REPRODUCTION OF EXPERIMENTAL LEPROSY IN MICE WITH PREVIOUSLY INDUCED INSUFFICIENCY OF THE MONONUCLEAR PHAGOCYTE SYSTEM

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The most adequate model of experimental leprosy is reproduction of the disease in the nine-banded armadillo (*Dasypus novemcinctus* Linn) [11]. The results obtained on armadillos have shown that experimental leprosy can develop according to the lepromatous and also the tuberculoid [4, 9] and borderline [10] type, and also that spontaneous ascending transformation of the process is possible, in the manner of a reversive reaction [7, 8]. Working with armadillos is complicated by the fact that they are difficult animals both to keep and, in particular, to obtain, they are available only in limited numbers and on the American continent, and they do not reproduce in captivity. There are also reports that armadillos may develop leprosy under natural conditions.

Shepard's model [14] has achieved widespread popularity for scientific purposes and, in particular, for screening compounds for antileprotic activity and for investigation of drug resistance of *Mycobacterium leprae*. To obtain this model, a measured number (10^4) of *M. leprae* cells from infected tissues of a leprosy patient is injected into the footpad of a mouse. The number of *M. leprae* cells at the site of inoculation rises to 10^6 after 8-12 months. During subsequent passages from paw to paw the number of mycobacteria increases up to 10^6 after 6-8 months. No lepromatous structures develop and the process does not become generalized. To stimulate the leprotic process in a mouse after intraplantar infection, immunity is depressed by preliminary (before infection) thymectomy and repeated irradiation by a cobalt cannon [12, 13]. Under these circumstances, after 5 months the number of *M. leprae* cells in one pore increased up to 10^6, and after 8 months lepromatous structures formed at the site of inoculation, and bacteriemia developed after 11-14 months, but without the development of lepromatous structures in the organs. The present investigation is the continuation of studies carried out by the Research Institute for the Study of Leprosy, in order to study the morphological and cytochemical properties of macrophages in leprosy [1-3]. The research is based on the view that the macrophage and the mononuclear phagocyte system (MPS) as a whole, are defective, and this is responsible for the development of the leprotic process. The working hypothesis is that reproduction of the infection in animals after preliminary induction of defectiveness of the MPS ought to lead to the more rapid development of the experimental infection and to its wider spread in the body.

EXPERIMENTAL METHOD

A model of defectiveness of the MPS was created by the method in [5, 6] by washing out peritoneal macrophages from the abdominal cavity daily for 10 days. Experiments were carried out on male CBA mice weighing initially 24-26 g. The mice were infected by injecting a suspension of *M. leprae* into a footpad in a dose of 10^4 bacterial cells. The source of infection was material from lepromas of untreated patients with the leprotamous type of leprosy, obtained directly from the patient or after passage from paw to paw. Altogether 150 mice with preliminary induction of insufficiency of MPS were infected. The control group consisted of 125 mice infected without any preliminary activity directed toward MPS. The control and experimental groups were infected with the same suspension on the same day. The mice were killed in mixed experimental – control batches after 2.5-3 months and after 6 months. Internal organs, skin, paws, and testes were taken for histological examination. The number of mycobacteria

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TABLE 1. Time Course of Multiplication of *M. leprae* at Site of Inoculation in Mice with Induced Insufficiency of MPS (experiment) and with Normal MPS (control)

Time after infection, months	Number of M. leprae cells per paw (M ± m)	
	experiment	control
2,5	$(1,4\pm0,9)\cdot10^5$	$(4,0\pm2,5)\cdot10^3$
3,0	$(3,3\pm1,1)\cdot10^6$	$(5,2\pm1,7)\cdot10^3$
6,0	$(2,9\pm0,5)\cdot10^6$	0,0
8,0	$(3,1\pm0,4)\cdot10^6$	$(1,1\pm0,35)\cdot10^6$

TABLE 2. Number of *M. leprae* Cells in Footpads of Mice with Induced Insufficiency of MPS (experiment) and with Intact MPS (control) 3 Months after Infection with Different Strains (M ± m)

