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Light and Ultrastructural Study of Sciatic Nerve Lesions Induced Using Intraneural Injection of Viable *Mycobacterium leprae* in Normal and Immunosuppressed Swiss White Mice[1]

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The present experimental model was developed with a purpose of depicting *in situ* the typical nerve lesions similar to the ones seen in human leprosy and to study the early sequence of events surrounding the entry of *M. leprae* into the peripheral nerve. To our surprise, freshly harvested *M. leprae* injected directly into the sciatic nerve, failed to retain its viability and multiply in both immunosuppressed (TR) and nonimmunosuppressed (non-TR) Swiss White (S/W) mice ([19]). A further detailed observation made with light and ultrastructural study described in the present paper also revealed that there was effective containment of the infection and regression of the lesion within the sciatic nerves of S/W mice.

MATERIALS AND METHODS

Animal. Randomly-bred, both male and female, Swiss White mice (S/W) maintained under standard laboratory conditions were used.

A total of four experiments were carried out. One experiment, each using human-derived *M. leprae* (freshly obtained nodule biopsy from two untreated lepromatous leprosy (LL) patients and armadillo-derived *M. leprae* (liver biopsy was obtained from Eleanor E. Storrs, ILEP-WHO, and was stored at –70°C) in groups of normal (non-TR) and immunosuppressed (TR) mice, respectively. Results, thus obtained, were compared.

The method of immunosuppression of mice ([2]), preparation of *M. leprae* suspension, and the intraneural injection procedure followed were as detailed in an earlier paper ([19]). In brief, 3 to 4 weeks-old, female S/W mice were thymectomized and immunosuppressed using 5 doses of 200 rad each at biweekly intervals. *M. leprae* suspension (10 µl) was macroinjected into the sciatic nerve using a Hamilton syringe.

Control. Normal S/W mice similarly injected into the sciatic nerve with 10 microliters of normal saline served as control.

Biopsy. The nerve biopsies were obtained at 24 hr, 72 hr, 1 week, 2 weeks, 3 or 4 weeks, 12 weeks, 24 and 48 weeks following intraneural injection. A minimum of 4 mice were biopsied (8 nerves) at each interval. Prior to the biopsy, mice were examined for clinical signs of hind limb weakness. The mice were anesthetized using 1% pentabarbitone. Sciatic nerves were exposed surgically and changes, such as adhesion, swelling and thickening if any at the site and along the length of the nerve, were recorded. Two cm length of the nerve to include the site of injection and equi-distance proximally and distally were biopsied. Four nerves, randomly chosen per interval, were fixed in Formal Zenker and glutaraldehyde and processed for light and electron microscopy, respectively.

After the nerve biopsy, both left and right foot pads, ear lobes, the liver and the regional lymph nodes collected at regular intervals, were homogenized and checked for bacterial presence, if any, using the standardized procedure ([23]).

Light microscopic studies. Nerve biopsies (4 nerves/interval) fixed in Formal

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THE TABLE *Average of cellular infiltrates and nerve damage seen between 24 hr and 1 yr. following intraneural injection of* M. leprae *in normal (N) and T200×5R (TR) mice.*

Biopsy interval	Mice	Polymorphs	'T' cells	Macrophages	Epithelioid	Plasma cell	Mast cell	Overall infiltrate	Bacilli[a]	Nerve damage[b]
24 hr	N	+	0	+	0	0	+	+	+	+
	TR	+	0	+	0	0	+	+	+	+
72 hr	N	2+	0	+	0	0	+	2+	+	+
	TR	2+	0	+	0	0	+	2+	+	+
1 wk	N	+	+	+	+	0	+	2+	+	2+
	TR	+	0	+	0	0	+	2+	+	+
2 wks	N	+	2+	+	2+	0	+	2+	+	2+
	TR	+	0	+	0					+
3 wks	N	+	3+	+	2+	0	+	3+	+	3+
	TR	+	1+	2+	0	+	+	3+	+	2+
4 wks	N	+	3+	1+	2+	0	+	3+	+	3+
	TR	+	2+	2+	0	+	+	3+	+	2+
3 mos.	N	0	3+	1+	2+	+	0	3+	+	3+
	TR	0	2+	3+	0	+	0	3+	+	3+
6 mos.	N	0	2+	2+	+	+	0	2+	+	3+
	TR	0	2+	2+	0	+	0	2+	+	3+
12 mos.	N	0	2+	2+	+	+	0	2+	+	3+
	TR	0	2+	2+	0	+	0	2+	+	3+

[a] In macrophages in the endoneurium.
[b] Loss/involvement of nerve fibers.

