bacilli, because they hydrolyze some of the basic constituents of all living things. Nonetheless, the relative proportion of each enzyme in macrophages should show some specificity for the type of intracellular microorganism involved, as they can be specifically induced (124). Such correlations, however, have not yet been made.

What has been clearly shown is that the tuberculous process affects some of these hydrolases more than others. After a mild stimulation by mineral oil, the lysozyme (16) and acid phosphatase (1) levels in peritoneal macrophages were higher in tuberculous animals than in controls. The increased levels of these hydrolases may be due to the fact that macrophages of the tuberculous host respond to nonspecific stimuli with greater intensity (see below). These increased levels may also be due to the specific stimulus of the infection (see below). Both the tuberculous animals and controls showed the same levels of proteases, esterases, nonspecific lipase, deoxy-

ribonuclease, and ribonuclease (16, 34; Meyer et al., in preparation; cf. 31).

BCG infection elevated the acid phosphatase, β-glucoronidase, and cathepsin levels (and protein content) of macrophages washed from the peritoneal cavities of mice without the prior injection of an irritant (138, 157). The very presence of a moderate number of free macrophages in the peritoneal cavities of normal mice suggests that a constant source of irritation exists there—perhaps caused by endotoxins diffusing from the gut.

In normal animals, the intravenous [or intratracheal (74)] injection of heat-killed tubercle bacilli increased in alveolar macrophages the levels of lysozyme, β-glucuronidase, acid phosphatase, β-galactosidase, a protease hydrolyzing N-benzoyl-D.L-phenylalanine β-naphthol ester, a “lipase” hydrolyzing naphthol laureate in the presence of taurocholate, ribonuclease, and deoxyribonuclease (28, 74, 116, 118; Meyer and Dannenberg, in preparation). It also increased their hexose monophosphate shunt activity and their bactericidal ability (53, 117). It did not increase their levels of nonspecific lipase, esterase, and a protease hydrolyzing hemoglobin (28, 116). [With a sufficient dose of heat-killed bacilli given intratracheally, the levels of this protease were elevated (74).]

These findings indicate that immunity in tuberculosis is associated with increased levels of certain hydrolytic enzymes in macrophages. The appropriate combination of such hydrolases could destroy the tubercle bacillus without the intervention of other factors (cf. 49). For example, if a specific lipase (yet to be discovered) hydrolyzes the lipid coat of the bacillus, macrophage lysozyme would probably hydrolyze its mucopolysaccharide framework (cf. 153–155, 168b). Tubercle bacilli without lipid coats are still viable, but are more readily destroyed by the host (10).

Macrophage Activation and Proliferation in Tuberculosis

Basic Mechanisms Involved in Macrophage Activation

We make a distinction between the excitation and adaptation phases of macrophage activation (39). Excitation is the immediate response of these cells to any irritant and should be rapidly reversible. It is characterized by increased oxygen consumption, glycolysis, lipid turnover, and other metabolic phenomena (39). These excited cells probably move faster and ingest particles more efficiently (39) than other cells. Adaptation refers to more permanent changes produced in macro-
phages in response to irritation. It is usually characterized by increases in the number of lysosomes and mitochondria, and in their enzymes. Adaptation is reversible (23), but is not as rapidly reversible as cell excitation. Throughout this report, activation refers only to this adaptation phase. Since many reviews (59, 71, 76, 77, 144, 156; cf. 3, 13, 50, 97) discuss activation of the reticuloendothelial system, this subject will not be included here.

Cohn et al. made thorough studies on factors involved in macrophage activation in vitro (7, 20–27, 48; cf. 39, 116). Lysosomal enzymes seem to be made in the endoplasmic reticulum and packaged by the Golgi apparatus into primary lysosomes (24). The primary lysosomes then fuse with phagocytic vacuoles which contain the material to be digested (24, 29). These secondary lysosomes (or phagolysosomes), now rich in enzymes, are evidently capable of fusing with more recent phagocytic or pinocytic vacuoles (14, 24, 66, 165), and may be more effective than primary lysosomes against recently ingested microorganisms.

