THE USE OF TUFTSIN IN EXPERIMENTAL LEPROSY

F. E. Vishnevetskii, I. S. Freidlin, A. A. Yushchenko, R. F. Vishnevetskii, V. V. Anokhina, N. G. Urlyapova, M. Yu. Yushin, and I. M. Eskina

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The writers noted previously [5] that synthetic oligopeptides can cause intensification of multiplication of *Mycobacterium leprae* in mouse footpads. Of all the synthetic oligopeptides, the one which has been studied the most is the tetrapeptide tuftsin, discovered in 1973 by V. Najjar [12]. In some outstanding reviews by G. I. Chipens [8, 9], devoted to tuftsin, its marked action on phagocytic activity of neutrophils and cells of the mononuclear phagocyte system (blood monocytes and tissue macrophages) was noted. In recent years most research on tuftsin has been devoted to its action on the CNS [2-4]. There is no information about the effect of tuftsin on leprosy.

EXPERIMENTAL METHOD

Experiments in vivo were carried out on immunologically intact CBA mice initially weighing 25 g. Experimental infection was carried out by injecting 0.3 ml of a leproma suspension into the hind footpad of a mouse in a dose of 10^4 mycobacteria per mouse [13]. Tuftsin (Thr-Lys-Pro-Arg), obtained from "Serva" (West Germany), was used in two modifications: intraperitoneal injection in a dose of 2 μg per mouse weekly through the period of the experiment; 2) a single injection of tuftsin as a component of the myocbacterial suspension, in the form of intraplantar infection in doses of 0.2, 2, and 20 μg/mouse. Animals receiving tuftsin in a dose of 2 μg (both intraperitoneally and by the intraplantar route) received antileprosy treatment throughout the experiment, in the form of a mixture of food and medication containing dapsone in a concentration of 0.01 g or rifampicin in a concentration of 0.05 g/100 g food [10]. Mice infected simultaneously with the same suspension, but not receiving tuftsin or treatment of any kind served as the control. The animals were killed by chloroform poisoning between two weeks and 7 months after injection. The intensity of multiplication of the mycobacteria was estimated from the time course of their number in the mouse footpad, counted by the standard method [10].

The experiments in vitro were conducted on a culture of macrophagallike cell line P 333 and on leproma tissue in culture. The time course of mycobacterial saturation was studied on a culture of the macrophagallike cell line P 388 by combined culture of these cells and *M. leprae* in medium RPMI-1540 ("Serva,") containing 10% fetal calf serum, 20 mM HEPES, 2 mM glutamine, and 100 U/ml penicillin. Tuftsin was added to the culture medium up to a final concentration of 1 μg/ml. In the control experiments the same medium was used but without the addition of tuftsin. Macrophagallike cells were cultured on coverslips in Leighton's tubes. Observations were made from 2 days to 4 weeks later. Leproma tissue was cultured by the method described previously [1]. The effect of different concentrations of tuftsin on mycobacterial saturation of the macrophages and their betaglucuronidase activity, and also the effect of a combination of tuftsin with dapsone and rifampicin on these parameters were studied. Tuftsin was added to the culture medium in concentrations of 0.01, 1, and 10 μg/ml. In the control, culture was carried out in medium without tuftsin. Dapsone and rifampicin were added to the medium with tuftsin in a concentration of 50 μg/ml. Culture with the same preparations in medium without tuftsin served as the control. The duration of culture was 44 days.

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TABLE 1. Effect of Multiple Intraperitoneal Injections of Tuftsin in a Dose of 2 μg Per Mouse on Time Course of Local Multiplication of *M. leprae*

Procedure	Number of mycobacteria ($\times 10^6$) in mouse footpad, at undermentioned times after infection									
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	4 months	5 months	6 months	7 months
Infection, tuftsin	0,26±0,02	0,34±0,06	0,55±0,04	0,83±0,05	0,98±0,05	1,26±0,10	3,80±1,07	5,10±0,26	22,9±2,0	19,0±1,0
Infection, tuftsin, treatment with ramification	0,07±0,02	0,64±0,03	0,74±0,05	0,86±0,03	0,93±0,09	1,18±0,11	1,23±0,03	1,10±0,07	1,85±0,66	1,00
Control. Infection without tuftsin and without treatment	0,17±0,02	0,22±0,03	0,36±0,03	0,39±0,01	0,61±0,04	0,64±0,07	0,63±0,05	0,60±0,05	0,90±0,01	1,0±0,01
	0,08±0,01	0,08±0,05	0,83±0,05	0,83±0,05	1,18±0,09	1,52±0,19	3,43±0,29	3,94±0,18	4,35±0,43	3,0±0,32

