Lep. Rev. (1967) **38**, 3, 189-192

Lysosomes—Their Relationship with Vitamin E and Leprosy

DR. MENY BERGEL
Director, Laboratorio de Investigaciones Leprológicas, Rosario, Argentina

INTRODUCTION

Leprosy is an infectious disease probably due to 'organic susceptibility'. Immunological studies of the disease have shown that, in the pair bacillus-host, the latter seems to be the main factor in its pathogenesis.

Therefore it will be interesting to investigate the biological, biochemical and immunological conditions that prepare the organic susceptibility which facilitates or impairs the growth of *M. leprae*. This paper is a partial attempt to unveil the pathogenic mechanism of the leprosy infection through modern knowledge on lysosomes.

LYSOSOMES

Lysosomes were discovered by De Duve in 1955, and the CIBA Foundation dedicated a symposium[1] to them. They are subcellular cytoplasmic organelles which enclose hydrolytic enzymes, mainly acid phosphatase, with only one membrane. These corpuscles are formed by enzymes, lipids, peptides, aminoacids, nucleotides, metals, etc.; they are found in most animal cells, especially in liver, kidney, spleen, intestine, leukocytes, macrophages, etc.

The enzymes contained in the lysosomes are of the hydrolytic type; they act mainly in acid pH and show their activity on biopolymers. These enzymes are generally proteases, phosphatases, glycosidases, etc.; the most important enzymes isolated were: acid phosphatases, cathepsin, ribonuclease, arylsulfatase, beta-glucuronidase and beta-galactosidase. The activity of the acid phosphatase is especially important as it indicates the lysosomic enzymatic complex.

Lysosomes are formed by 2 elements: the membrane and the hydrolytic enzymes contained inside it.

The membrane differs from that of other subcellular corpuscles as mitochondria—with double membrane—because of its simple structure. This single membrane of lipoproteic nature acts as a barrier between lysosomic enzymes and substrates. Thus the enzymes remain inactive as long as the lipoproteic membrane is untouched.

It has been proved lately that lysosomes are involved in the mechanism of morphologic changes, catabolic disorders, autolytic processes, necrosis, and infections and inflammatory processes, etc. They initiate intracellular digestion and a great number of catabolic processes, being therefore connected to the pathogenesis of several morbid processes. They would be mainly connected with phagocytosis and the destruction of alien substances.

LYSOSOMES AND VITAMIN E

The lipoproteic nature of this single membrane in the lysosomes suggested a relationship between these corpuscles and the compounds connected with the auto-oxidation of lipids such as Vitamin E—well known biological antioxidant—as the attack of subcellular particles is generally initiated through their membranes.

Damage of the lysosomic membrane and lysosomes themselves were connected to the conditions which cause auto-oxidation of lipids, such as irradiation peroxides, systems that form free radicals, vitamin E deficiency, etc.

Experimental studies[2 3 4] have shown that these conditions damage the lysosomic membrane. Besides these, other alterations as osmotic pressure, pH, detergents, freezing, etc., cause the previously mentioned damage.

These studies led to experiences on nutritional Vitamin E deficiency and muscular dystrophies in relation to lysosomes.

Tappel *et al.*[5] have shown that in muscular dystrophies, genetic as well as nutritional ones, caused by Vitamin E deficiency, there is a high proportion of lysosomic enzymes originated in the macrophages, in direct relationship to the catabolic processes of the muscle. These, in turn, are evidenced in histological manifestations of muscular disorganization and excretion of products of tissular degradation. Lysosomic alterations have also been shown to occur in other alterations caused by Vitamin E deficiency, such as encephalomalacia in chickens. Homogenates of chicken brains in chickens with Vitamin E deficiency and symptoms of encephalomalacia show important increase in lysosomal activity.

The explanations of these facts would be as follows: Vitamin E deficiency causes an increase in peroxidation of lipids, damaging cells and its subcellular components, among which lysosomes are counted. As these are broken, lysosomal enzymes are freed, and they alter cells causing an invasion of macrophages and muscular catabolism.

On the other hand, Desai *et al.*[2] have shown that the lysosomes isolated in rat livers are extraordinarily labile to the auto-oxidation produced by systems that form free radicals, such as linoleate emulsions, ultra-violet radiation, and gamma radiation.

Sawant *et al.*[6] have proved that irradiation frees lysosomal enzymes in *in vitro* systems such as arylsulfatases, beta-glucuronidase, acid phosphatases and ribonucleases.

In conclusion, both peroxidation and irradiation cause selective damage on the lipoproteic membrane of lysosomes through the activity of the free radicals generated by the mentioned systems.