Zenker were embedded in paraffin. Five micron thick transverse and longitudinal sections were cut and stained using Trichrome Modified Fite Farracco (TRIFF) ([8]). These sections were used for studying the gross cellular and structural changes, as well as the localization of *M. leprae* within the nerve. The type and quantum of cellular infiltrate were also graded and recorded for each nerve.

Ultrastructural studies. The glutaraldehyde fixed nerves (4 nerves/interval) were divided into three parts. The proximal (½ cm) segment, the middle (1 cm) length of the nerve to include the site of injection and the remaining distal segment of the nerve were post-fixed in osmium tetraoxide and embedded separately in araldite. One micron thick sections, stained with toluidine blue were examined using a light microscope to record changes, such as fiber loss, type of nerve degeneration and cellular infiltrate. The ultra thin sections, stained using uranyl acetate and lead citrate, were examined using a JEOL 100S transmission electron microscope to record the fine structural changes, as well as to spot the *M. leprae* bearing cells.

RESULTS

Functional deficit and local changes. Hind limb weakness, as assessed by an inability to stretch the toes on holding the mice vertically, was noted in 23/65 (35%) cases at the later intervals, i.e., at the 6th and 12th months. On dissection, adhesion of the nerve to the bed at the site of injection was noted at 72 hr and later. A definite elliptical thickening was noted at the site in the majority of the nerves, two weeks onwards in both non-TR and TR mice. The nerve thickening remained restricted to a length of 2 cm ± 1 cm suggesting that there was no further expansion of the lesion either proximally or distally.

In the entire series of experiments, the occurrence of a large abscess near the site of injection was noted in two cases. One each in a non-TR (at 6th month) and a TR (at 9th month) mice, respectively. The mass was seen lying on the dorsum of the sciatic nerve at the thigh region, but no direct link with the nerve could be established. Biopsy and histopathological examination of the same revealed presence of caseating granulomatous infiltrate. No neural tissue or acid-fast bacilli were seen in the mass. It was noted that in both these cases there was marked weakness in the respective limbs. The pressure exerted by the abscess mass might have resulted in an additional damage to the nerve.

The cell types and their dynamics in the sciatic nerve lesions following intra-

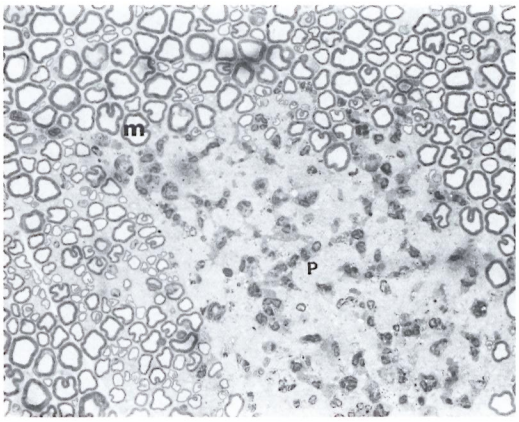

FIG. 1. Sciatic nerve lesion at 24 hr in a TR mouse showing a large aggregate of polymorphs (P) in the endoneurium at the site of *M. leprae* seeding ('middle' segment). The surrounding myelinated nerve fibers (m) appear normal. Transverse one micron thick, araldite embedded tissue section, stained with toluidine blue. ×650.

neural injection of *M. leprae* in non-TR vs. TR mice (refer to Table 1 for summary). An influx of neutrophils (polymorphs) were seen at 24 and 72 hr in both the non-TR and the TR mice (Fig. 1). Nearly 2 cm length of the nerve around the site of injection showed presence of polymorphs in the endo and epineurial region. The numbers decreased subsequently and no polymorphs were seen beyond 4 weeks. In TR mice, well-differentiated macrophages with mild foamy changes were seen at the 4th week with a peak noted at the