Material	Experiment	Control
Directly from patient	$(0,7\pm0,19)\cdot10^5$	$(0,23\pm0,14)\cdot10^5$
3rd passage	$(3,5\pm0,58)\cdot10^6$	$(0,03\pm0,02)\cdot10^6$
4th passage	$(53,59\pm13,96)\cdot10^6$	$(1,66\pm1,26)\cdot10^6$
7th passage	$(0,12\pm0,03)\cdot10^6$	0

was counted by the method in [14]. A suspension was prepared from the tissue of the footpad, the mycobacteria were stained by the Ziehl–Nielsen method, and their number counted on special "Cook Microprint Slides," obtained from "Dynatech" (Switzerland).

EXPERIMENTAL RESULTS

Analysis of the time course of reproduction of the mycobacteria at the site of inoculation showed that their number in mice with induced insufficiency of MPS (experiment) was one or two orders of magnitude higher than in mice with an intact MPS (control). It will be noted that the number of *M. leprae* cells at all times in the control series and after 2.5 months in the experimental series either was not greater than the number injected (in the control), or the increase was not significant. A clear tendency was found for the significance of the mean values of the parameters to increase with an increase in the time elapsing after infection (Table 1). The most interesting results are those obtained 3 months after infection. By this time a significant increase by 2 orders of magnitude was observed in mice of the experimental group compared with the number of *M. leprae* cells injected. In the control series of experiments, the number of microbial cells after 3 months was almost an order of magnitude less than the number injected, and they were not found in all the animals. Thus by taking deliberate action against MPS it is possible to obtain reliable proof of multiplication of the organism in the footpad, i.e., under conditions essential for screening compounds for antileprotic activity, by the end of the 3rd month after infection, which is 3-5 months earlier than with Shepard's method. The rate of reproduction of the mycobacteria in the paw was found to depend on the character of the infective material. Mycobacteria obtained directly from a leper multiplied 3 months later than strains maintained by passage from paw to paw, and the "passage strains" could also differ considerably from each other in their rate of reproduction toward the end of the 3rd month. However, the experiment: control ratio was preserved, i.e., the number of mycobacterial cells in mice infected after induction of MPS insufficiency was an order of magnitude or more greater than after infection of mice with an intact MPS (Table 2).

At autopsy and histological examination of the mice killed after 3 months no changes were found in the experimental or control groups. In animals infected without insufficiency of their MPS, and killed after 6 months, no macroscopic or microscopic changes likewise were observed. In a high proportion of mice infected after induction of insufficiency of MPS, macroscopic and microscopic changes were found in the organs. A very characteristic feature was tumescence of the paws into which the infected material had been inoculated. Measurement of

