The Pathogenesis of Leprosy

Ciba Foundation
Study Group
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PATHOGENESIS OF LEPROSY

In honour of Professor V. R. Khanolkar

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Preface

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Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, some lasting three or four days, others for one day only. The proceedings of the former are published in book form, and the latter in the manner of the present booklet. Each of these one-day study groups is being organized in honour and around the work of an eminent member of the Foundation's scientific advisory panel, on which there are one or more representatives for most of the countries of the world.

The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

It is hoped that the rapid publication of these study groups in a compact form and at a popular price will permit readers in more distant centres, away from easy reference to the latest literature, to feel more closely in touch with recent developments, many of which have important clinical significance, and be stimulated to make their own topical contributions to international research.

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for the promotion of international co-operation in medical and chemical research

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Study Group on the Pathogenesis of Leprosy, 16th January, 1963

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Contents

CHAIRMAN'S OPENING REMARKS

DR. J. A. DOULL

IT is a rare privilege for me to preside over this study group. Firstly, it is being held as a tribute to Professor Khanolkar, whose contributions are a stimulus to workers in general pathology, in oncology and in leprology. Secondly, the symposia of the Ciba Foundation have achieved an eminent position in medical science under its distinguished Council and Director. Finally, the purpose of this meeting is to further research on leprosy, to which the Leonard Wood Memorial has been devoted for more than 30 years and to which I have given my full time since 1948.

Although it is clearly essential for the study group to restrict its discussions to the development of leprosy in the individual, a word regarding its importance to mankind may not be amiss. In any case I suppose that its distribution in various population groups is determined fundamentally by the peculiarities in its pathogenesis. The disease is chiefly one of warm climates and of under-developed countries. The number of cases in the world is variously estimated at from 5 to 12 millions. Although the disease is not usually a direct cause of death, life is shortened in the lepromatous type, and in probably more than 20 per cent of all cases a physical disability of a serious or permanent nature is present. As far as is known the source of leprosy is exclusively the human case. There is no relationship between human leprosy and the so-called leprosy of animals. The very much higher risk of attack for persons living in household association with lepromatous patients than those in similar association with patients suffering from tuberculoid leprosy indicates the importance of the open case, as in tuberculosis, although the possibility that family susceptibility is a factor has not been excluded.

Although more than 88 years have elapsed since the announcement of the discovery of the leprosy bacillus, an accepted method of *in vitro* cultivation has not yet been reported, nor has anyone succeeded in producing disseminated leprosy in an experimental animal. These are obviously the great handicaps to progress and

it is interesting to speculate on why greater progress has not been made. Some of the reasons are as follows. There are inhibitions resulting from the failures of able scientists in the past. New prospects, especially in the study of rickettsia and viruses, appear to offer quicker returns. There is a public demand for the investigation of diseases of greater magnitude, such as cancer, heart and mental diseases. There is the significant fact that leprosy is uncommon in most countries that have become leaders in scientific endeavour. Add to these the world-wide shortage of scientific personnel and the increase in demand of the physical sciences and we have a formidable list of obstacles to be overcome before any comprehensive attack can be made on the basic problems of leprosy.

I think our real need is the attention of a large number of interested and highly trained scientists. To secure this it is essential that there be adequate financial support. We are making some progress in the United States in that in recent years the National Institutes of Health have made generous grants to investigators, not only in the United States, but also in certain foreign countries. Several pharmaceutical manufacturers have also made substantial contributions to leprosy research. One of the best ways of arousing and stimulating the interest of scientists is by such meetings as this. It seems to me that a succession of small conferences on leprosy held through the 1950's, including the Addendum to the Symposium on Experimental Tuberculosis held by this Foundation, at which five of this group were present, and the Symposium on Research in Leprosy sponsored by Johns Hopkins and the Leonard Wood Memorial, in which six members of this Study Group participated, were significant events. I am sure that this meeting will also have a profound effect and one that will be world-wide.