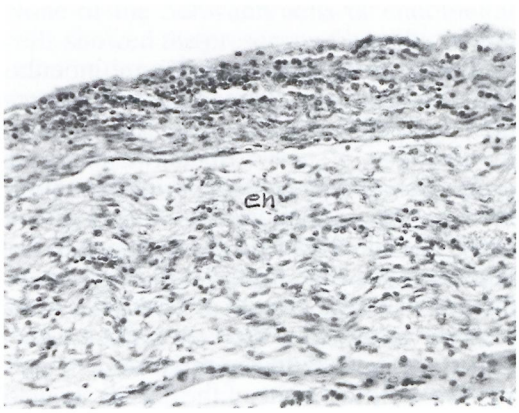

FIG. 2. Sciatic nerve lesion at 3 months in a TR mouse showing scattered lymphocytes as well as macrophages with mild foamy changes in the endoneurium (en). Paraffin-embedded tissue section stained with TRIFF. ×650.

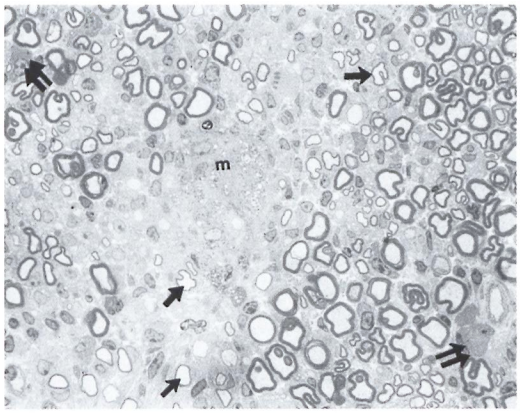

FIG. 3. Sciatic nerve lesion at 3 months in a TR mouse. The 'middle' segment showing mainly macrophages (m) with mild foamy changes and bacterial globi. Note the presence of thinly myelinated fibers (→) and few plasma cells (→→). One micron thick semithin section stained with toluidine blue. ×650.

12th week (Figs. 2 and 3). At the 6th month and 1-year intervals, macrophages with extensive foamy cytoplasmic changes were seen as aggregates in the endoneurium and epineurium (Fig. 4) thus indicating regression. Most of the macrophages were seen with clumps of bacilli all throughout. Unlike this, in non-TR mice, epithelioid-type of macrophages and formation of giant cells were seen at one and two weeks (Fig. 5). Cellular activity showed a peak between the 3rd and 4th week and remained active until 3 months. At the 6th and 12th month, however, there was aggregation of macrophages that showed vacuolated cytoplasm, thus indicating regression similar to that of the TR mice (Fig. 6). The bacilli were seen all throughout only in the macrophages. Lymphocytes were the predominant cell-types seen in both non-TR and TR mice. In non-TR mice, the T cells were mainly seen around blood vessels and the sub perineurial zone. Small groups of epithelioid and occasional giant cells were also seen surrounded by lymphocytes at the 2nd and 3rd week. On the other hand in TR mice the lymphocyte migration was delayed, the peak was seen at 3 months and the distribution was both perivascular, as well as scattered (Fig. 2). At the 6th and 12th month intervals, the lesions in both non-TR and TR mice showed a decrease in the number of lymphocytes. A good number of well differentiated plasma cells were seen in nerve le-

