Advances in Tuberculosis Research

Fortschritte der Tuberkuloseforschung
Progrès de l'Exploration de la Tuberculose

17

S. KARGER · BASEL (SWITZERLAND) · NEW YORK

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Vol. 17

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With 33 figures and 71 tables

19 70

BASEL (Switzerland) S. KARGER NEW YORK

Published simultaneously as / Erscheint gleichzeitig als / Paraît simultanément comme

Bibliotheca Tuberculosea, No. 26

Previously published

No. 1 = Fortschr. TuberkForsch./Adv. Tuberc. Res./Progr. Explor. Tuberc. Vol. 1
No. 2 = Fortschr. TuberkForsch./Adv. Tuberc. Res./Progr. Explor. Tuberc. Vol. 2
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S. Karger AG, Arnold-Böcklin-Strasse 25, 4000 Basel 11 (Switzerland)

Printed in Switzerland by Kohlhepp AG, Basel
Blocks: Steiner & Co., Basel

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Adv. Tuberc. Res., vol. 17, pp. 1–27 (Karger, Basel/New York 1970)

The Macrophage in Tuberculosis

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Contents

I. Introduction

From the earliest of histopathologic studies, it was apparent that in mammalian hosts the primary cells of response to tubercle bacilli were

mononuclear cells: the small lymphocytes and the large mononuclear phagocytes. The latter, on contact with tubercle bacilli, were seen to become transformed into epithelioid cells and multinucleated giant cells of the Langhans type. It was recognized that an aggregate of the large mononuclear cells which had been converted to epithelioid cells and multinucleated giant cells, often surrounded by lymphocytes, formed the histologic tubercle which gave the name to the bacillus and to the disease. When resistance to tuberculosis was shown not to be the consequence of circulating antibodies, it was only natural that many workers believed that demonstrable resistance was largely the result of the action of the same mononuclear cells and predominently that of the large mononuclear phagocyte or macrophage. Experimental inquiry into the proof of this assumption and into the mechanisms of that cellular resistance to the tubercle bacillus has occupied the efforts of generations of scientists. Their observations on the functions of the macrophage in tuberculosis will be the subject of this review.

II. The Macrophage

Literally the macrophage is the large phagocyte in contrast to the microphage, the small phagocyte or neutrophilic polymorphonuclear leukocyte. Macrophages are the wandering, free-living phagocytes of all tissues and also are known as histiocytes, clasmatocytes, endotheliocytes, primitive wandering cells, polyblasts, adventitial cells, perithelial cells, transitional cells and in the brain, the microglial cells. The many names given to the same cell over the years apparently resulted from its different appearances during different functions in different locations as well as from its distribution in so many tissues. Today it seems clear that all of these terms apply to the same cell: the free or nonfixed reticuloendothelial cell of all tissues. The cell will be considered in this review simply as the *macrophage*. Different environments may impart to this cell greater or lesser functional capacities but in all organs und under all stimuli this cell seems *qualitative* the same.

Furthermore, the circulating blood monocyte is undoubtedly the same free reticuloendothelial cell as the tissue macrophage. The circulation serves to transport this cell from its place or places of origin to other areas of the body. Indeed, transformation of the blood monocyte into

morphologically typical macrophages and then into epithelioid cells and multinucleated giant cells has been carefully observed with both light and electron microscopy by SUTTON and WEISS [77].

In many functions as well as in morphology, the *fixed* reticuloendothelial cells of the sinusoidal vascular spaces of the bone marrow, liver, spleen and lymph nodes are similar to the freely motile tissue macrophage. Thus it is logical to believe that the tubercles that so rapidly form in the liver, spleen, lymph nodes and bone marrow in hematogenous tuberculosis are the result of phagocytosis of tubercle bacilli by fixed reticuloendothelial cells with subsequent conversion of these cells into epithelioid cells and multinucleated giant cells just as with free tissue macrophages and blood monocytes. Evidence proving this probable chain of events is lacking and free tissue macrophages and circulating monocytes could provide cells for such miliary tubercles as well as the fixed reticuloendothelial cells in these organs.