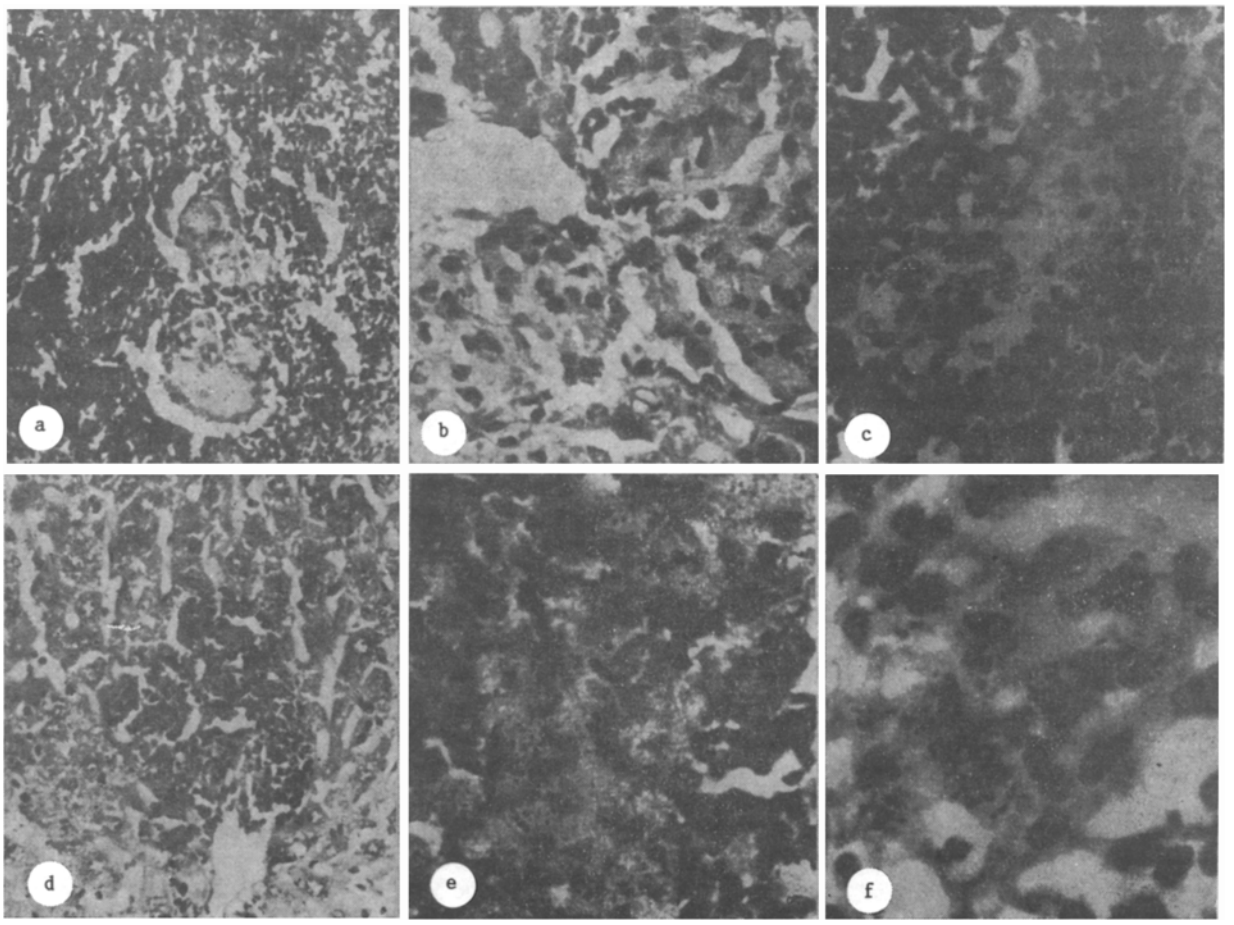

Fig. 1. Lepromatous structures in spleen and liver 6 months after infection with *M. leprae* after preliminary induction of insufficiency of MPS. a) Lepromas in paracortical layer of spleen. 200×; b, c) Spleen. Lepromatous infiltration. 400×; d, e) Liver. "Pale-cell" and "dark-cell" lepromatous granulomas along the course of central veins. 500×; f) Liver. *M. leprae* in macrophages of a granuloma. 1250×. a-e) Hematoxylin and eosin; f) Ziehl—Nielsen's stain.

of their volumes showed significant enlargement of the paws of the experimental groups of mice compared with the controls (more than twice as large). Tumescence of the paws after intraplantar infection also was found in mice subjected to preliminary thymectomy and irradiation with a cobalt cannon, 11 months after injection of the infected material in a dose of 10^4 microbial cells per paw [13]. In a high proportion (up to 60%) of mice infected after induction of insufficiency of MPS, changes in the internal organs, chiefly the spleen and liver, were found at autopsy. The spleen was enlarged to twice or 3 times its normal size, it was firmly elastic in consistency, and multiple gray millet-seed nodules were found in the capsule and in the depth of the organ, and in some cases they replaced 40-60% of the splenic tissue. The liver usually was not enlarged, its consistency was firmer, and gray millet-seed nodules were found on the capsule of the liver and in the depth of its tissue, and were clearly distinguishable from liver tissue. No distinct macroscopic changes could be found in the other organs.