The macrophages of the tuberculous host respond with greater intensity than controls to a variety of stimuli (97). These cells, already partially activated, can probably undergo further activation more easily than unprimed cells. Possibly their cell membranes are more sensitive; possibly the synthesis of new lysosomes and enzymes, once begun, is more readily continued. The activation of macrophages induced by the specific agent, namely the tubercle bacillus and its tuberculin-like products, probably occurs with a smaller dose or occurs faster and to a greater degree than their activation by nonspecific agents (cf. 108). These same relationships would apply to the stimulation of macrophage proliferation (82).

**Correlation of Macrophage Activation with Cellular Immunity**

Activation seems necessary for the development by macrophages of an increased cidal capacity for tubercle bacilli. In other words, macrophage activation seems necessary for cellular immunity. Macrophages from immune rabbits with increased power to inhibit the growth of tubercle bacilli (95, 97) ingested carbon and colloidion particles, tubercle bacilli, and staphylococci more readily (93, 97). Rabbit alveolar macrophages with a greater content of enzymes as a result of stimulation with heat-killed BCG (28, 74, 116) could destroy Listeria more readily in vitro (53, 117). Extracts of these cells showed increased antibacterial activity for *Mycobacterium smegmatis* (53, 117). Similar alveolar macrophages from granulomatous lungs of mice (cf. 175) were apparently responsible for the resistance of these animals to tubercle bacilli administered by the airborne route (2, 6, 133).

Three pertinent studies from other laboratories involve mouse peritoneal macrophages. (i) Those from mice vaccinated with high concentrations of viable BCG showed increased size, active pinocytosis, large numbers of lysosomes (dense bodies), and high microbicidal activity against *Salmonella typhimurium*, an unrelated intracellular bacterium (Blanden, Lefford, and Mackaness, in preparation; see 108). (ii) Mouse peritoneal macrophages, rich in enzymes because of activation in vivo by the intraperitoneal injection of fetal calf serum, possessed high microbicidal activity against *S. typhimurium* (Blanden, unpublished data, quoted in 109). (iii) Peritoneal macrophages of mice activated by *Escherichia coli* lipopolysaccharide showed increased levels of acid phosphatase and increased cidal activities for the unrelated bacillus *S. typhimurium* (4).

Our histochemical findings also support an inverse correlation between the content of enzymes in macrophages and their content of bacilli (see below).

**Macrophage Proliferation**

The number of defense cells, especially macrophages, that accumulates in a tuberculous lesion plays a role in determining its fate. Large numbers of cells seem to limit bacillary growth better than small numbers. Lurie's studies with cortisone (101, 102) and triiodothyronine (TTT) (100, 103) support this contention (but by no means prove it, because in these studies many other factors are also involved). He observed little perifocal inflammation and few defense cells in the pulmonary tubercles of cortisone-treated rabbits, where the growth of the bacilli within macrophages was marked. In contrast, there was more perifocal inflammation and more defense cells in the pulmonary tubercles of TTT-treated rabbits, where the bacillary growth was diminished.

The origin of these defense cells has been the subject of several investigations, reviewed by Spector (146) under the heading of chronic delayed hypersensitivity reactions. Evidently, circulating monocytes (macrophages) and lymphocyte-like premacrophages (83, 160–162) emigrate from the blood stream into the granulomatous lesions where they proliferate. Initially, the blood is the major source of macrophages in these lesions, but, as chronicity is established, local proliferation becomes more important. Evidence for macrophage emigration from the blood comes from Spector's group (146–148), Kosunen et al. (83), Volkman and Gowans (160–162), and
Gell and Hinde (63). Evidence for local macrophage proliferation also comes from Spector's group (146–148), Gell and Hinde (63), and Kosunen et al. (83), and from work in progress in our own laboratory involving the incubation of biopsies of dermal BCG and tuberculin reactions in vitro with tritiated thymidine and hyperbaric oxygen. Waksman and Matolsky (163) showed that tuberculin caused an increase in the total number of macrophages in cultures of peritoneal exudates from sensitized guinea pigs. Both differentiation from an intermediate lymphocyte-macrophage and macrophage proliferation seemed involved.