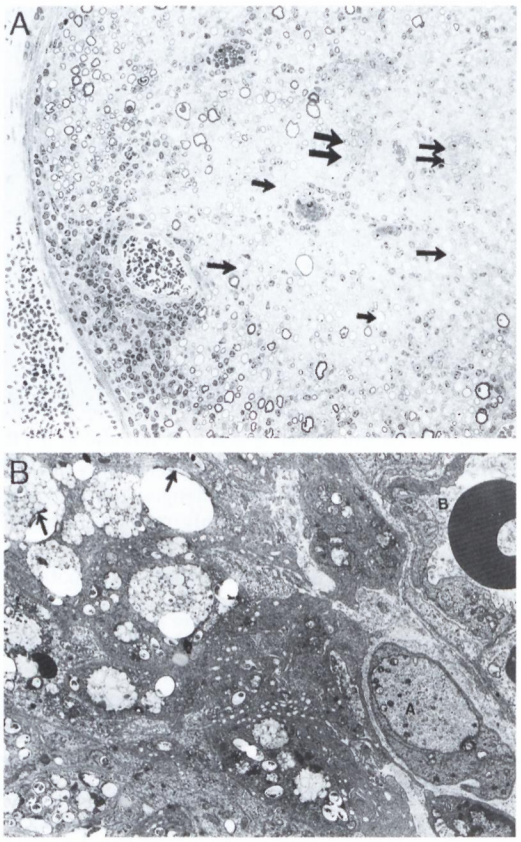

FIG. 4. **A.** Sciatic nerve lesion at one year in a TR mouse. The 'middle' segment showing extensive involvement. Several aggregates of foamy macrophages (→→) are seen along with evenly distributed demyelinated axons (→). Transverse section. ×650. **B.** Above nerve (4a), electron micrograph showing one of the aggregate of foamy macrophages, containing large number of highly granular bacilli (→), suggesting loss of viability. B—a blood vessel, A—a large axon devoid of myelin. ×4500.

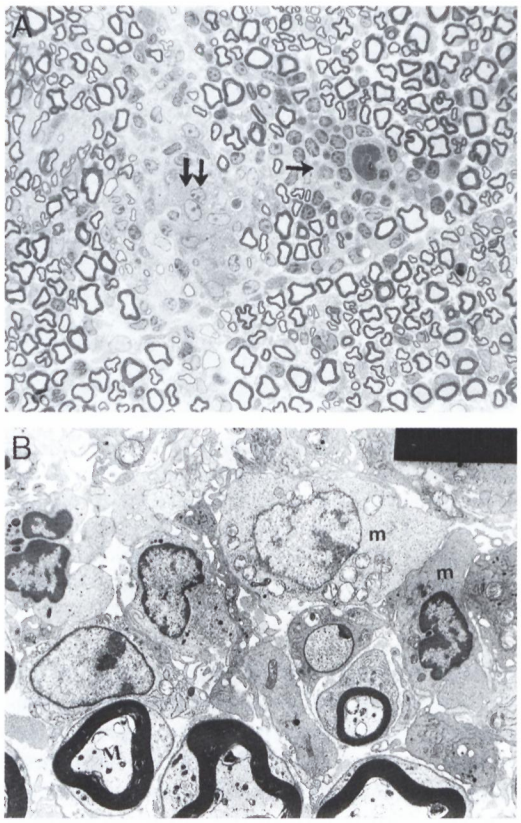

FIG. 5. **A.** Sciatic nerve lesion at two weeks in a non-TR mouse. The 'middle' segment showing perivascular lymphocytic infiltration (→), a multinucleated giant cell (→) and involvement of myelinated fibers around. Transverse semithin section. ×650. **B.** Part of the same nerve (Fig. 5a) showing group of interdigitating well differentiated epithelioid type of macrophages (m). M—myelinated fibers. ×4500.

sions of non-TR mice at thr 3rd month interval onward, whereas in TR mice they were seen at the 3rd and 4th week interval onward.

Nerve damage. The features of nerve damage were best studied using one micron semithin sections stained with toluidine blue and subsequent ultrathin sections of the same.

At 24 hr a mild disturbance and degeneration of a few fibers were seen at the site where the inocula was seeded (Fig. 1). The distal segment of the nerves also showed a mild increase in inter-fiber space suggesting edema and occasional fibers showed degenerative changes. Subsequently, a localized area around the site of *M. leprae* inoculation showed acute demyelinating changes in both non-TR and TR mice. The extent of involvement of nerve fibers was directly proportional to the extent of inflammation seen in the endoneurial area of a given nerve. Predominance of segmental demyelination involving a short segment of the nerve around the site of *M. leprae* inoculation was noted. This was further ascertained, because both the proximal, as well as the distal, segments of the nerves showed consistently a near normal fiber density. The occurrence of remyelination was also seen more or less simultaneously with demyelination. The lesions studied at the later intervals, i.e., the 6th and 12th month,

