A Study of the Biology of *M. lufu* and Prospects for Using It in Leprosy Investigations

Although presently a possibility of acquiring leprosy infection from various sources (from nine-banded armadillos, mangabey monkeys, etc.) is proved, yet a patient with leprosy is considered the main source of infection of epidemiological significance.

But such postulates do not give answers to a lot of questions posed by practical leprology: cases of spontaneous leprosy when the source of infection is unknown; prevalence of lepromatous type of leprosy on the territories with fading endemic. It is difficult to explain occurrence of leprosy infection in wild nine-banded armadillos in the USA and mangabey monkeys in Zaire and to ascertain the role of these animals in maintenance of the infection on the above territories. Ways of transmission and entry for *Mycobacterium leprae* infection as well as the causes of persistence of leprosy endemic regions also remain unclear.

At the end of the 19th century a hypothesis of acquiring leprosy infection from the environment was put forward by Biedencap, Beaven-Rack and Lutz [4]. Our studies were based on the assumption that leprosy bacilli are a part of the biosystem of a given region (contrary to the generally accepted view of obligate intracellular parasitism of *M. leprae*). In order to validate this hypothesis a series of investigations was carried out using *M. lufu* isolated from the soil by Prof Francoise Portaels. *M. lufu* are able to grow on the artificial nutrient media and show sensitivity to sulfones similar to that in *M. leprae* [3].

In vivo experiments with introduction of *M. lufu* into mice foot pads (Shepard's method) [6] showed a significant growth of *M. lufu* cultivated on Levenstein-Jensen medium. Intraplantar multiplication of mycobacteria was inhibited by dapsone administered to mice in their diet. Mice infected with *M. lufu* against the background of introducing synthetic tetrapeptide tuftsin showed a generalized infection involving internal organs like human lepromatous leprosy and lepromatoid disease in armadillos [7]. The most marked changes were mainly observed in lungs, spleen and liver.

Histological investigation showed infiltrates around splenic lymphatic follicules and red pulp. The infiltrates consisted of fused granulomas presented by large macrophages with pale eosinophilic cytoplasm and a small, bean-shaped, basophille nucleus. There were numerous acid-fast bacteria (AFB) and fuchsinophilic grains in macrophage cytoplasm. Similar granulomas were observed along central veins, in the stroma of portal tracts and in the middle zones of hepatic lobes. Besides macrophages, granulomas included a few lym-

phocytes and histiocytic elements, as well as fibroblasts delimiting the granulomas from surrounding tissues. In the lungs, alveoli and interalveolar septa were densely infiltrated with large macrophages with AFB in their cytoplasm.

The results of this experiment suggested that under certain conditions *M. lufu* were capable of spreading the internal organs of *M. lufu*-infected animals inducing in them a leprosy-like disease. The inoculation of the infection taken from the tissues and footpads of *M. lufu*-infected animals onto Lewenstein-Jensen medium yielded no growth. This finding is of great importance, in our opinion. It seems as if mycobacteria underwent some changes resulting in the loss of their ability to grow on artificial nutrient medium after their first passage to the animals.

Thus, experimental evidence (a morphological substrate of the infection) for pathogenic nature of *M. lufu*, i.e., their ability to induce a leprosy-like disease in mice under certain conditions, have been obtained ([11]). Morphological study of *M. lufu* showed that they are gram-positive acid-fast rods with a golden luminescence in luminescent microscopy. *M. lufu* dimensions range 1–5 μm in length and 0.3 μm to 0.5 μm in diameter. There were also more elongated forms reaching 10 μm in length.

M. lufu showed mixed growth in the form of light cream or bright-yellow R- and S-colonies on the solid Lewenstein-Jensen medium. A weak pigmentation did not depend on lighting. The growth rate was 5–7 days. *M. lufu* grew at 25°C and 37°C, but at 45°C growth was absent. When cultivating on Shkolnikova's medium uniform turbidity of the medium and formation of particulate sedimentation was observed.

Cultural and biochemical identification allowed us to establish that *M. lufu* were able to grow on the medium enriched with tiophen-2-carboxyl hydrazide (TCH), reducing nitrates into nitrites. This finding conformed to a positive niacin test and set *M. lufu* apart from atypical mycobacteria known. A comparison of *M. lufu* with 27 slow-growing and 33 fast-growing mycobacterial species in 20 parameters could not reveal their affiliation to either group. *M. lufu* most closely resembled *M. fortuitum;* however, the latter are distinctive in

niacin negativity, ability to PASA-degradation and TWEEN-80 hydrolysis.