The studies of Mackaness et al. (see below) also provide evidence that macrophage proliferation is involved in cellular allergy and cellular immunity (82, 105, 109; cf. 58). Correlations were found among (i) the percentage of mouse peritoneal macrophages that incorporated tritiated thymidine, (ii) the development of delayed hypersensitivity, and (iii) a decrease in the number of bacteria in the spleen (82). Similar correlations (105) were found among the mitotic activity of macrophages in the lesions and factors ii and iii above.

Local proliferation of macrophages not only occurs in chronic delayed hypersensitivity reactions, but also in granulomata induced by colloidal carbon, paraffin oil, and other macromolecules (146; cf. 32). Undoubtedly, the lipids and perhaps other components of tubercle bacilli, as well as other irritants, can stimulate macrophage proliferation without the intervention of delayed hypersensitivity. [Macrophages of tuberculous animals show a greater rate of mitotic and amitotic division than controls in response to the nonspecific stimulus of intrapleural India ink and aleuronat-starch (93).] However, in a host possessing delayed hypersensitivity, the tubercle bacillus would cause local macrophage activation, proliferation, and immunity to occur at a faster rate and to a greater degree than in nonsensitive hosts (58, 97, 108, 109). This has been termed accelerated tubercle formation.

The macrocytogenic humoral factor of Wloughby, Coote, and Spector (170) from lymph nodes apparently causes the bone marrow to produce or release macrophages or premacrophages. It may also contribute to local macrophage accumulation, activation, and proliferation.

**Correlation of Macrophage Proliferation with Activation**

A direct correlation was found by Wiener (168a) between the rate of DNA synthesis, estimated by tritiated thymidine incorporation, and the degree of macrophage activation, measured as acid phosphatase activity. In these studies, mouse peritoneal macrophages were activated in vivo and also in vitro. A similar correlation was made with Kupffer cells by Kelly, Brown, and Dobson (81a), who used thymidine incorporation as evidence of cell proliferation, and phagocytic activity as evidence of cell activation. Thus, macrophages respond to stimuli both by increasing their number and by increasing their individual capabilities.

This correlation, however, may exist only during the activating process. In healing tuberculous lesions, the very mature epithelioid cell, which has become extremely rich in enzymes (cf. 37), is probably so differentiated for digestive function that it no longer divides. Studies begun in our laboratory will attempt to substantiate this hypothesis: biopsies of BCG lesions, incubated in vitro with tritiated thymidine, are being sectioned, processed for $\beta$-galactosidase activity, and stained for acid fast bacilli (cf. 37).

**Immunological Mechanisms and Systemic Nature of Cellular Hypersensitivity in Tuberculosis**

Several excellent reviews (3, 12, 49, 62, 80, 97, 109, 129, 158, 159) indicate that the ultimate nature of delayed hypersensitivity is still unknown. It seems reasonable to assume that the macrophages of tuberculous animals (i) are directly or indirectly sensitive to tuberculin (see below), (ii) are injured by high concentrations of this bacillary product (3, 97, 134, 135, 159), and are stimulated (iii) to divide (see above) and (iv) to produce more lysosomes and enzymes by low concentrations (see above). These four effects of tuberculin largely determine the behavior of macrophages during the pathogenesis of tuberculosis.

Lymphocytes respond to tuberculin with blast cell formation (6b, 33, 110, 115, 123, 140; cf. 73, 121a), which is associated with their multiplication (68) and with the production of antibodies (cf. 68, 78) and other factors (66). Such factors produced by lymphocytes influence the function of macrophages, perhaps by sensitizing them to tuberculin.