Legend. All animals were infected simultaneously by a suspension prepared from a mouse footpad (6th passage, primary isolation from leproma) in an untreated patient with the lepromatous type of leprosy. Each mouse received 0.3 ml of suspension, containing 10^4 mycobacterial cells.

TABLE 2. Effect of a Single Intraplantar Injection of Tuftsin on Local Multiplication of *M. leprae*

Procedure	Number of mycobacteria ($\times 10^6$) in mouse footpad, at under-mentioned times after infection		
	3 months	4,5 month	6 months
Infection with simultaneous injection of tuftsin in a dose of 2 μg/mouse, treatment			
0,2	4,30±0,49	69,28±1,09	62,13±2,16
	1,54±0,15	45,24±8,57	54,61±1,43
20	3,28±0,16	69,4±0,42	67,38±1,77
Infection with simultaneous injection of tuftsin in a dose of μg/mouse, treatment			
with dapsone	0,83±0,26	1,94±0,03	1,96±0,05
with rifampicin	0,03±0,00	0,08±0,00	0,43±0,15
Control. Treatment without tuftsin:			
Without treatment	0,67±0,2	8,10±1,5	16,6±1,83
Treatment with dapsone	0,36±0,19	0,1±0,00	0,93±0,18
Treatment with rifampicin	0,03±0,00	0,08±0,00	0,43±0,15

Legend. All animals were infected simultaneously with one suspension prepared from the footpad of a mouse (4th passage, isolated initially from a leproma in an untreated patient with the lepromatouse type of leprosy). Each mouse was given 0.3 ml of suspension containing 10^4 mycobacterial cells.

EXPERIMENTAL RESULTS

Experiments in Vivo. In control experiments with intraplantar infection, a significant increase in the local concentration of *M. leprae* was observed after the 4th month, indicating multiplication of the microorganism [11]. The use of tuftsin in the form of multiple intraperitoneal injections in a dose of 2 μg/per mouse during the first four months had no marked effect on local multiplication of *M. leprae*. Some increase in the number of *M. leprae* cells was observed after 5 months. After 6 months the number of *M. leprae* cells in the footpad was more than five times greater than in the control experiments (Table 1). Thus repeated intraperitoneal injection of tuftsin in a dose of 2 μg/per mouse leads to intensification of multiplication of *M. leprae* in the case of intraplantar infection. The use of antileprosy drugs in this series of experiments showed that their efficacy was preserved in mice receiving tuftsin. The therapeutic action of rifampicin was clearly marked after 12 weeks, and that of dapsone after 4 months. In the experiments with a single intraplantar injection of tuftsin, the number of *M. leprae* cells in the early stages was already significantly higher than in the experiments without tuftsin (control) and in experiments with intraperitoneal injection of tuftsin. The peak of the action of tuftsin was observed after 5 months and it was significantly higher. The number of mycobacteria reached 50.08 ± 0.14, i.e., ten times greater than after intraperitoneal injection of tuftsin, and 12.7 times greater than without tuftsin. The study of dose-dependence of tuftsin after intraplantar injection showed that a tenfold increase and also a tenfold decrease in the dose of tuftsin lead to some degree of intensification of local multiplication of *M. leprae*. The action of antileprosy treatment was similar in principle to that observed after intraperitoneal injection of tuftsin (Table 2). The most logical explanation of these results is the suggestion that the action of tuftsin, as an activator of phagocytosis in leprosy, is mainly connected with the ingestion phase, when it leads to the fullest degree of incorporation of the inoculated material by macrophages, promoting survival and multiplication of a larger number of *M. leprae* cells.

