some of them were more likely to be a manifestation of intracellular edema than of phagocytosis. In AL, as a result of the action of the pathogenic agent, a distinctive type of "paralysis" of the microglia may take place, with the result that they lose their ability to ingest degenerating cells. Incidentally, some investigators also failed to find any evidence of functioning of the microglia as macrophages in other diseases and experimental situations [1, 6]. Experimental AL in guinea pigs is thus a convenient model with which to study both the mechanism of death of the central axons and functioning of the glial cells under conditions when the integrity of the blood-brain barrier is preserved.

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MORPHOLOGICAL CHARACTERISTICS OF HUMAN AND EXPERIMENTAL ANIMAL LEPROMA CELL CULTURES AND EFFECT OF ANTILEPROSY DRUGS ON THEM*

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KEY WORDS: leprosy; tissue culture; morphology

In view of the absence so far of any reproducible methods for the culture of *Mycobacterium leprae* in cell-free media, the World Health Organization has recognized the need for a search for other approaches to the screening of drugs for antileprosy activity in vitro [8]. The main direction of these investigations is the study of the action of test compounds on various parameters of cells containing *M. leprae* in culture. For this purpose various methods of culture of leproma tissue from human leprosy patients [1, 6, 15] and leproma tissue from animals with experimental leprosy infection [1], infection of cultures of macrophages from blood monocytes of leprosy patients with *M. leprae* [10], cultures of rat and mouse peritoneal macrophages [11] and also of macrophages during culture of nerve tissue, schwannomas, and gliomas [7, 14], have been used. Changes in the quantitative parameters of the dopa reaction [6], the concentrations of thymidine [10, 12] and ATP [9] in the culture, mycobacterial saturation of macrophages [2], and also the ability of *M. leprae*, when subjected to the action of the test compounds, to reproduce in the plantar tissues, were used as criteria of antileprosy activity.

A test system for rapid screening of drugs for antileprosy activity based on their action on a culture of leprous tissue, has been developed at the Research Institute for the Study of Leprosy [2]. In this paper we describe the morphological and functional characteristics of leprous tissue taken from lepromas of varied origin (from patients with leprosy, from experimentally infected nine-banded armadillos and mice) in culture, and the results of their exposure to basic antileprosy drugs.

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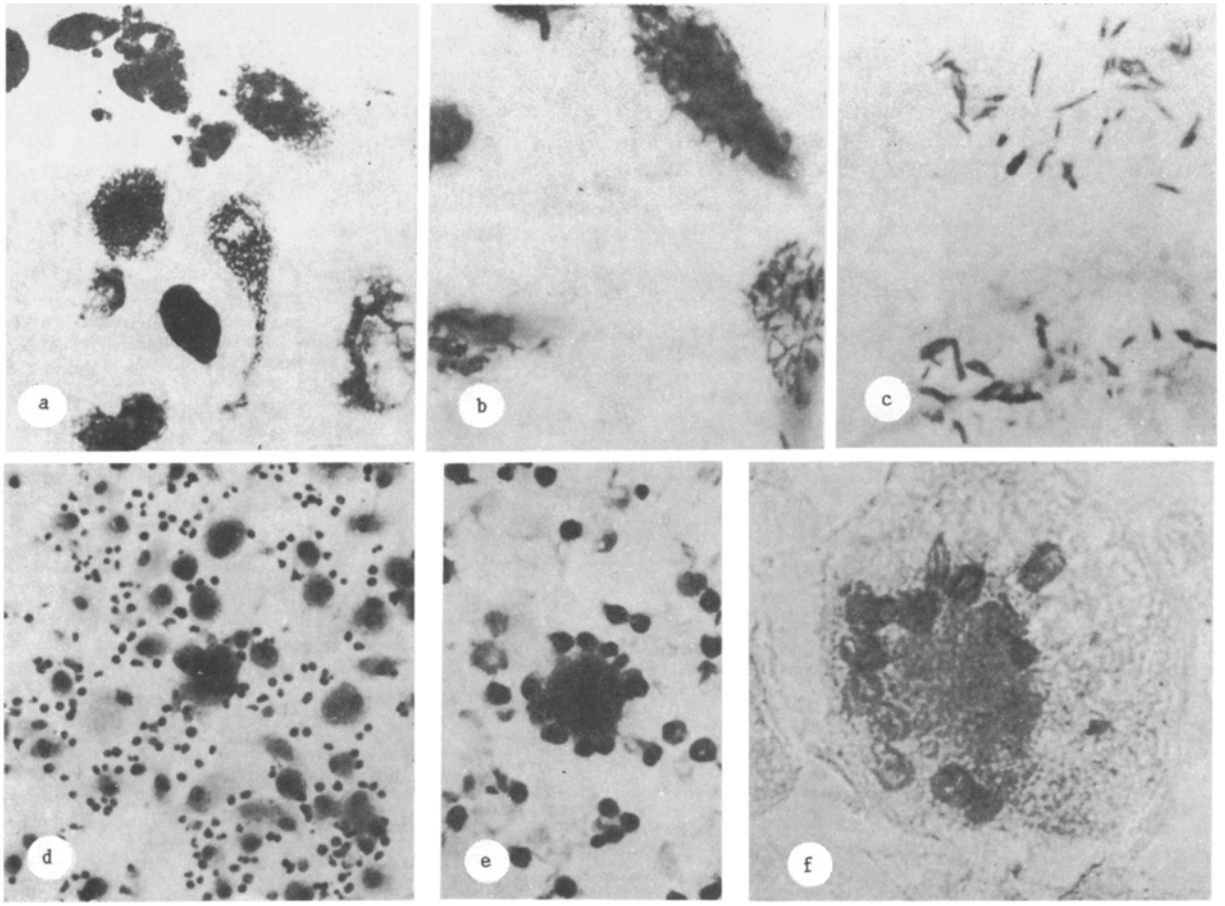