The effect of lymphocytes on macrophages is currently a very active field of investigation (3, 12, 78). At the present time, the following data seem pertinent.

Culture supernatant fluids from sensitive purified peritoneal lymphocytes, incubated with tuberculin (purified protein derivative, PPD) for 24 hr, contain a factor (presumably a protein) which inhibits the migration of normal guinea pig exudate cells, but does not kill them (66,
The sensitive lymphocytes produce migration inhibitory factor (MIF) only when stimulated by the specific antigen (11, 11a, 46, cf. 39a–43, 65, 151b), and they may subsequently undergo blast-cell transformation (6b, 11a, cf. 71a). This factor may directly inhibit the migration of the macrophages or may sensitize them so that the tuberculin present causes their inhibition. The intradermal injection of partially purified MIF (not necessarily tuberculin-free) produces skin reactions that histologically resemble delayed hypersensitivity reactions (6c).

Tuberculin specifically enhanced the effect of dialyzed MIF on normal peritoneal exudate cells (6b, 11a, cf. 151b). The MIF in these experiments may have sensitized the exudate macrophages to tuberculin, or may have sensitized the lymphocytes also present (see below). In the latter case, the added tuberculin might have caused these newly sensitized lymphocytes to release a substance toxic to macrophages.

"Pure" populations of macrophages from sensitized animals are not inhibited by the allergen (11, 11a, 46), but a sensitizing factor produced by lymphocytes could have been removed from these macrophages during their isolation (see 120).

Heise and Weiser (75) provided evidence that the inhibition of macrophage migration by tuberculin (PPD) can result from a type of antigen-antibody complex on the macrophage surface [see Benacerraf (6a) for a discussion of such phenomena].

Ruddle and Waksman (137, 137a; see also Federation Proc. 27: 45, 1968) and Holm and Perlmann (77a) showed that sensitized lymphocytes, incubated with allergen and subsequently washed (to remove excess allergen), are toxic for fibroblasts or Chang cells in culture. Pincus (to be published) considers this effect to be due to the formation of a cytotoxic factor (cf. 126, 127).

Thus, the question remains unanswered whether the macrophages become specifically sensitive to tuberculin, or whether they are affected nonspecifically by a toxic or irritating product produced by the interaction of sensitive lymphocytes and tuberculin. As mentioned in the introduction, the effect of tuberculin on the pathogenesis of tuberculosis would be the same in either case, because lymphocytes and macrophages exist side by side in every tuberculous lesion.

Cellular hypersensitivity is systemic in nature. Tuberculin elicits an inflammatory response of macrophages and lymphocytes almost anywhere in the body of the tuberculous host (3). Polymorphonuclear leukocytes, when present, are considered a nonspecific response to tissue damage and necrosis (3).] Lymphocytes and their progeny (and/or certain of their immunologic products) circulate throughout the body (67) and seem to be responsible for the systemic nature of delayed hypersensitivity (158a). The circulation of macrophages and premacrophages has not yet been studied (cf. 160–162).

Interrelations among macrophages, their rapidly dividing lymphoid progenitors in the bone marrow (160–162), cells intermediate between lymphocytes and macrophages (83), long-lived recirculating small lymphocytes (67), short-lived lymphocytes (67), and lymphocytes originating in the thymus or gut (cf. 32) remain to be clarified with respect to the induction and expression of delayed hypersensitivity (cf. 53a, 67, 78).

In human beings, Lawrence (85, 86) transferred delayed hypersensitivity to tuberculin with extracts of peripheral blood leukocytes (see also 54a, 54b, 155a, 155b). This transfer factor was probably produced by the lymphocytes present. It seems to have two active components (5, cf. 47). One is like ribonucleic acid (RNA); the other is protein. Conceivably, the RNA-like component (with or without traces of antigen) transfers the ability to produce the protein component, and the latter actually sensitizes lymphocytes (and perhaps macrophages). Thus, a few lymphocytes may manufacture both components; a greater number may manufacture the protein component; and a still greater number may only be sensitized and may be unable to manufacture either. These possibilities remain to be investigated in both man and animals. The relation of Lawrence's transfer factor to the migration inhibitory factor discussed above also requires investigation.