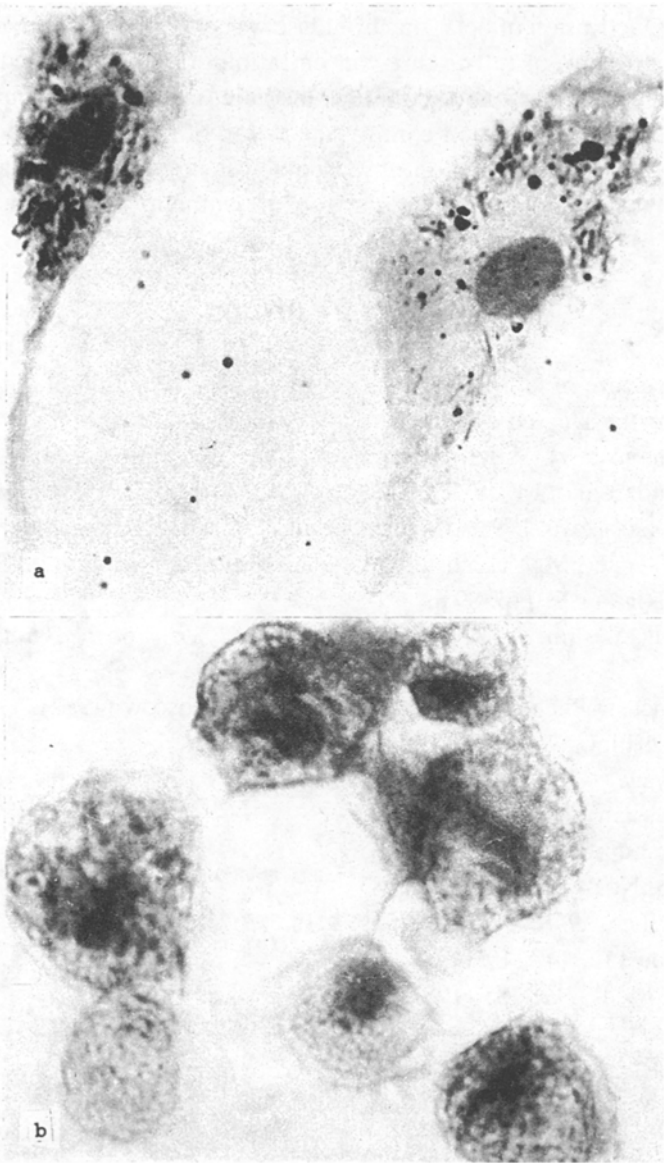

Fig. 1. Leproma tissue culture: a) *M. leprae* in macrophagallike cells of line P 388. Medium with addition of tuftsin. 4 weeks after addition of *M. leprae* to medium. Ziehl-Nielsen stain, 1260×; b) High degree of reaction to beta-glucuronidase activity in macrophages of leproma tissue culture. Medium with addition of tuftsin in a dose of 0.01 μg/ml. Hajashi's reaction, 1260×.

In experiments on a culture of macrophagallike line P 388, addition of tuftsin to the medium lengthened the time of persistence of *M. leprae* in the macrophages. In control experiments (without addition of tuftsin) *M. leprae* cells were found intracellularly only during the first week, and they were completely absent at later times. In medium containing tuftsin, mycobacteria were found in the cytoplasm of the macrophages (Fig. 1a) one month after the beginning of culture (period of observation).

In experiments on leproma tissue culture addition of tuftsin to the culture medium, both combined with antileprosy drugs and without them, had no effect on mycobacterial saturation of the macrophages (compared with the control — culture without tuftsin). Interesting results were obtained by the study of beta-glucuronidase. This enzyme, constantly found in intact macrophages [7], could not be seen in macrophages of lepromatous infiltration and leproma tissue culture, containing *M. leprae* cells [1, 14]. In experiments with the addition of tuftsin to the culture medium but

without the use of antileprosy drugs, activation of beta-glucuronidase was observed in the macrophages containing mycobacteria, and was most marked in the presence of tuftsin in a concentration of 0.01 μg/ml medium (Fig. 1b). On the addition of tuftsin together with dapsone and rifampicin to the culture medium, no beta-glucuronidase could be detected in the macrophages. The results of the in vitro investigation confirm the action of tuftsin on macrophagal cells in leprosy, which is manifested most clearly as activation of incorporation of M. leprae cells by the macrophages.

The results demonstrate the potential value of the use of synthetic tuftsin when studying various aspects of experimental leprosy.

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