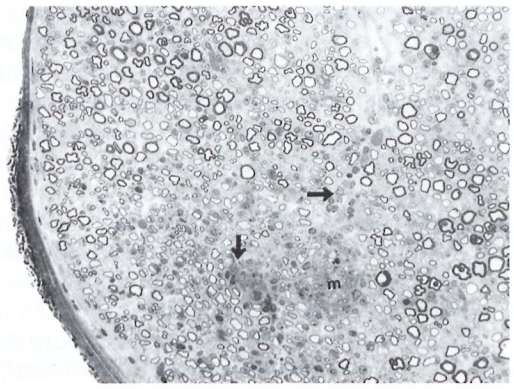

FIG. 6. Sciatic nerve lesions at 6 months in a non-TR mouse, part of the middle segment. Note the presence of aggregates of macrophages (m), few lymphocytes (→) around the blood vessels and uniformly distributed thinly myelinated fibers. One micron thick semithin section. ×310.

showed evenly distributed thinly myelinated fibers and a large number of naked axons suggesting that the remyelination was grossly incomplete until one year in both non-TR and TR mice. Macrophages were seen with myeloid bodies in the cytoplasm. However, active participation of the macrophages in the myelin stripping was not seen in any of the nerves studied.

Study of cell types carrying *M. leprae* using electron microscopy (EM). At 24 hr the majority of bacteria were seen intracellularly. In the nerve lesions of both non-TR and TR mice, the only cell-type that showed the presence of bacilli in the endo- and epineurial area were the macrophages. None of the Schwann cells or endothelial cells showed the presence of bacillus at any point in time. The bacteria persisted within the macrophages but appeared increasingly beaded (degenerate) at the 6th and 12th month intervals.

One other interesting incidental finding was that there were a number of nerves at different time intervals where bacilli and infiltrating cells were seen only in the epineurial region. It was deduced that in these cases there was a failure to transfer the inocula into the endoneurium. Conspicuously, the lesion remained confined to the epineurial region at the 3rd, 6th and 12th month intervals.

Controls. The nerve biopsies obtained from saline injected mice showed the presence of polymorphs and occasional monocytes at the site until 2 to 3 weeks. No other gross abnormalities or demyelination were noted in the nerves studied.

Baseline viability of *M. leprae* used in the intraneural experiments. Both the human-derived *M. leprae* inoculas (obtained from two untreated LL patients' nodule biopsy) on inoculation into the foot pads of normal Swiss White mice gave a maximal yield of $5 \pm 2 \times 10^6$ per foot pad and the take was 100%. The armadillo-derived *M. leprae* used in the remaining two experiments were similarly tested at three intervals, once on arrival (fresh biopsy airlifted) and once each time before the use in the first and the last experiments. On arrival, it gave a maximal yield of $6 \pm 2 \times 10^6$ per foot pad and the take was 100% like that of human derived *M. leprae*. The viability test carried out at the second and third time, i.e., after 2 and 2½ years of storage at –70°C showed a maximal yield of $4 \pm 1 \times 10^5$ at the 12th month interval and the take was 100%, nevertheless suggesting a decline in viability on prolonged storage.

Check for bacterial dissemination. No detectable *M. leprae* counts were obtained in the homogenates of foot pads, ear lobes and liver biopsies that were harvested. The regional lymph nodes showed some counts only during the initial stages (up to 3 weeks) (results not shown). Thus, it was concluded that there was no dissemination of *M. leprae* following intraneural injection in these mice.

DISCUSSION

Mycobacterium leprae is the only known bacteria that invades the peripheral nerve in man. It is also one of the least toxic, obligate intracellular parasites, with a long generation time ([9]). The route by which leprosy bacilli enter the peripheral nerves, their spread, and the unique relationship between *M. leprae* and the Schwann cell remain unclear. For obvious reasons the findings in human are largely dependent on circumstantial evidences. Nevertheless, the bulk of information derived from the studies of cutaneous nerve lesions in early untreated cases of leprosy in particular, show that in man, *M. leprae* primarily find a footage in the Schwann cells of nonmyelinated fibers ([3, 4, 15, 16, 17]). Though it is not known why there is such a selective lodgement, once