**SYSTEMIC INFECTION AND SYSTEMIC IMMUNITY**

Pulmonary tuberculosis in man and animals varies from an extensive disease to a small solitary focus of infection. With extensive disease, the bacilli and especially their tuberculin-like products are released into the blood stream and reach many parts of the body. With minimal disease, both are more confined to the local area. The severity of the infection, i.e., the amount of tuberculin, bacilli, and other products released, determines the degree of activation of systemic macrophages and the degree of nonspecific systemic immunity that results. Tuberculin is probably the main activator of systemic macrophages, because these cells are directly or indirectly sensitive to even minute quantities of this substance.
In clinical practice, BCG vaccine is the most widely used activator of systemic macrophages, including those of the reticuloendothelial system (45, 97). Endotoxins, which produce a similar effect, are used more frequently in experimental animals (97, 144).

Macrophages that have become richly endowed with enzymes in the local tuberculosis lesion may perhaps migrate to other sites in the host and contribute to systemic immunity. This possibility is more likely when the host contains a hundred, rather than merely a few, local lesions.

Mackaness and his associates (9, 30, 105–109) have demonstrated that systemic infection makes an ideal experimental model for elucidating basic principles of cellular hypersensitivity and cellular immunity, many of which are difficult to study in a local infection. The use in mice of other facultative intracellular bacteria, such as Listeria, Salmonella, and Brucella (in addition to BCG), has been a further aid, because these bacteria and their antigens are more readily destroyed by the macrophages of the host. The main conclusions derived from these experiments are listed below. The recent editorial by Mackaness (108) is recommended for a more complete review.

(i) Cellular immunity once developed is not specific for the bacteria that induced it. It is also effective against heterologous bacteria.

(ii) Cellular immunity disappears when the antigen is eliminated.

(iii) Cellular immunity is rapidly (and specifically) recalled by homologous bacteria; heterologous bacteria produce immunity only at the original rate.

(iv) The specificity of this rapid recall is correlated with the existence or rapid development of cellular allergy.

(v) The amount of cellular immunity remaining after recovery from a primary infection is related to the infecting dose and the persistence and type of bacilli. Mycobacterium (and its antigens) persists longer than Brucella. Brucella persists longer than Listeria.

(vi) Systemic immunity is relative, not absolute. A large number of reinfesting microorganisms will usually cause disease.

(vii) Systemic immunity is reflected by the ability of peritoneal macrophages to inhibit in vitro the intracellular growth of Listeria (105). The rise and fall of immunity in these macrophages probably involve the development and regression of cell activation. This rise and fall might also involve the selection and subsequent multiplication of a population of cells most responsive to the allergic stimulus, followed by a decline in their number when immunity regresses (82, 105).

(viii) Systemic cellular immunity and hypersensitivity appeared to be passively transferred by lymphoid cells, but not by peritoneal macrophages or by serum (108). Such immunity was specific for the bacteria that induced it. It is therefore likely that only cellular hypersensitivity was transferred, and that because of this hypersensitivity cellular immunity developed at a more rapid rate upon challenge with homologous bacteria. Only after such challenge was the immunity nonspecific.

(ix) Thus, the presence of cellular hypersensitivity does not mean that the host already possesses immunity. It only means that the host can develop immunity at a faster rate and to a greater degree when specifically challenged (108).

(x) A larger dose of bacteria is required to stimulate immunity in peritoneal macrophages, which represent "uninvolved" systematic macrophages, than to stimulate immunity in the local macrophages of the spleen, which ingest a large proportion of the intravenously injected microorganisms (108).