We record with deep regret the death of Dr. Doull, which occurred while this book was in press. We are all the more grateful that it was possible for him to lead these discussions in a subject to which he had made so many contributions and to which he had devoted much of his life.

EXPERIMENTAL OBSERVATIONS RELATED TO THE HISTOPATHOLOGY OF LEPROSY

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THE density and patterned arrangement of nerves terminating in normal human skin varies widely from region to region, and although the most striking difference is between hairy and non-hairy skin (e.g. palm of hand), each region of hairy skin has a well-characterized pattern of innervation. For instance, skin from the back of the trunk contains comparatively few hairs and thus there are few bundles of large myelinated axons to be seen. It is also worthy of note that there are very few non-myelinated axons per unit area. Indeed, only about 2 per cent of the papillae counted in the skin from the back contained nerve bundles, and of those that did, the number of axons per bundle was minimal. By contrast, skin from the dorsum of the ear is lavishly innervated. This difference is well matched by the results of testing the sensory acuity of the skin. Tactile stimuli are found to be most accurately located when the ear skin is stimulated and least accurately when the skin covering the back of the trunk is stimulated. The innervation of the skin cannot, however, be regarded as static. For various reasons, e.g. trauma due to scratching, insect bites etc., the nerve fibres are constantly being damaged, and they continually degenerate and subsequently regenerate. In almost every skin biopsy from normal healthy persons it is possible to find a few nerve fibres at some stage within this cycle (Weddell and Miller, 1962). The changes affect not only the axons but more particularly some of their Schwann cells which undergo a very striking series of alterations in appearance and behaviour, for, as will be shown later, it is they which engage in the task of removing the products of degeneration and prepare a pathway along which regenerating

axons can travel with minimal obstruction. Turnover of this kind increases with age and is also excessive in conditions such as psoriasis (Cowan, Ramaswamy and Weddell, 1962).

How precisely is neural debris removed to leave a clear pathway for regenerating nerve sprouts? Are all Schwann cells equally capable of assuming the rôle of macrophages and are those which do the job capable of removing any kind of particulate matter in their vicinity impartially? In the sensory nerves in non-lepromatous leprosy, Schwann cells in affected nerves are apparently able to ingest both neural debris and *Mycobacteria leprae*, although it is impossible to say whether the mycobacteria were ingested along with the axonal debris or came from elsewhere (Nishiura, 1960). In view of the close connexion which has been established beyond doubt between cutaneous nerves and Hansen's bacillus, it seemed to us worth while to determine in a series of animal and human experiments the behaviour of Schwann cells when confronted with particulate matter at various times following nerve damage (Palmer, Rees and Weddell, 1961). We used widely different forms of particulate matter, ranging from fine carbon particles of standard size (200 Å in diameter) to *M. leprae* and *M. lepraemurium*.

Rats were used in two series of experiments. In the first the sciatic nerve on one side was cut and after periods ranging from 0 to 14 days particulate matter was injected between the cut ends of the nerve. In the second the nerve was crushed, leaving only the epineurium intact (to reduce invasion by extraneous phagocytes to a minimum), and at the same time particulate matter was injected into the nerve in the region where it had been crushed.

After the sciatic nerve had been cut and carbon particles injected between the stumps it was found that the uptake of the particles by the Schwann cells was dependent on the length of time which elapsed between nerve section and the injection of particles. Histologically, using light microscopy, it became clear that the uptake after both cutting and crushing was maximal when Schwann cells were most actively ingesting neural debris (see Table I).