enclosed, a vertical spread along the length of the Schwann column would be facilitated due to their overlapping abundance of cell processes that are continuous ([17]). However, supporting evidence to this effect is not forthcoming in two of the extensively studied animal models for leprous neuropathy, mice ([11, 12, 13, 14]) and armadillo ([6, 10]). In the present experimental model freshly harvested *M. leprae* were microinjected into the sciatic nerve in normal and TR mice to bypass the barrier ([20]). Albeit artificial, this allows a direct interaction of *M. leprae* with the Schwann cells *in vivo*. Characteristic epithelioid and macrophage granulomas were seen in non-TR and TR mice, respectively, thus depicting the borderline tuberculoid and lepromatous lesions.

There was no significant difference in the response of polymorphonuclear cells and mast cells, in non-TR vs. TR mice. Migration and peaking of lymphocytes were delayed by nearly three weeks in TR mice, but the density of lymphocytes at the peak interval were comparable in both. The plasma cells appeared early in TR mice. The lesions in non-TR mice showed differentiation of macrophages to epithelioid cells and formation of few giant cells depicting a borderline tuberculoid (BT) leprosy. In TR mice, on the other hand, the macrophages showed foamy cytoplasm and features of borderline lepromatous (BL) leprosy. However, the lesions studied at the 6th and 12th month intervals in both non-TR and TR mice showed definite signs of lowering activity and regression. The presence of *M. leprae* were seen consistently in the macrophages in the lesions of both non-TR and TR mice. Thus, while there was some difference in the kinetics of migration and peaking of cells in non-TR vs. TR mice, subsequently, the lesions regressed slowly and steadily in both non-TR and TR mice. Contrary to our expectation, there was no expansion of the lesion along the length of the nerve, either proximally or distally until one year in both non-TR and TR mice, using either human- or armadillo-derived *M. leprae*. The histopathological evidence of regression noted in this study was in keeping with the loss of viability of *M. leprae* that were exposed to the neural environment *in vivo* ([19]). Collectively, these findings confirm that there was a self restricting containment of infection, as well as the lesion thus produced, in the sciatic nerves of mice. It can be stated that *in vivo*, the neural environment was not conducive for the survival and multiplication of *M. leprae* which is unlike the foot pad in the same host. In the present study, utmost precaution was taken to insure that the *M. leprae* inocula used had the best baseline viability. The possibility of the higher inocular size used ($10–20 \times 10^6$) serving as an immunogen cannot be ruled out in the non-TR strain of mice. However, the same does not hold true for the TR mice. Local temperature could be yet another factor that may be playing some role.

Lack of Schwann cell bacillation. The Schwann cells unlike macrophages lack significant phagocytic activity. The presence of basal lamina could act as a barrier as well as prevent formation of microvilli. Therefore, intraneurally-injected *M. leprae* which were primarily phagocytosed by the invading macrophages in both non-TR and TR mice was not found to be surprising. However, the fact that they remained confined to the macrophages and that at no point in time were *M. leprae* located in the Schwann cells is a most important paradox. This is also unlike the *in vitro* findings where *M. leprae* were readily phagocytosed, but only by the free Schwann cells ([7]).

To this effect, Ridley ([9]) opined that the role of nerves in making possible establishment of leprosy bacilli is due to a delayed recognition of antigen, that allows the bacilli to reach a higher level in the nerve and that it is not due to any special affinity. In the mouse model, *M. leprae* are not seen in the Schwann cells, nevertheless, there is nerve damage. The observations made in the present set of experiments also leads us to a similar conclusions, i.e., that regardless of the route of infection, the peripheral nerve and the Schwann cells, in particular, are not receptive to *M. leprae* infection. This does not find an analogy in human leprosy ([3, 4, 15, 17]). We propose, therefore, that the answer to why peripheral nerves are not a favored site for *M. leprae* infection in animals, may lie in the apparent resistance of the Schwann cells to *M. leprae* infection and, thus, a species-related difference in tissue tropism. Well-documented findings that are in favor of this proposition are: a)

following foot pad inoculation, *M. leprae* colonize and multiply in the striated muscle fibers and not in the nerve or endothelial cells at this site ([21]); and b) in the armadillo, on the other hand, there is a predominance of bacillation in the endothelial cells ([10]). Therefore, we believe that the species-related difference in bacterial colonization influence the subsequent course of infection and its spread in a given species.