LYSOSOMES AND LEPROSY

The electronic-microscopic study of the leprosy cell allowed the discovery by Yamamoto, Imaeda and Nisihura[7 8 9 10] of subcellular elements called 'opaque droplets' or 'opaque bodies'. The histochemical study of these elements identified them as lysosomes. At this stage of knowledge on these subcellular corpuscles, study of their connection with the histogenesis, pathogenesis and chemotherapy of leprosy was started.

Cytochemical techniques show clear evidence of the existence of acid phosphatases in those 'opaque droplets' that Brieger and Allen[11] identify as lysosomes.

The lysosomes, in the appearance of a thin granular substance, are found in the leprosy cell around the leprosy bacillum. They are also found in other infections, such as those by tuberculosis bacillus and staphylococci.

Chemotherapy with DDS and similar compounds alters the contents of this lysosomal substances, in the sense that administration of such chemotherapy increases the contents.

It was also seen that bacilli disintegrate in that substance of abundant hydrolytic enzyme content. When bacterial disintegration occurs a foamy structure appears around the lysosomal substance.

The lysosomal activity of the leprosy cell has also been connected with the clinical evolution of leprosy patients, with the degree of ease with which the leprosy bacillus develops, and with the natural immunity to this disease.[12]

Karat[13] suggests that DDS acts through the lysosomes, which, by freeing hydrolytic enzymes, kill the *M. leprae* and help the removal of bacillar rests.

Recent experiences tend to show that Schwann cells are rich in lysosomes and immunity to leprosy rests partially in them. There can also be a connection between the lack of response to the antileprotic chemotherapy and the deficient content of lysosomes in the cells or the decrease or inhibition of lysosomic activity that occurs in certain cases.

Interpretation of these facts is as follows: lysosomes are subcellular corpuscles with a lipoproteic membrane, very sensitive to auto-oxidation processes which have the important function of destroying *M. leprae*. Therefore any element that tends to protect the lipoproteic membrane and also the lysosome, indirectly helps destroying *M. leprae*. That is the case of the biological antioxidants, potent inhibitors of

auto-oxidation, and of DDS, biological antioxidant with Vitamin E-type of activity, as shown by Bergel.

VITAMIN E AND LEPROSY

In 1951 Bergel[14] made the first complete description of the possible relation between leprosy and Vitamin E. Later on, this investigator carried out many experimental trials[15 16 17 18 19 20] which have confirmed this type of relation. Other authors have also confirmed[21 22 23] part of these trials.

Of this series of experimental trials, the most important facts that show the connection between Vitamin E and leprosy are summarized below: (*a*) development of *M. leprae* inoculated in rats and mice under prooxidant and severely prooxidant diets; (*b*) biological antioxidant activity, manifested as anticeroid activity, of chemotherapeutic antimicrobial agents, especially antileprotics. Bergel[24] has proved the biological antioxidant activity—similar to that of Vitamin E—of the DDS, both *in vitro* and *in vivo*, which would be due to the presence of its 2 free amino groups in position 1-4; (*c*) therapeutic activity of a great number of biological antioxidants as those already mentioned and of Vitamin E. It has also been established that prooxidant diets favour the development of experimental infections caused by other mycobacteria as BCG, *M. tuberculosis* Vallee, *M. fortuitum* Penso, etc.[25]

COMMENTS

This reveals the importance of lysosomes in the pathogenesis and cure of leprosy and also the important role of antioxidants, especially Vitamin E in the protection of the lipoproteic membrane of lysosomes. An increase in the biological oxidations through the formation of free radicals destroys the lipoproteic membrane of lysosomes and therefore they cannot destroy *M. leprae*, which develops freely and originates the disease. The administration of DDS and other biological antioxidants, by impairing auto-oxidant processes would protect the lipoproteic membrane of lysosomes increasing therefore the defensive capacity of the organism against *M. leprae*. Hence, the antileprous chemotherapy acts on *M. leprae* indirectly through the lysosomes. In this sense it differs from antituberculosis chemotherapy which, according to Rist, acts directly on *M. tuberculosis*.

This confirms the fact that leprosy needs a special biological susceptibility for its development, which is conditioned by the lysosomal activity. This explains why *M. leprae* experimentally inoculated does not develop in normal animals under normal conditions, as Bergel has sustained.

SUMMARY

This is a review of the experimental facts that connect lysosomes with leprosy and Vitamin E. Leprosy infection develops in an auto-oxidant susceptibility which favours destruction of the lipoproteic membrane of lysosomes. Lysosomes, due to their destructive activity of *M. leprae*, represent one of the natural mechanisms of defence against leprosy infections.