There is much evidence that local tissue macrophages when appropriately stimulated are capable of active multiplication [69, 70]. Mitotic figures are commonly seen in these mononuclear phagocytes at sites of inflammation. EBERT and FLOREY [17] initially showed and recent evidence has further indicated that the majority of local tissue macrophages originally are derived from the pool of circulating blood monocytes [69, 68, 70]. It has also been shown that blood monocytes originate primarily from the bone marrow rather than from other reticuloendothelial organs such as the thymus or lymph nodes [80]. In these studies, VIROLAINEN employed the chromosomal marker technique which indicated that intravenous injections of thymus, lymph node and peritoneal exudate cell suspensions from normal donor animals into lethally irradiated animals did not provide precursor cells for peritoneal macrophages as did donor bone marrow cell suspensions similarly injected. VAN FURTH and COHN also demonstrated the bone marrow origin of blood monocytes and, thereafter, of peritoneal macrophages using tritiated thymidine techniques as well as partial or total body irradiation [23]. The latter workers' evidence also indicated a 22-hour half-life in the circulation of blood monocytes with a long life of nondividing tissue macrophages up to 4 to 8 weeks.

The possible origin of macrophages from lymphocytes has been proposed for many years. Present evidence indicates that almost certainly blood monocytes and tissue macrophages are derived solely from precursor cells of the primitive reticuloendothelial cell type of

the bone marrow and that lymphocytes do not transform into such cells [23].

The stimuli which lead to increased numbers of macrophages in tissues are many. In general, the stimulus is one which has not resulted in acute cell necrosis. Lipid substances free in tissues are particularly apt to call forth a macrophage response. In addition to mycobacterial disease, macrophages and probably the fixed reticuloendothelial cells are the predominant cell of inflammatory response to infections by the *Salmonellae, Brucellae, Histoplasma capsulatum, Blastomycetes, Coccidiodes immitis,* certain parasitic helminths, in the disease sarcoidosis, in certain virus disease such as lymphopathia venereum and cat-scratch disease as well as in a large number of foreign body reactions. Many of these are diseases where delayed hypersensitivity reactions are prominent but not all; most are diseases where resistance is not governed by antibodies and in which cellular resistance is demonstrable or is postulated; in all, granulomata of epithelioid cells with or without giant cells commonly or occasionally form. Extracts of tubercle bacilli in much larger amounts than would be necessary if the bacilli were whole will result in macrophage accumulation, epithelioid cell transformation, and Langhans giant cell formation, all in a tubercle aggregate [63, 9]. The role which macrophages play in protecting the host from the pathogenic organisms eliciting the macrophage response has been experimentally investigated in animals for many years particularly using mycobacteria. *In vitro* techniques newly developed have resulted in recent more extensive studies on the macrophage resistance in tuberculosis and other intracellular bacterial infections.

A. Phagocytosis by Macrophages in Tuberculosis

Even in the most susceptible of hosts, tuberculosis has a biologic course of many weeks so that one would not anticipate that phagocytosis would be an important factor in resistance unless in the more susceptible animal it were extremely prolonged or restricted altogether.

In the course of experimental tuberculosis extreme prolongation of the act of phagocytosis has not been observed. Most workers have reported that macrophages of susceptible species or susceptible indi-

viduals phagocytosed tubercle bacilli as promptly and as effectively *in vivo* as did macrophages of resistant species or specifically immunized animals [58]. LURIE, however, detected *in vivo* enhancement of both specific phagocytosis of tubercle bacilli and of nonspecific phagocytosis of staphylococci and carbon particles following prior immunization with BCG and especially during active tuberculosis in rabbits [32]. HENDERSON *et al.* [25] also demonstrated that nonimmunized but natively resistant rabbits possessed alveolar macrophages which were able to ingest up to 2 times more bovine tubercle bacilli *in vitro* than macrophages from susceptible strains. Recently, MAXWELL and MARCUS showed that alveolar macrophages from immune guinea pigs ingested more virulent $H_{37}R_v$ tubercle bacilli *in vitro* in one hour than did nonimmune cells although this was not observed with peritoneal macrophages from the same animals and not with avirulent $H_{37}R_a$ bacilli [45]. Indeed immune alveolar macrophages in their experiments ingested less than $^1/_6$ as many avirulent bacilli as did control alveolar macrophages thus casting considerable doubts on the significance of their results with the virulent bacilli.

In our own tissue culture experiments employing peritoneal macrophages and suspensions of isolated virulent tubercle bacilli in proportions of approximately 1:1, we could not demonstrate significant differences between immune and normal macrophages in rates or quantities of phagocytosis of tubercle bacilli whether living or dead or whether the suspending medium was serum from immunized or nonimmunized guinea pigs [5].