Local Nature of Cellular Immunity in Tuberculosis

Local Macrophage Activation

In contrast to cellular allergy, cellular immunity in tuberculosis is mainly local in nature. Marked degrees of activation take place in the local lesion, where macrophages become epithelioid cells extremely rich in lysosomal and mitochondrial enzymes (37). The high activation of macrophages in local areas is associated with the destruction of tubercle bacilli. The same degree of activation is not common in macrophages found elsewhere in the body (Dannenberg, unpublished data).

Present in the local lesion are both antigenic material and defense cells: macrophages, lymphocytes, and later even plasma cells (cf. 63, 145). Because of the proximity of the antigen and its high concentration, local lymphocytes and macrophages should become more sensitive to tuberculin and become more activated by it than cells at a more distant site. Also present locally are nonspecific substances which activate macrophages, such as agents released during inflammation (cf. 26, 69), tissue debris, and bacillary lipids, e.g., cell walls or Wax D (cf. 60, 61, 128, 133, 168, 173). Thus, in the local lesion, the macrophages become highly activated, probably because of both immunological factors, like stimulation by tuberculin, and nonspecific factors, like stimulation by bacillary and tissue lipids and other substances.

Histochemical Studies

Histochemical techniques have been used to
study dermal BCG lesions, biopsied at various times during their growth and regression (37). With the indolyl substrates (125), enzymes can be demonstrated in the same tissue section as acid-fast bacilli. The local presence of the bacillus was associated with the formation of epithelioid cells and an increase in their levels of $\beta$-galactosidase, acid phosphatase, $\beta$-glucuronidase, cytochrome oxidase, and succinic dehydrogenase (Fig. 1). In areas without bacilli, macrophages did not form epithelioid cells nor show increased enzyme levels. Furthermore, epithelioid cells with the highest enzyme activity ($\beta$-galactosidase) never contained as many bacilli as some of the epithelioid cells with lower activity (Fig. 2). This relationship was statistically highly significant (37).

These observations suggest that tubercle bacilli and their components in the local lesion stimulate both the development of epithelioid cells and an increase in their enzymes, and that this increase is associated with bacillary destruction. Histochemical studies in other laboratories are consistent with these conclusions. Macrophages in granulomatous lesions were found to be rich in acid phosphatase (60, 61, 70), $\beta$-glucuronidase (60, 61, 70), succinic dehydrogenase (61, 173), cytochrome oxidase (61, 173), aminopeptidase (60, 61), phosphamidase (60, 61), nonspecific esterase (61), and nicotinamide adenine dinucleotide diaphorase (61).

Studies with Cell Cultures

In vitro experiments (3, 8, 13, 49, 95, 151, 159) with macrophages are also consistent with the concept of local immunity. In these experiments, systemic macrophages from tuberculous and control animals were maintained in culture, or in the anterior eye chamber of a normal rabbit. The cells from the tuberculous groups showed an increased ability to inhibit the growth of tubercle bacilli within their cytoplasm. Such cellular immunity is probably the result of (i) greater initial activation of systemic macrophages by tuberculin and perhaps other products, which reached these cells via the circulation, and (ii) greater ability of these “primed” macrophages to become further activated locally in the culture when stimulated by the tubercle bacillus and its products, by the ingestion (and digestion) of serum proteins (22, 23), or by other substances present. Control macrophages would be rela-

![Fig. 1. $\beta$-Galactosidase activity of a BCG lesion from a rabbit injected intradermally 21 days previously. A group of epithelioid cells with high enzyme activity is seen in the tuberculous granulation tissue. At the lower right is a portion of the lesion's liquefied center. The black cells in the photograph are in reality colored bright blue from the indigo formed when the substrate 5-bromo-4-chloroindol-3-yl-$\beta$-D-galactopyranoside is hydrolyzed (125). The section was lightly counterstained with hematoxylin to demonstrate the other cells. $\times$ 200.](http://mmbr.asm.org/)

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resistantly insensitive to tuberculin and would respond more slowly to other stimuli.