The antileprosy activity of the sulfones and other antileprotics would be indirect and through its biological antioxidant capacity which protects the lipoproteic membrane of lysosomes, natural agents of destruction of *M. leprae*.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Carlo Erba (U.K.) Ltd. for their valuable co-operation.

He also thanks the Medical Division of Pfizer S.A.C.I. for the English translation of this paper.

REFERENCES

1. Lysosomes-Ciba Symposium 1963. J. & A. Churchill Ltd., London, 1963.
2. DESAI, D., SAWANT, P. L. and TAPPEL, A. L. Peroxidative and radiation damage to isolates lysosomes. *Biochim. Biophys. Acta*, 1964, **86**, 277.
3. SAWANT, P. L., DESAI, D. and TAPPEL, A. L. Factors affecting the lysosomal membrane and availability of enzymes. *Arch. Biochem. and Biophys.*, 1964, **105**, 247.
4. TAPPEL, A. L. Free-radical lipid peroxidation damage and its inhibition by vitamin E and selenium. *Federation Proceedings*, 1965, **24**, 1.
5. TAPPEL, A. L. Increased lysosomal enzymes in muscular dystrophy of vitamin E-deficient rabbits. *J. Biol. Chem.*, 1962, **237**, 2678.

6. SAWANT, P. L., SHIBKO, S., KUMTA, U. S. and TAPPEL, A. L. *Biochim. Biophys. Acta*, 1964, **85**, 82.
7. YAMAMOTO, T., NISHIURA, M., HARADA, N. and IMAEDA, T. Electron microscopy of ultra-thin sections of lepra cells and *Mycobacterium leprae*. *Int. J. Lepr.*, 1958, **26**, 1.
8. NISHIURA, M. The electron microscopic basis of the pathology of leprosy. *Int. J. Lepr.*, 1960, **28**, 357.
9. IMAEDA, T. Electron microscopic analysis of the components of lepra cells. *Int. J. Lepr.*, 1960, **28**, 22.
10. IMAEDA, T. Electron microscopy. Approach to leprosy research. *Int. J. Lepr.*, 1965, **33**, 669.
11. BRIEGER, E. M. and ALLEN, J. M. Cytopathology of the Virchow cell of human leprosy. The Pathogenesis of Leprosy. Ciba Foundation Study Group N.15, London. J. & J. Churchill Ltd., 1963, 31.
12. COCHRANE, R. G. The need for bringing leprosy research into universities. *Int. J. Lepr.*, 1965, **33**, 403.
13. KARAT, A. B. A. Advances in immunology and biochemistry in leprosy in the next decade. *Lep. Rev.*, 1967, **38**, 97.
14. BERGEL, M. El sindrome de carencia de tocoferoles y las dietas prooxidantes considerados desde el punto de vista de la infección lepromatosa. *Rev. méd. de Rosario*, 1951, **41**, 191.
15. BERGEL, M. Inoculacion del *M. leprae* a ratas alimentadas con dietas prooxidantes. *Semana Méd.*, 1957, **111**, 480, 1148 and 1313.
16. BERGEL, M. Influence of various prooxidant nutritional conditions on the growth *in vivo* of *M. leprae*. *Lep. Rev.*, 1959, **30**, 153.
17. BERGEL, M. Animal lepromin with leprosy bacilli from infected rats. *Int. J. Lepr.*, 1959, **27**, 59.
18. BERGEL, M. Actividad tipo vitamina E de la 4 butoxi 4′ dimetilaminotiocarbanilida (CIBA 1906). *Semana Méd.*, 1958, **112**, 704.
19. BERGEL, M. Lepra lepromatosa producida por la inoculación del *M. leprae* en la almohadilla plantar de ratas con dietas proosidantes. *Derm. Trop.*, 1964, **3**, 115.
20. BERGEL, M. Leprosy and nutrition. *Lep. Rev.*, 1966, **37**, 163.
21. MASON, K. E. and DJU, M. Y. Inoculation of *M. leprae* in animals under special dietary conditions. Symposium on Research in Leprosy. Baltimore, 1961, 264.
22. EISMAN, P. C., BARKULIS, S. S. and GEFTIC, S. Attempts to infect rats on a prooxidant diet with *M. leprae*. Proceedings of the Carville Conference on Leprosy, 1961, 4.
23. MOORE, T. and SHARMAN, I. M. Nutritional studies in rats deficients in vitamin E. *Amer. Rev. Tuberc. Resp. Dis.*, 1959, **80**, 223.
24. BERGEL, M. Mecanismo de la actividad antileprotica de las sulfonas. *Semana Méd.*, 1957, **111**, 164.
25. DUBOS, R., SCHEIDLER, R. and BERGEL, M. To be published.