Under the electron microscope it was possible to analyse the process in greater detail. In rats killed five days after the sciatic nerve was cut or crushed and injected with either carbon or

colloidal gold particles, Schwann cells, which normally possess a basement membrane, can be seen divesting themselves of this membrane in stages and then putting out processes which ingest both neural debris and the foreign particles placed in their neighbourhood. An additional and significant finding was the appearance at this time of many pre-collagen or collagen fibres, seemingly sprouting from the Schwann cells themselves, to fill the gaps which had formed between them. These gaps were the results of the death of some of the Schwann cells forming a

TABLE I

RAT SCIATIC INJECTED WITH CARBON PARTICLES (200 Å DIAMETER)

Number of animals	*Time of injection (post-op.—days)*	*Time: injection to death (days)*	*Results*
Nerve cut			
10	0	10	+ve
1	0	24	+ve
2	0	35	+ve
4	2	10	+ve
2	7	10	+ve
2	14	10	−ve
1	14	19	−ve
Nerve crushed			
1	0	10	+ve
2	0	35	−ve

particular pathway and of the complete removal of neural debris from other pathways. The electron microscopy observations not only confirmed those seen under light microscopy but provided additional evidence for the way in which phagocytic Schwann cells tackle their job. The ultimate fate of most of the ingested foreign particles, as well as the neural debris, is certainly removal into blood vessels lying within the perineurium of the nerve bundles.

The behaviour of Schwann cells of rat sciatic nerves is quite different towards mycobacteria. *M. lepraemurium* were only freely ingested by the Schwann cells of uninfected rats when

they were injected into crushed nerves, not when they were placed between the cut ends of the sciatic nerve (see Table II). The Schwann cells of rats already infected with murine leprosy, however, did ingest *M. lepraemurium* placed between the cut ends of the nerve almost as freely as they ingested carbon particles

TABLE II

RAT SCIATIC INJECTED WITH MURINE LEPROSY BACILLI (RATS NOT INFECTED WITH MURINE LEPROSY)

Number of animals	*Time of injection (post-op.—days)*	*Time: injection to death (days)*	*Results*
Nerve cut			
2	0	10	— ve
1	0	19	— ve
1	0	5	weak + ve
1	0	5	+ ve
2	0	5	— ve
2	0	9	— ve
4	2	10	— ve
1	2	20	— ve
5	7	10	— ve
1	7	20	— ve
4	14	10	— ve
1	14	19	— ve
Nerve crushed			
3	0	5	+ ve
2	0	12	+ ve
1	0	35	— ve
1	0	35	weak + ve

(see Table III). On the other hand, Schwann cells from cut nerves of rats either infected or uninfected with murine leprosy rejected both fresh and autoclaved *M. leprae* in the same way as the uninfected rats rejected *M. lepraemurium*. *M. leprae* and *M. lepraemurium* were accepted in small numbers following injection into crushed nerves, when the Schwann cells behaved in a comparable way to those of uninfected rats towards *M. lepraemurium*.

These findings were unexpected, but they suggested that the

effect of injecting carbon particles as well as autoclaved *M. leprae* and *M. lepraemurium* into sensory nerves of human volunteers who already had leprosy might be very rewarding. Since *M. leprae* primarily affect sensory nerves, we started by injecting carbon particles into the skin of five volunteer patients with lepromatous leprosy. We chose this form of the disease because we knew that the skin was well innervated yet subject to a considerable amount of neural "turnover". In each case carbon

TABLE III

RAT SCIATIC INJECTED WITH MURINE LEPROSY BACILLI (RATS INFECTED WITH MURINE LEPROSY)

Number of animals	*Time of injection (post-op.—days)*	*Time: injection to death (days)*	*Results*
Nerve cut			
2	0	5	+ve
1	0	5	−ve
3	0	12	+ve
2	0	35	−ve
Nerve crushed			
2	0	5	+ve
2	0	12	+ve
2	0	35	−ve

particles were seen, seven days after injection, in Schwann cells in those bundles in which there were degenerating nerve fibres. The same thing occurred in the radial nerve in the wrist. Schwann cells containing carbon particles were seen in biopsies removed five days after the particles had been injected between the cut ends of a small fasciculus removed from one of the bundles in each of three volunteers.

Next we offered either autoclaved *M. leprae* or *M. lepraemurium* to Schwann cells by injecting them between the cut ends of small fasciculi of the radial nerve at the wrist in four volunteers, two with lepromatous and two with an indeterminate form of the disease (all lepromin-negative), and we assessed their uptake by removing material from between the cut ends five days later.