Primary segmental demyelination. It was interesting to note that in spite of differences noted in the types of infiltrating cells in non-TR vs. TR mice, the resultant degeneration of the nerves were closely comparable. In both the cases, there were predominance of demyelination. The demyelination was restricted to a short segment of the nerve around the site of *M. leprae* inoculation and ensuing inflammation. In the absence of Schwann cell bacillation and active participation of the macrophages in myelin stripping, it can be deduced that the demyelination was brought about by the inflammatory cytokines ([22]). Notably at the end of one year, there was poor bacterial clearance, as well as remyelination, in the involved segment which was barely two centimeters in length. This was in keeping with the functional deficit noted in the form of hind limb weakness in 35% of the mice. Focal demyelinating lesions associated with cellular infiltrate is one of the characteristic features in human leprous nerves ([5, 17]). Prolonged persistence of bacterial antigens is also a common feature in the human nerve lesions ([18]). One striking difference between the nerve lesions in humans vs. the present experimental model, however, is the lack of Schwann cell and endothelial cell bacillation in the latter. Therefore, this could be one of the key factors governing the containment of infection.

One important incidental finding worth noting was that there were a number of nerves at different time intervals, where the presence of bacteria and inflammatory cells were seen only at the epineurial region. Conspicuously, the lesion remained confined to the epineurial region at the later intervals, i.e., the 3rd and 6th month intervals. This finding indicates that there was no subsequent migration or spill-over of the cells carrying bacteria across the perineurium, or via the blood vessels into the endoneurium, thus this fails to support the earlier proposition, that *M. leprae* find their way to the endoneurium via the perineurium ([1]) in this system.

SUMMARY

Freshly harvested *M. leprae* were microinjected into the sciatic nerves of non-immunosuppressed (non-TR) and immunosuppressed (TR) mice using the technique described by Wisniewski and Bloom. The lesions thus induced, on bypassing the blood-nerve barrier, were biopsied at regular intervals beginning 24 hr and followed up to one year. The fate of *M. leprae* and the ensuing inflammation and nerve damage were studied using light and electron microscopy. The lesions in both non-TR and TR mice at 24 hr showed an influx of polymorphonuclear leukocytes and an increase in mast cells. The influx and peaking of lymphocytes were delayed by two weeks and 6 weeks, respectively, in TR mice, but the density of lymphocytes at the peak intervals was comparable in both. The plasma cells denoting the humoral response were seen in both, but there was a delay of 3 weeks in non-TR mice. The lesions in non-TR mice showed differentiation of macrophages into epithelioid cells and the formation of giant cells depicting borderline tuberculoid leprosy (BT), Whereas in TR mice, the macrophages showed foamy cytoplasmic changes depicting borderline lepromatous leprosy (BL). Other significant observations common to both non-TR and TR mice were: a) The lesions remained highly localized and showed signs of regression at the 6th and the 12th month intervals. b) The characteristic segmental demyelination and some attempt at remyelination were seen at the site. c) The influx of lymphocytes concorded well with demyelination. d) Bacteria were only seen in the macrophages and never in the Schwann cells or endothelial cells. e) Bacteria persisted in the macrophages, but appeared progressively degenerate at the 6th and 12th post-inoculation months, suggesting loss of viability. The study shows that there was a very effective containment of the infection and that the Schwann cells were resistant to *M. leprae* infection in the neural milieu. Nerve damage and Schwann cell bacillation do not go hand-in-hand.

