The Epithelioid Cell in Tuberculosis is Secretory and Not a Macrophage

TO THE EDITOR—In their editorial, Lugo-Villarino and Neyrolles [1] refer to the publication by Feng et al [2] and conclude that these authors have come “closer . . . to solving the enigma of how the macrophage population undergoes changes in response to Mycobacterium tuberculosis.” Jones Williams et al [3], however, have demonstrated by electron microscopy that the epithelioid cell in tuberculosis is “primarily biosynthetic rather than phagocytic.” These epithelioid cells, therefore, are not transformed macrophages as claimed by Feng et al, and the term epithelioid macrophage is misleading.

This model described by Feng et al [2] does not reproduce the features of human tuberculosis. Similarly, in recent reviews, referring to animal experiments, the epithelioid cell granuloma of tuberculosis has not been reproduced. Moreover, a specific mycobacterial antigen has not been identified, and it is therefore unlikely that a specific diagnostic test or vaccine can be developed.

Feng et al [2] have detected lipid in human tuberculous granulomas and assume that this lipid is derived from mycobacteria. However, if fragments of mycobacteria are not being phagocytosed, then this cannot be the explanation for the presence of lipid. In the B-type epithelioid cell described by Jones Williams et al [3], the cells often fuse to form giant cells containing many mitochondria. In insects, mitochondria can lose their outer membrane and lipid can be demonstrated at their surface. Ultrastructural studies should help clarify the position, but special stains should be used [4].

An autoimmune animal model of human tuberculosis leprosy has been produced by injecting rabbits with a homogenate of human sensory peripheral nerve plus adjuvant. Some of the rabbits have developed a state of granulomatous hypersensitivity; that is, skin testing using a dilute suspension of sensory nerve in saline has produced an epithelioid cell granuloma [5, 6]. The cytoplasm contains extensive rough endoplasmic reticulum filled with an electron dense product, similar to the A-type epithelioid cell described by Jones Williams et al [3] and also reproducing the ultrastructural features of human tuberculous leprosy. Andrade et al [7] have recently demonstrated that epithelioid cells are CD123 positive in human leprosy, indicating that plasmacytoid dendritic cells are present in the granulomas of leprosy. The antigen is a nonmyelin protein in doses of 1 µg.

In other models of granulomatous hypersensitivity using beryllium and sensitivity to zirconium, the antigen is specific. For example, in patients with berylliosis, skin testing with zirconium produces a foreign body reaction rather than an epithelioid cell granuloma. [6] Granulomatous hypersensitivity can be induced only in humans and is unsuccessful even in nonhuman primates [8].

The Kveim reagent contains granulomatous tissue taken from the spleen of patients with sarcoidosis. Results of skin tests with this reagent are negative in patients with sensitivity to zirconium [8]. Although its results are positive in only 50% of patients with the disease, the test does not give false-positives so is probably specific. Biopsy reveals that the epithelioid cells show “marked secretory activity” [9] and are identical to those seen in human sarcoidosis. It may be possible to induce granulomatous hypersensitivity by injecting Kveim material plus adjuvant into rabbits and performing skin testing with dilute Kveim material. If this procedure is successful, isolation and purification can be performed, and the molecular weight of the active antigen can be determined [10].

Epithelioid cell granulomas rarely occur in the spleen of patients with tuberculosis but are common in lymph nodes. In tuberculous lymph nodes, plasmacytoid monocytes are CD123 positive, producing large amounts of interferon α, indicating that plasmacytoid dendritic cells are present. Lymph node tissue could be obtained from patients who have died. It would be essential to exclude human immunodeficiency virus infection in these patients; a Kveim-type reagent could be prepared and then injected with adjuvant into rabbits. If granulomatous hypersensitivity can be induced, an antigen can be isolated, according to the procedure adopted for sarcoidosis. Adding phenol to the preparation and heating it to 60°C should make it sterile without losing its activity [10]. This antigen should be specific and lead to a diagnostic test.

Note

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References

to progress into an epithelioid phenotype and the terminology "epithelioid macrophage" has been regarded as misleading. We disagree with these comments.

First, we did not invent "epithelioid macrophages," but carefully assigned platelet-transformed macrophages as epithelioid-like cells based on their transcriptional/genetic hallmarks and their cellular morphology. Second, the fact that macrophages develop into epithelioid phenotypes is well documented in granulomatous diseases and particularly in tuberculosis [2, 3]. Multiple lines of evidence, including various animal models, indicate that macrophages with epithelioid appearance (high cytoplasm/nucleus ratios and diffusely eosinophilic cytoplasm [4, 5]) are induced during tuberculosis/bacille Calmette-Guérin (BCG) infection [6, 7] or stimulation with mycobacterial lipids [8]. Such epithelioid macrophages have been described in mice infected with BCG [7] and Mycobacterium tuberculosis [9, 10]. They are recruited into tissue upon stimulation with BCG-coated/mycobacterial lipid-coated beads or mycobacterial glycolipids [8, 11]. More recently, epithelioid macrophages were thoroughly characterized in a nonhuman primate model of tuberculosis. The cells stained positive for macrophage/myeloid cell markers (CD11c, CD68) and for HAM56, a foamy cell marker, thereby indicating their origin and occurrence of mixed phenotypes [6]. Epithelioid macrophages contained M. tuberculosis and at the same time expressed secretory abilities (propensity to release nitric oxide based on the immunostaining pattern). The platelet-transformed macrophages in our ex vivo set-up similarly released cytokines and chemokines, thus acting as secreting cells. Notably, our analyses were performed on bulk-transformed human macrophages and, as such, the capacity to internalize bacteria or release mediators could not be fractionated for foamy, giant, or epithelioid-like cells. While acknowledging this technical limitation of our study, we strongly disagree with the assertion of neglecting the existence and myeloid origin of epithelioid macrophages.

In sum, we maintain that epithelioid macrophages are phenotypes with distinct ontogeny and are endowed with several functions relevant for tuberculosis outcome. Additional new roles of such transformed macrophages remain to be uncovered by future studies.

Our investigations did not aim at the development of diagnostic tests or vaccines, but rather at identifying roles of platelets in tuberculosis pathogenesis and characterizing transformed macrophages in this disease. Moreover, we do not exclude the presence of cells of lymphoid origin, such as some plasmacytoid dendritic cell subsets in tuberculous granuloma, which may express an epithelioid appearance [12]. In fact, our group recently reported the presence of granzyme B-positive plasmacytoid dendritic cells in lymph node granulomas from tuberculosis patients [13]. Rather, we acknowledge the complexity of cellular types involved in granuloma formation and stability. In this context, macrophages and their transformed subtypes are critical for understanding mechanisms underlying disease progression or protection within granulomas [14].

On behalf of all authors (see [1]), and editorial authors: G. Lugo-Villarino and O. Neyrolles (see [15]).

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