SUTER has reported similar observations [73]. Furthermore, when the *in vitro* infection levels were increased in our experiments to considerably greater bacillus-macrophage ratios, normal cells were able to ingest as many virulent bacilli as immune cells within the same period of time. The length of times used in our experiments on macrophage phagocytosis of tubercle bacilli were short, with only slight or no increases of phagocytosis occurring after three hours. Phagocytosis, however, was not complete at one hour in our system, the interval used by MAXWELL and MARCUS [45] to report comparisons of immune and control cell phagocytosis. At one hour, however, we could detect no consistent differences in rate or quantity of phagocytosis between immune and control peritoneal macrophages.

In summary, we strongly suspect that the differences which have been observed in the rates and quantities of phagocytosis of tubercle

bacilli by macrophages from immunized and control animals, while perhaps measurable in some experimental systems, are of such magnitudes as to be insignificant in a disease of such slow evolution as in even the most acute tuberculosis. This is in marked contrast to the situation in disease produced by *Diplococcus pneumoniae* in which polymorphonuclear leukocytes of highly susceptible animals can readly destroy the organism once phagocytosis has occurred but in which phagocytosis is markedly restricted in the absence of type specific antibody.

B. Nonspecific Activation of Macrophages

There is much evidence that a wide variety of stimuli may activate the entire reticuloendothelial cell system and thus macrophages [24]. LURIE's BCG immunized rabbits had greater phagocytosis of staphylococci and carbon particles [32]. More rapid clearance of blood of bacteria or other particulate matter may well indicate a more active system but in a chronic process such as tuberculosis such activity does not necessarily indicate greater resistance. Indeed, miliary tuberculosis in the highly susceptible patient or experimental animal clearly indicates by the very myriads of tubercles in all organs and especially in the reticuloendothelial organs the highly effective clearance of the blood of tubercle bacilli by patients about to die!

Increased numbers of macrophages whether due to specific immunization or nonspecific activation, however, could be of value to resistance since this would reduce the numbers of tubercle bacilli per macrophage after phagocytosis had occurred. Increase in total numbers of macrophages could be achieved by both the initial presence of more cells in the invaded tissues as well as by a more rapid multiplication of those cells and a more rapid arrival of blood monocytes after invasion by tubercle bacilli had occurred. LURIE believed that BCG-vaccinated and tuberculous animals mobilized macrophages more rapidly at sites of nonspecific irritation and that rates of mononuclear mitotic division at such sites was also faster than in normal animals [32]. In this regard, RAUCH recently demonstrated more mitoses in mononuclear leukocytes from tuberculous or BCG vaccinated persons than in normals when tuberculoprotein was added *in vitro* [56]. Further comments will be made concerning nonspecific activation of macrophages with reference to intracellular organelles and enzymes in a later section.

III. Contribution of Macrophages to Resistance in Tuberculosis

Since the tubercle bacillus possesses no toxic substances of consequence to explain the massive destruction found in many organs in progressive human or other mammalian tuberculosis, the best available explanation for that destructiveness is the effects of cellular or delayed hypersensitivity on cells and tissues in the presence of increasing amounts of antigen which results from multiplying bacilli. Many workers, including RICH [61] and LURIE [34], have repeatedly emphasized the simultaneous development of delayed hypersensitivity and caseation necrosis in experimental tuberculosis. All pathologists will attest to the tremendous importance of caseation necrosis in tuberculosis. Serious spread of this disease, whether local, by way of bronchi or through the vascular bed, is essentially dependent on the development of significant necrosis. Thus the multiplication of the tubercle bacillus in the susceptible and the inhibition or restriction of that multiplication in the resistant may be the most important feature in susceptibility or resistance in tuberculosis and the elimination or killing of bacillus relatively unimportant.

When tubercle bacilli are injected into living animal hosts, multiplication within the macrophages occurs for several weeks, when, if the host is resistant, multiplication ceases within those cells, bacilli diminish or disappear and the lesion is arrested. Because of these repeated observations, RICH many years ago recognized the need to isolate the macrophages from the rest of the host responses to the tubercle bacillus and to study the macrophage-tubercle bacillus relationship. Techniques then available permitted maintenance of such macrophages cultures for only three days. Consequently RICH and LEWIS were unable to detect differences in cell resistance to intracellular bacilli in susceptible and immune animals [59]. With the improvement of cell culture techniques and with the important demonstration by MACKANESS [37] that small concentrations of streptomycin in the medium could inhibit extracellular but not intracellular multiplication of tubercle bacilli *in vitro,* studies to elucidate this fundamental cell-bacillus relationship have been possible. Throughout the following discussion which will be largely concerned with the relationship of macrophages to tubercle bacilli, it must be remembered that in the living animal, both susceptible and resistant, tubercle bacilli are sometimes capable of significant and, in case of the former, massive *extracellular* growth.