We were again surprised to find that not only had the Schwann cells ingested the *M. leprae* in the two injected cases (fortunately autoclaved bacilli have a distinct morphology which allows them to be distinguished from those infecting the patient) but were in a state of activity usually found seven rather than five days after nerve section. On the other hand *M. lepraemurium* were not seen in the Schwann cells in the second biopsies from two volunteers with the same forms of the disease in whom the same procedures had been carried out. They had been ingested by macrophages, which were present in large numbers together with other cells commonly found in zones of acute inflammation. These results seemed to imply that autoclaved *M. leprae* stimulated the Schwann cells in sensory nerves of lepromin-negative patients suffering from leprosy to become specifically attracted towards them, whereas *M. lepraemurium* did not do this.

These observations, even when taken in isolation, suggest that Schwann cells must have a very significant function in human leprosy; our problem was to determine its precise nature. This, we felt, might become clearer if we assumed that the defence mechanisms of the body against infection by one species of mycobacteria would broadly resemble that against another species.

To this end 132 randomly selected schoolchildren (living in Northern Nigeria where there was a high incidence of lepromatous leprosy) were examined for enlargement of the main nerve trunks which can be easily felt in the limbs. This was analogous with mass radiography in the case of infection with *M. tuberculosis*. We found that as many as 21 per cent of the children had one or more enlarged nerves but no other clinical signs of leprosy. We also found that a high percentage of the adult population in the same area of country had one or more enlarged nerves. In other areas of the country, however, where the incidence of leprosy of all forms was low, few of the adults had enlarged nerves, and the same was true in over 100 schoolchildren, in whom the rate was only 6·5 per cent. We also discovered that if biopsies from nerve trunks serving skin lesions in non-lepromatous patients were taken at points far removed from the areas of skin which they supplied, *M. leprae* were still present, although none could be found outside the epineurium or in biopsies taken from the skin lesion itself. This was also found by Nishiura (1960).

This observation, combined with the two series of observations reported above, particularly when added to the fact that in some forms of polyneuritic leprosy no skin lesions can be found (Cochrane, 1959; Dharmendra, 1960), forced us to the conclusion that whatever their portal of entry, *M. leprae* are likely to reach the sensory nerve trunks by way of the bloodstream at some stage in the disease. If this is so, then logically it should be possible to find an occasional bacillus in most of the sensory nerve bundles in the body in cases having single circumscribed skin lesions. Fortunately, a case presented itself which enabled us not only to confirm this, but also to ascertain more precisely the rôle played by the Schwann cell in leprosy.

A 27-year-old male of mixed Malay and European stock stated that he had first noticed an area of slight numbness of the skin just above his left elbow ten years previously; three months before he was examined he noticed that the patch was getting larger and that there was a thickened cord above the left elbow which could not be felt on the right side. On examination, there was a poorly outlined macule centred around the point of the left elbow, measuring approximately 12 cm. proximo-distally and 6 cm. in width, which the patient outlined as the area of numbness. Sensory testing revealed a small oval patch ($2\frac{1}{2} \times 1\frac{1}{2}$ cm.) with significantly reduced acuity to touch, warmth, cold and pin-prick in the upper outer zone of the macule. Despite a careful examination of the whole of the rest of the body only one other small ill-defined area of diminished sensory acuity was found, on the medial margin of the left great toe. A comprehensive physical examination revealed no other abnormalities except that the left ulnar nerve was larger than the right just above the elbow. A skin biopsy was taken to include a portion of the zone of diminished acuity as well as skin of normal sensory acuity. The patient also permitted a small fasciculus to be excised from a bundle of his right radial nerve at the wrist to provide a sample of nerve which could be fixed under optimum conditions for electron microscopy.

The skin biopsy showed two quite different histological pictures; in the affected skin no nerve fibres, Schwann cell pathways (silver staining) or acid-fast bacilli were seen; the normal zone, on the other hand, contained a surprisingly large number of axons, many of which were either degenerating or