On histological examination lepromatous granulomas were found most constantly in the spleen and liver, and less frequently in other organs. Granulomas in the spleen were located in the paracortical and intrafollicular zones, where they had the appearance of nodular formations and conglomerates of nodules, or sometimes of small clusters of cells. The component cells were macrophages with vacuolated cytoplasm, containing numerous acid-fast mycobacteria, and lymphocytes with, in some cases, small numbers of fibroblasts and solitary polymorphonuclear leukocytes (Fig. 1a-c). Marked hyperplasia of the lymphatic follicles was present in some mice. Granulomas in the liver were usually arranged along the course of the central veins; the number of granulomas varied from single ones, consisting of several macrophages, to multiple

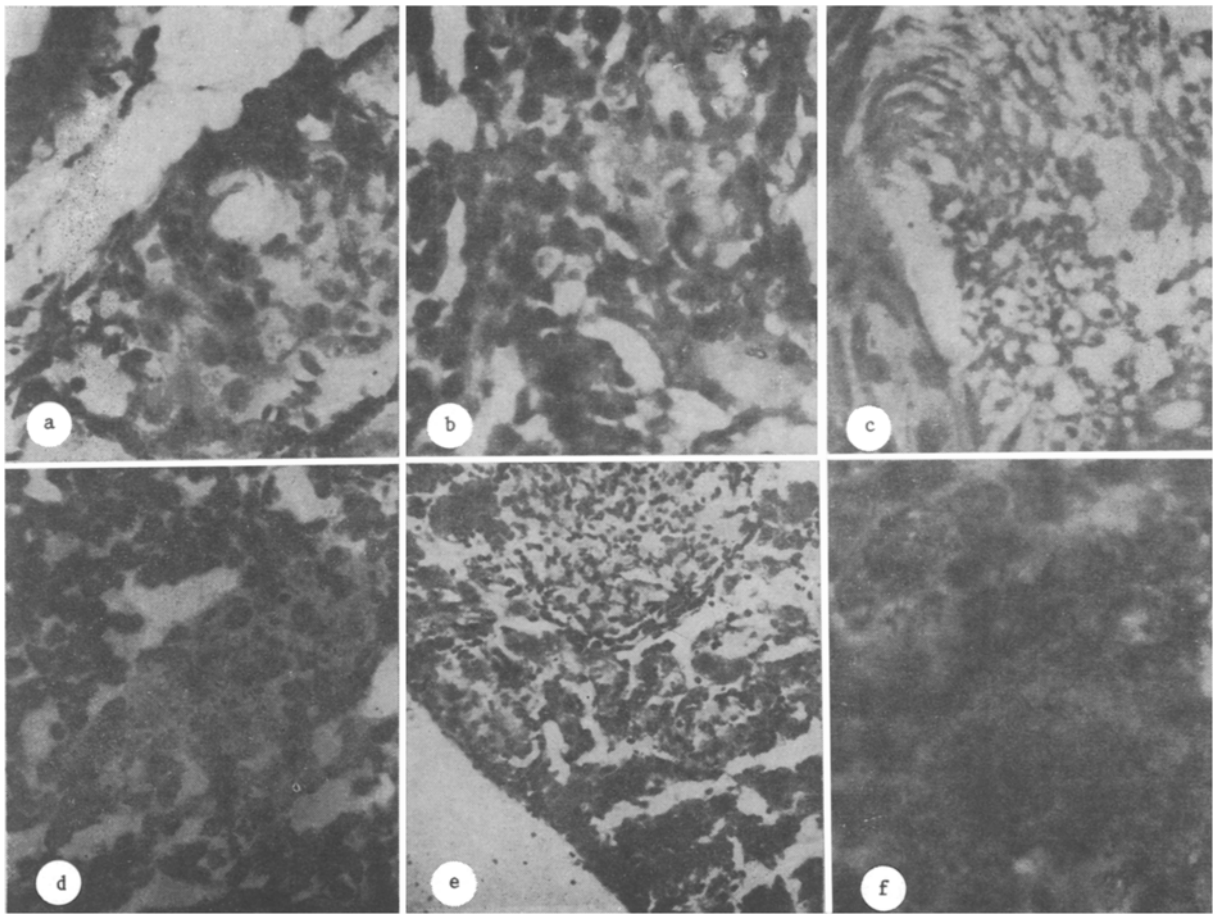

Fig. 2. Generalization of lepromatous structures 6 months after infection with *M. leprae* preceded by induction of insufficiency of MPS. a) Lepromatous granuloma in dermis. 200 ×; b) Lepromatous granuloma along course of nerve. 200 ×; c) Lepromatous granuloma in lung. 200 ×; d) Lepromatous granuloma in testis. 320 ×; e) Lepromatous granuloma in lymph node. 320 ×; f) *M. leprae* in macrophages of granuloma in a lymph node. 1250 ×; a-e) Hematoxylin and eosin; f) Ziehl–Nielsen's stain.

forms, surrounding the vein like a cuff. Separate granulomas located beneath the capsule also were seen. Granulomas in the liver were of two types: "pale," consisting mainly of vacuolated macrophages, and "dark," containing many lymphocytes, fibroblasts, and giant cells. Macrophages of the granulomas contained many acid-fast mycobacteria (Fig. 1, d-f). Specific structures were found much less frequently in other organs. In the lungs granulomas consisting of macrophages and lymphocytes were seen, and similar granulomas were present beneath the epidermis at the site of inoculation of the infected material and in the lymph nodes. In the testis and in nerve, lepromatous structures consisted of small concentrations of macrophages with vacuolated cytoplasm; the macrophages of all granulomas contained acid-fast mycobacteria (Fig. 2).

It can be concluded from these results that insufficiency of the macrophagal component of immunity may be the basis of predisposition to the development of infection with leprosy.