Fig. 1. Inversive (a-c) and reversive (d-f) types of cell culture from human leproma tissue. a) Monolayer of round and fibroblast-like macrophages with vacuolated cytoplasm. 600 ×; b) *M. leprae* in cytoplasm of macrophages. 1250 ×; c) *M. leprae* cells located extracellularly. 1600 ×; d) abundance of lymphocytes in cell culture. 256 ×; e) Rosette-like arrangement of lymphocytes. 500 ×; f) giant cell without *M. leprae*. 1250 ×; a, c, e) Stained with hematoxylin and eosin; b, d, f) stained by Ziehl–Nielsen method.

EXPERIMENTAL METHOD

The material for culture consisted of fragments of cutaneous lepromas from 13 patients with the lepromatous type of leprosy, from two nine-banded armadillos, and 100 mice. Six of the patients were admitted to hospital for the first time, having not previously been treated, in seven patients lepromas appeared during recurrence of the disease. The nine-banded armadillos (*Dasypus novemcinctus* Linn.) were studied under the auspices of Project No. 780259 of Section II of the MMLEP Program of WHO (responsible executive A. A. Yushchenko). The armadillos were infected experimentally by the method described previously [5]. Male CBA mice weighing 24-26 g were infected by injection of $t \cdot 10^5$ mycobacteria obtained from an untreated patient with the lepromatous type of leprosy, and maintained by passage through the mouse footpad [4], into the inguinal fold. The mice were killed by decapitation under ether anesthesia. Culture was carried out by primary explantation on coverslips in special perfusion chambers and in Leighton's tubes. The explant was fixed to the coverslip in a plasma clot and cultured in nutrient medium No. 199 and RPMI 1640 ("Serva," West Germany) with the addition of penicillin (10 U/ml) at 37°C for 7-30 days. The culture medium was changed every 3-7 days. To detect antileprosy activity the test drug was added to the culture medium during the change. In control experiments a solution of dimethyl sulfoxide in a concentration of 0.3%, used as the solvent for the test drugs, was added to the culture medium.

EXPERIMENTAL RESULTS

Comparison of cultures from lepromas of different origin revealed certain general features and also significant differences typical of each species. A common feature of all cultures of

leprous tissue was the presence of a monolayer of macrophages, distinguished by high mycobacterial saturation, much greater than that in macrophages of infiltrates of histological preparations of the explanted tissue. As a rule the macrophages were round, but ovoid, fibroblastlike macrophages, not observed in the explanted tissue, also were observed. In a high proportion of cases a varied number of lymphocytes and of multinuclear giant cells, of various shapes, were found. Observations over a period of time showed that migration of round macrophages with high mycobacterial saturation was observed during the first days of culture. A few days (3-5) later, giant cells of various kinds and fibroblast-like, ovoid macrophages with mycobacteria in their cytoplasm appeared or were formed from round macrophages. The formation of the culture from human and armadillo lepromas was complete after 7 days, whereas in the case of lepromas from mice, it was complete after 3-4 days. The state of the permanent structure of leprous tissue of plateau type in culture could be observed for a period of 10-15 days. By this time a capsule had formed at the periphery of the plasma clot from fibroblasts not containing mycobacteria, fibrocytes, and fibrous structures. In the later stages involution of the culture was observed, in the form of destruction of the cells and overgrowth with fibroblasts. Meanwhile the structure of the leprous tissue as a whole in culture and also the morphology of the cells, cultured in different objects, had distinguishing features so that to some extent it was possible to speak of the species specificity of the cultured leprous tissue.