Because of the absence of a marked ability to form pigment, *M. lufu* might belong to Group III of non-photochromogenic mycobacteria (according to Runyon's classification). This is supported by thermostability of *M. lufu* catalase. Active catalase is lost in niacin-positive *M. tuberculosis* after they have been heated. In atypical mycobacteria belonging to Groups I, II and IV of Runyon's classification, catalase activity is not lost after heating. Mycobacteria of Group III are weakly positive for catalase thermostability. The same is true for *M. lufu.*

A protein profile of *M. lufu* was studied in SDS-polyacrylamide gel electrophoresis (PAGE) according to Laemmli ([2]). The assay showed absence of high molecular triplet characteristics for pathogenic mycobacteria. There were distinct fractions in a zone of average molecular-weight proteins, non-typical of acid-fast saprophytes. This observation suggests that *M. lufu* should be included into the group of opportunistic mycobacteria ([1]). The data obtained confirm the results of *in vivo* experiments when *M. lufu*-infected animals developed pathological process only after previous treatment with tuftsin.

Because the presence of DOPA-oxidase activity in mycobacteria is one of the identification tests for *M. leprae*, recommended by the World Health Organization (WHO), we tested *M. lufu* for DOPA-oxidase activity with using biochemical and electron cytochemical methods. Electron microscopy of *M. lufu* showed "homogenous bodies" of strong electron density, suggesting the presence of DOPA-oxidase in mycobacterial cells. Enzyme granules of 10 nm to 50 nm were found in the cytoplasm of bacterial cells, mainly around cytoplasmatic membranes. The presence of D-DOPA-oxidase activity in *M. lufu* indicates the similarity of *M. lufu* and *M. leprae* in this parameter ([10]).

The study of shared properties of *M. lufu* and *M. leprae* was extended using six clones of monoclonal antibodies (WHO Bank). Species-specific antigenic determinants of *M. leprae* were detected in *M. lufu*, suggesting antigenic affinity of *M. lufu* and *M. leprae* ([5]).

We tried to study protective properties of

M. lufu in experimental leprosy infection. In addition, we attempted a comparative study of the protective properties of *M. lufu* and those mycobacteria used currently as antileprosy vaccines (BCG, heat-killed *M. leprae, M. vaccae*). It was established that preparations from *M. lufu* effectively inhibited *M. leprae* multiplication in footpads of mice infected by the Shepard method. Maximal rates of protection were achieved with single intracutaneous or intraperitoneal doses of *M. lufu* preparation 28 days earlier than the *M. leprae* inoculation. Protective properties of *M. lufu* were not dependent on administration schemes being much higher than the vaccines from BCG, *M. vaccae,* and viable and killed *M. leprae* ([9]).

We developed an ELISA-based test for the serodiagnosis of leprosy using *M. lufu* preparations as test antigens. The plates were sensitized with antigens from *M. lufu* and *M. leprae*. Sera from active and cured leprosy patients, tuberculosis patients and patients with syphilis and other acute infectious and skin diseases were tested. Healthy subjects' sera were used as controls. It was found that when using either *M. lufu* or *M. leprae* antigen average antibody levels in patients with active leprosy significantly exceeded baseline levels, while in cured leprosy patients as well as in healthy donors and patients with other diseases they did not exceed baseline titres. With sera from patients with active pulmonary tuberculosis, high values of optical density (OD) would be expected because of antibodies towards shared crossreacting antigens of *M. leprae* and *M. tuberculosis*. Nevertheless, average OD values did not exceed baseline titres in this group either. Thus, the results of testing *M. lufu* were similar to the results of testing with *M. leprae*. A comparison of these tests showed that at 100% sensitivity both systems were comparable in specificity and efficacy. Water-soluble preparations from sonicated *M. lufu* had antigens interacting in ELISA with antibodies specific for antigens of leprosy sera. This finding suggests antigenic affinity of *M. leprae* and *M. lufu* ([8]).

All the characteristics found through our investigations permitted to state some relationship between *M. leprae* and *M. lufu* and infer that *M. lufu* might be a strain of *M. leprae*. Thus, we have a culture of leprosy mycobacteria suited for developing diagnostic tools and vaccines in leprosy (as evidenced by our investigations).

Our results suggest that *M. leprae* penetrating the tissues of warm-blooded animals (as was shown in *in vivo* experiments with *M. lufu*) should lose their ability to grow on artificial media even after the first passage. This peculiarity of leprosy agent is considered as a failure of *M. leprae* to survive outside their host.

Based on novel notions and views of *M. leprae* biology, namely, their ability to survive in the environment irrespective of presence or absence of leprosy-susceptible animals or humans in a given country, we might look at epidemiology of leprosy differently. If terms of the endemicity of leprosy, one should admit that leprosy incidence could be contributed not only by leprosy patients discharging numerous mycobacteria but by microorganisms being equivalent members of an association of diverse organisms living with each other (a biocoenosis) that was formed long ago. In leprosy-endemic areas infection via soil, water and etc. competes with another route, i.e., from human to human. With sporadic cases of leprosy the chance of catching leprosy from the environment seems to be a more workable hypothesis as one if not the only method for the transmission of the disease.