A. Intracellular Multiplication of Bacilli of Different Virulence

In experimental animals, virulent tubercle bacilli multiply in the cells of the host much more rapidly than do attenuated bacilli and avirulent bacilli may not multiply at all. In *in vitro* studies using peritoneal macrophages, MACKANESS *et al.* [38] showed similarly that virulent tubercle bacilli grow rapidly within normal cells and attenuated bacilli multiplied slowly or, if the cultures were old, not at all. SUTER [72] and we [6] have also demonstrated that *in vitro* intramacrophage growth rates of tubercle bacilli varied directly with virulence. In our experiments when initial infection by BCG organisms of normal cells was very heavy, further multiplication during the 10–14 days of the experiments led to death of the cells. When infection levels of 1 to 5 BCG bacilli per cell were reached, multiplication was either imperceptible or so slow that cells were not injured. Although SHEPARD used HeLa cells rather than macrophages, he also demonstrated the same relationship of virulence to intracellular multiplication [67].

B. Multiplication of Virulent Tubercle Bacilli in Resistant and Normal Macrophages

In experimental tuberculosis workers have long noted that virulent tubercle bacilli in macrophages of natively resistant species of animals, after a brief period of increasing numbers, were seen to cease multiplication and to disappear [60]. This has also been noted in immunized animals of the same species as compared to controls and in resistant as compared to susceptible strains of the same species. In the latter incidence LURIE has particularly well demonstrated the inhibition of multiplication of human and bovine tubercle bacilli of moderate virulence *in vivo* in macrophages of resistant strains of rabbits compared to susceptible strains [36]. Furthermore, LURIE many years ago [33] showed that mononuclear cells from immunized rabbits which had ingested tubercle bacilli either *in vivo* or *in vitro* and were then cultured in the anterior chambers of eyes of normal rabbits inhibited the bacilli as demonstrated by colony count techniques more than mononuclears from normal rabbits similarly studied.

Using *in vitro* techniques, MACKANESS was not able to demonstrate differences in rates of multiplication of bacilli within macrophages of

immune and control animals [39]. He had infected macrophages with relatively large numbers of bacilli perhaps in some way 'overwhelming' cellular resistance or, by the addition of large amounts of antigen, causing a predominantly injurious effect on the macrophage through delayed hypersensitivity thus obscuring resistance. SUTER [73] who very lightly infected monocytes and we [7] who infected 30–60% of cells in the cultures with from 1 to 5 bacilli/cell have both demonstrated that immune macrophages inhibited the intracellular multiplication of virulent tubercle bacilli compared to control macrophages. In our experience, subcutaneous injection of living BCG vaccines did not result in guinea pig peritoneal macrophages as significantly immune as did airborne infection of the lungs with attenuated human tubercle bacilli. We also observed that while counting the changes in total numbers of intracellular bacilli in immune and control cells at intervals during the experiment yielded significant results, a more definite end-point was the marked reduction in formation of cords and solid clumps of bacilli in immune cells. The formation of colonies of intracellular bacilli caused more rapid 'bursting' of the macrophages in the control cell cultures. Since infection was induced by filtered single cell suspensions of virulent tubercle bacilli and since the medium contained streptomycin, more rapid development of cords and solid clumps of tubercle bacilli within the macrophages of normal animals was unequivocal evidence of more rapid intracellular multiplication than in the macrophages of immunized animals.

MAXWELL and MARCUS [45] using bacteriologic culture plating techniques which gave total numbers of viable tubercle bacilli which remained within macrophages 72 hours after ingestion, showed that immune guinea pig peritoneal macrophages inhibited multiplication of 85% of the ingested $H_{37}R_v$ bacilli as compared to 10% inhibition by the control cells. Of great interest was the notable difference in their experiments between pulmonary macrophages, probably largely from the alveoli, and peritoneal macrophages. Alveolar macrophages from immune animals inhibiting further growth of 98% of ingested bacilli as compared to the inhibition of 77% of virulent bacilli by normal alveolar macrophages! Why normal alveolar macrophages should be almost as effective against virulent bacilli as BCG immunized peritoneal macrophages is puzzling but may represent a nonspecific state of activation in which alveolar macrophages are normally found. Others have also shown the increased antibacterial activity of alveolar macro-