The culture from lepromas of leprosy patients was distinguished by great diversity in different patients. According to the main criteria on which the modern classification of leprosy [13] is based, cultures can be divided into two types. Type 1 is characterized by the presence of numerous multinuclear giant cells and a high content of lymphocytes. The latter are diffusely arranged in the form of nodules and rosettes around macrophages. The giant cells possess 6-8 nuclei, located in the center or eccentrically, and their cytoplasm contains no mycobacteria. Macrophages of fibroblast shape are distinguished by their relatively low mycobacterial saturation; bi- and trinuclear large macrophages were seen with eccentrically located nuclei, moderate mycobacterial saturation, and large vacuoles around the periphery of the cell. Compared with the histological structure of the explant, lymphocytes and, in particular, giant cells, not found in the explant in some cases, were more numerous. These are characteristic features of reversion of the process of transition into a higher region of the immunologic spectrum. This structure of leprous tissue in culture can be defined as the reversive form. In the other type of leprous tissue in culture lymphocytes and giant cells were almost completely absent and the mycobacterial saturation of the fibroblast-like macrophages also was higher. Quite often groups of macrophages of different sizes, round in shape, and distinguished by their finely vacuolated cytoplasm, resembling lepra cells, were found. Compared with the histological picture of the explant, no signs of reversion (an increase in the number of lymphocytes and giant cells) were observed, but in some cases signs of inversion were discovered in the form of reduction of the number (or their total disappearance) of lymphocytes and giant cells. In these cases the inversive form of leprous tissue culture was identified (Fig. 1). Retrospective comparison of the morphological features of the tissue cultures with the time course of the clinical, histological, and bacteriological parameters of the patients from whom the lepromas were moved for culture, revealed quite distinct correlation with the times of onset of regression under the influence of antileprosy treatment. It was discovered that patients which were the source of lepromas of which the tissue cultures developed in accordance with the reversive type responded much more rapidly and effectively to treatment than patients with lepromas of which the tissue cultures were of the inversive type. This is particularly clearly seen if cultures polarized with respect to their morphological characteristics, i.e., having a well-marked reversive and well-marked inversive character, are compared.

The results obtained can be explained as follows. During culture in vitro a tendency was observed toward ascending transformation of cells of the mononuclear phagocyte system (monocytes into macrophages and giant cells), which may be regarded as a phenomenon of macrophagal and giant-cell transformation of precursors of cells of the mononuclear phagocyte system, and may be recommended for evaluation of the immunologic status of the patient [3]. From this standpoint the reversive type of development of a culture of leproma tissue can be regarded as evidence of the preservation of this tendency, whereas if the structure of the culture is of the inversive type, it characterizes inhibition of the immunologic mechanisms responsible for ascending transformation of cells belonging to the mononuclear phagocyte system, i.e., a most important component of the delayed-type hypersensitivity reaction.