Evidence has been obtained that macrophage preparations (containing lymphocytes) from BCG-vaccinated animals have a factor, probably RNA, that can passively transfer immunity to macrophages of an unvaccinated animal (56, 57). This transferred immunity may involve hypersensitivity, which could increase the rate of macrophage activation in cell culture in the presence of tubercle bacilli. It may also involve non-specific activating factors.

Studies with Tuberculous Animals

The concept of local immunity is supported by the fate of individual tuberculous lesions in the host. Reinfection tuberculosis in the skin near the site of the primary infection progresses more slowly than tuberculosis in the skin on the opposite side of the animal (143). [A similar phenomenon occurs in the lung (142).] Even healing of tuberculosis at the primary site in the skin (and draining lymph nodes) can occur when there is progression of the disease at other sites in the body (92). This finding is partly explained by the fact that tuberculous lesions in the different organs of the host heal at different rates (89, 90).

Recapitulation

Cellular immunity in tuberculosis is mainly a local manifestation developing in macrophages of the tubercles that may be present in lung, kidney, spleen, liver, or other organs. The rate of development of such immunity is dependent on (i) local conditions provided by the organ in which the tubercles reside (89, 134), (ii) native (or genetic) resistance of the host (94), and (iii) acquired resistance, which includes systemic hypersensitivity (107, 108).

Conclusions

Cellular hypersensitivity in tuberculosis is a typical immunological phenomenon in which most, if not all, of the macrophages and lymphocytes of the host become directly or indirectly sensitive to tuberculin. It is highly specific, of long duration, and systemic as well as local in nature. Because of this hypersensitivity, tuberculin activates macrophages (and lymphocytes) and stimulates them to proliferate.

Cellular immunity is presented in activated macrophages. These cells are able to destroy tubercle bacilli in their cytoplasm, presumably owing to their high content of lysosomes and associated enzymes and cidins. Cellular immunity is
not a typical immunological phenomenon, because, once developed, it is effective against many microorganisms and is no longer specific for the tubercle bacilli that induced it.

Macrophages develop the most cellular immunity in the local tuberculous lesion. Here the tuberculin-like products of the bacilli are in the highest concentration. Thus, the specific stimulus for cell activation is greater in the local lesion than elsewhere in the host. The concentration of non-specific stimulatory products from the bacilli and injured tissues is also higher locally. Local immunity is economical; the macrophages become most effective at the sites where they are most needed, whereas the majority of them remain relatively inactive and in reserve.

The immunity developed in systemic macrophages is significant in preventing secondary foci of infection, even though it is an order of magnitude less than the immunity developed in many macrophages of the local lesions. Tuberculin (and perhaps other products) released into the circulation activates directly or indirectly systemic macrophages. The degree of immunity developed in these macrophages is roughly proportional to the amount of tuberculin released, and this amount is roughly proportional to the extent of disease present. After the disease is healed, the release of tuberculin almost entirely ceases, and the immunity of systemic macrophages tends to disappear. However, because of persisting cellular hypersensitivity, such immunity can be rapidly and specifically recalled by exogenous or endogenous reinfection.

In tuberculosis, delayed hypersensitivity is both beneficial and detrimental. In low concentrations, tuberculin stimulates the development of immunity in macrophages. Therefore, the presence of hypersensitivity is an asset in preventing pulmonary tuberculosis, for only small units of one to three bacilli reach the alveolar spaces where the infection begins. In high concentrations, tuberculin kills macrophages and thus is responsible for caseation and much of the tissue injury accompanying the disease. In addition, delayed hypersensitivity is at least partly responsible for the liquefaction of caseous foci. This process results in tremendous extracellular multiplication of tubercle bacilli followed by their spread throughout the bronchial tree and to other people. Liquefaction is a major factor in the perpetuation of tuberculosis in mankind.

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