With high prevalence of leprosy, the transmission through human-to-human contact dominates because in this case people are infected with an adapted-to-humans strain of *M. leprae*. Also, this is the reason that the foci of leprosy infection shows the whole spectrum of leprosy manifestations—from lepromatous to tuberculoid forms. Mycobacteria existing in the environment and able to induce leprosy are not adapted to humans. They can induce the disease only in those people with high susceptibility to leprosy. This seems to be the reason of prevalence of the lepromatous type of leprosy at when the incidence is sporadic, since it is generally accepted that the type of leprosy depends on immune defect of the diseased individual rather than on virulence of leprosy pathogen. Prolongation of the leprosy incubation period under conditions of sporadic incidence could also be explained as a longer period of adapta-

tion of the agent entering a human organism from the environment. "Spontaneous" cases of leprosy in human beings and leprosy-like disease in some animals (nine-banded armadillos, monkeys) can also be explained by having acquired the infection from the environment. "Relapses" of the disease in some patients who do not receive further treatment after cure might be considered as repeated infection of leprosy-susceptible people.

Thus, the causes of persistent leprosy endemic "pockets" include a combination of natural factors, the main of which is the existence of opportunistic microorganisms (as a part of biocenosis) capable of inducing leprosy infection in susceptible human beings and in animals under certain conditions.

—M. Yu. Yushin, M. D.

Volzhskaya, 62, app. 7
414045 Astrakhan, Russia

REFERENCES

1. KALYANINA, O. V., YUSHIN, M. YU., DYACHINA, M. N., KOMEYEV, A. A., CHERNOYUSOVA, L. N., KALININA, O. A. and SALAMATINA, O. S. Cultural, biochemical and immunochemical characteristics of laboratory strains M.01, M.011 and *M. lufu.* Materials of Symposium "Immunodiagnosis and Immunorehabilitation in Leprosy, Tuberculosis and Other Chronic Diseases." Astrakhan, 1998, pp. 68–69 (in Russian).
2. LAEMMLI, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227** (1970) 680–685.
3. PORTAELS, F. Study of unclassified dapsone sensitive mycobacteria isolated from the environment in Zaire. Ann. Soc. Belg. Med. Trop. **60** (1980) 381–386.
4. RESHETILLO, D. F. *Prokaza.* St. Petersburg, 1904 (in Russian).
5. SALAMATINA, O. S., DYACHINA, M. N., KALININA, O. A., CHERNOUSOVA, L. N., KALILLANINA, O. V. and YUSHIN, M. YU. Detection of species-specific antigenic determinants of *M. leprae* with using monoclonal antibodies. In: *Topical Problems of Skin and Sex-Transmitted Diseases.* Astrakhan, 1998, pp. 115–117 (in Russian).
6. SHEPARD, C. C. The experimental disease that follows the infection of human leprosy bacilli into foot pads of mice. J. Exp. Med. **112** (1960) 445–454.
7. VISHNEVETSKY, F. E., FREYDLIN, I. S., JUSENKO, A. A., VISHNEVETSKY, R. F., ANOKHINA, V. V., URLYAPOVA, N. G., YUSHIN, M. YU. and ESKINA, I. M. Use of tuftsin in experimental leprosy. Bulletin of Experimental Biology **8** (1991) 181–183 (in Russian).
8. YUSHIN, M. YU., DYACHINA, M. N., DEGTYAREV, O. V. and KALYANINA, O. V. Leprosy serodiagnosis with using *M. lufu* antigen. Materials of Symposium "Immunodiagnosis and Immunorehabilitation in Leprosy, Tuberculosis and Other Chronic Diseases." Astrakhan, 1998, pp. 35–39 (in Russian).
9. YUSHIN, M. YU and KALILLANINA, O. V. Protective properties of *M. lufu* in experimental leprosy infection. Problems of Tuberculosis **2** (1995) 47–48 (in Russian).
10. YUSHIN, M. YU., MASLOV, A. K., BOCHANOVSKY, V. A., KALYANINA, O. V. and MIKHAILOVA, M. N. Biochemical and electron cytochemical study of DOPA-oxidase activity in *M. lufu.* Abstracts of Concluding Scientific Conference of ASPU. Astrakhan, 1997, p. 26 (in Russian).
11. YUSHIN, M. YU., VASILYEV, A. E. and KALYANINA, O. V. A study of some properties of *M. lufu in vivo* experiments. Transactions of Conference "Epidemiology, Clinics, Diagnosis, Treatment and Prevention of the Main Infectious Diseases. Tambov-Astrakhan, 1994, pp. 137–138 (in Russian).