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MONOCLONAL ANTIBODIES TO α-ENDORPHIN EFFECTIVE IN IMMUNOHISTOCHEMISTRY AND IMMUNOBLOTTING

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The endorphins include groups of endogenous opioid peptides, which are synthesized mainly in the anterior and intermediate lobes of the pituitary [1, 2, 11]. α-, β-, and γ-endorphins are known: β-endorphin consists of 31 amino-acid residues, and α- and γ- endorphins are fragments 1-17 and 1-16 of β-endorphin [1, 2, 11]. The concentration of endorphins in the pituitary does not exceed 1 μg/mg wet weight of tissue. To identify such quantities of biologically active substances in tissues, sensitive immunochemical methods based on the use of highly specific antibodies to the substances assayed are nowadays widely used.

In the investigation described below monoclonal antibodies to α-endorphin were obtained and characterized, for subsequent use in immunochemical methods of α-endorphin assay in tissues and culture fluids.

EXPERIMENTAL METHOD

BALB/c mice were immunized by repeated subcutaneous injections of α-endorphin (synthesized and generously provided by Professor M. I. Titov, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), conjugated with bovine serum albumin with the aid of bis-diazotized benzidine [5]. Splenic lymphocytes from immune mice (with a titer of not less than 1:200) were fused with mouse myeloma X63-Ag8.653 cells [8], using the fusion technique described in detail in [10]. The clones were tested for the presence of antibodies by radioimmunoassay [3]. Positive hybridomas were cloned by the limiting dilutions method, using mouse peritoneal or splenic cells as the nurse layer. To obtain ascites fluid, 10^6 hybridoma cells were injected intraperitoneally into young male BALB/c mice, into which Pristane had been injected 2 weeks previously, or Freund's incomplete adjuvant on the previous day. Cross reactivity of the monoclonal antibodies to α-endorphin relative to β- and γ-endorphins was determined by studying the ability of the antibodies to bind equivalent quantities of ^{125}I-β- and γ-endorphins, with specific activity equal to that of ^{125}I-α-endorphin. Radioimmunoassay

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