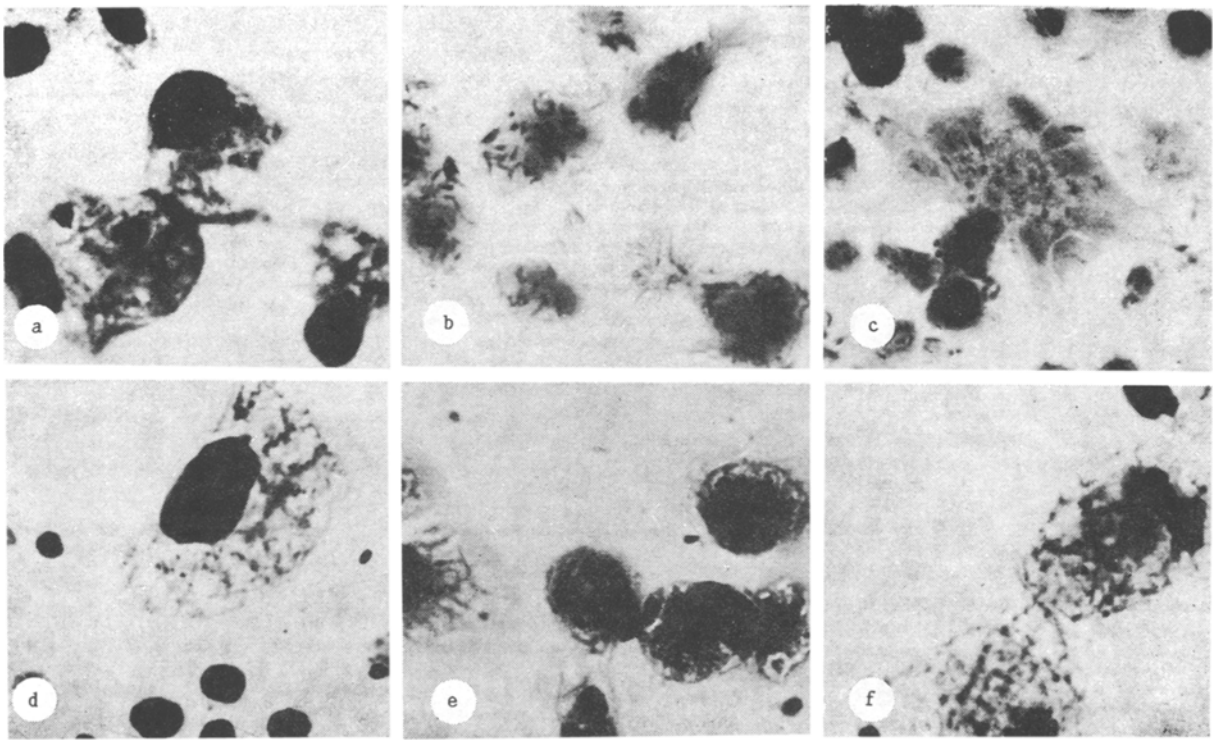

Fig. 2. Cell culture from leproma of nine-banded armadillo (a-c) and mouse (d-f). a) Characteristic signet-ring macrophages; b) high mycobacterial saturation of macrophages; c) polygonal giant cell containing *M. leprae*; d-e) characteristic peripheral vacuolation of macrophage cytoplasm; f) *M. leprae* cells in macrophages. 1250×. a, d, e) stained with hematoxylin and eosin; b, c, f) stained by Ziehl–Nielsen method. Magnification: a-f) 1250 ×.

In tissue cultures obtained by explantation of lepromas from mice and nine-banded armadillos the picture was more uniform, but certain differences were observed between the species. In cultures from mouse lepromas most of the cells were round macrophages, distinguished by the characteristic peripheral vacuolation of their cytoplasm. Fibroblast-like macrophages and lymphocytes were rarely found. Solitary multinuclear giant cells containing up to 10 pale nuclei, diffusely arranged in their cytoplasm, and large numbers of homogeneous mycobacteria, were found. Tissue cultures from armadillo lepromas showed the formation of a special kind of giant cells, not observed in the explant, which were polygonal in shape with three or four long processes and 10-15 round nuclei, arranged around the periphery of the cell in the form of a ring or horseshoe. The bulk of the culture consisted of round macrophages, some of them with finely vacuolated cytoplasm. Fibroblast-like macrophages and lymphocytes were rarely seen (Fig. 2).

The effect of the basic antileprosy drugs was evaluated with respect to their action on the round macrophages containing large numbers of mycobacteria, which constituted the great majority of cells in cultures of leprous tissue both from lepromas of human leprosy patients and from lepromas of mice with experimental leprous infection. The results of this investigation showed that rifampicin, in concentrations of 20, 50, and 100 μg/ml, caused a considerable decrease in mycobacterial saturation of the macrophages — by 20-40-60% proportionally to its concentration. With a concentration of above 100 μg/ml a cytopathic effect was noted. Diaminodiphenylsulfone in concentrations of 32, 64, 128, and 256 μg/ml led to a moderate (by 12-35%) reduction of mycobacterial saturation of the macrophages proportionally to the concentration of the compound in the culture medium. The cytopathic effect also was proportional to concentration and was found even when the concentration of the drug was very low (64 μg/ml).

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ACCUMULATION OF BIOLOGICAL AND NONBIOLOGICAL CORPUSCULAR PARTICLES IN MAMMALIAN LUNGS

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KEY WORDS: lungs; capillary lumen; accumulation of particles

The phenomenon of selective phagocytosis of autologous erythrocytes, treated with glutaraldehyde solution, by leukocytes located in the capillaries of the lungs has been described [3]. It is natural to suggest that this phenomenon reflects the presence of a special function, characteristic of the mammalian lung, connected with the elimination of foreign particles circulating in the blood stream. The investigation described below was undertaken to study this function.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 260-300 g. All painful procedures and removal of the animals from the experiment were performed under ether anesthesia. Altogether 36 rats were divided into nine groups. Animals of group 1 received an intravenous injection of 1 ml of a 1% suspension of yeast cells, used for making dough, suspended in isotonic salt solution. Animals of group 2 received an injection of a suspension of staphylococci ("Zhaev" strain) into the blood stream, in a dose of 10^9 microbial cells to 1 ml of isotonic salt solution; animals of group 3 received an injection of 0.5 ml of commercial black ink, free from preservatives and other coarse particles [1]. Animals of groups 4, 5, and 6 received an injection of 1 ml of polyacrolein microspheres 3 μ in diameter into the blood stream. The microspheres were labeled with a fluorochrome (pyronine). In group 4, the microspheres were

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