RESUMEN

Utilizando la técnica de Wisniewski y Bloom, se inyectaron suspensiones recién preparadas de *M. leprae* en los nervios ciáticos tanto de ratones inmunosuprimidos (TR) como de ratones no inmunosuprimidos (no-TR). Las lesiones inducidas de esta manera, sobrepasando la barrera sangre-nervio, fueron estudiadas a intervalos regulares que comenzaron a las 24 horas y se continuaron durante un año. El destino de *M. leprae* y la inflamación y daño a nervios resultantes, fueron estudiados utilizando la microscopía de luz y la microscopía electrónica. A las 24 horas, las lesiones tanto en los ratones TR como en los no-TR mostraron un influjo de leucocitos plimorfonucleares y un aumento en el número de células cebadas. Sin embargo, comparando con los ratones no-TR, el influjo y el pico de linfocitos aparecieron retardados por dos y seis semanas, respectivamente, en los ratones TR, aunque la densidad de linfocitos en los intervalos pico fueron comparables en ambos grupos. Las células plasmáticas, reflejo de la respuesta humoral, fueron aparentes en ambos grupos pero se observó un retardo de 3 semanas en los ratones no-TR. En las lesiones de los ratones no-TR se observó la diferenciación de los macrófagos en células epitelioides y la formación de células gigantes características de la lepra tuberculoide subpolar (BT) mientras que en los ratones TR, los macrófagos mostraron cambios citoplásmicos espumosos característicos de la lepra lepromatosa subpolar (BL). Otras observaciones significativas, comunes tanto en los ratones TR como en los no-TR, fueron: a) las lesiones permanecieron altamente localizadas y mostraron signos de regresión a los intervalos del 6° y 12° meses, b) en el sitio de inoculación se observaron la característica desmielinización segmental y un intento de remielinización, c) el influjo de linfocitos correlacionó bien con la desmielinización, d) las bacterias sólo fueron vistas en los macrófagos y nunca en las células de Schwann o en las células endoteliales, e) las bacterias persistieron en los macrófagos pero aparecieron progresivamente degeneradas a los 6° y 12° meses post-inoculación, sugiriendo la pérdida de viabilidad. El estudio muestra que hubo un eficiente confinamiento de la infección y que las células de Schwann fueron resistentes a la infección por *M. leprae* en el microambiente neural. El daño a nervios y la presencia de bacilos en las células de Schwann son dos fenómenos no correlacionados.

RÉSUMÉ

Des *Mycobacterium leprae* fraîchement récoltées furent microinjectées dans les nerfs sciatiques de souris non immunodéprimées (non-TR) et immunodéprimées (TR), en utilisant la technique décrite par Wisniewski et Bloom. Les lésions ainsi induites, qui court-circuitent la barrière hémato-nerveuse, furent biopsiées à intervalles réguliers commençant à 24 heures et allant jusqu'à un an. L'évolution de la charge bacillaire, de l'inflammation et de l'atteinte nerveuse fut étudiée par microscopie optique et électronique. Les lésions des souris tant TR que non-TR à 24 heures étaient caractérisées par un recrutement de leucocytes polynucléaires et une augmentation des mastocytes. Le recrutement de lymphocytes et le pic de celui-ci furent retardés de deux semaines et 6 semaines, respectivement, chez les souris TR mais la densité lymphocytaire pendant ce pic de recrutement fut comparable entre les deux groupes de souris. Les plasmocytes signant la réponse humorale étaient présents dans les deux groupes de souris, mais il y eut un délai de 3 semaines chez les souris non-TR. Les lésions des souris non-TR étaient caractérisées par des macrophages montrant une morphologie épithélioïde et la présence de cellules géantes multinucléces signant une lèpre tuberculoïde borderline (BT), tandis que celles des souris TR montraient des macrophages au cytoplasme spumeux signant une lèpre lépromateuse borderline (BL). Les autres observations importantes présentes à la fois chez les souris TR et non-TR furent les suivantes : a) Les lésions restèrent hautement localisées et montrèrent des signes de régression au 6ème et au 12ème mois. b) Une démyélinisation segmentaire caractéristique et des tentatives de remyélinisation furent observées au site d'inoculation. c) Le recrutement lymphocytaire était en bon accord avec la démyélinisation. d) Les bactéries ne furent observées que dans les macrophages et non dans les cellules de Schwann ou les cellules endothéliales. e) Les bactéries persistèrent dans les macrophages, mais présentèrent un aspect dégénéré au 6ème et 12ème mois suivant l'inoculation, suggérant une perte de viabilité. L'étude montre que, dans ces conditions expérimentales, l'infection est restée localisée de façon très efficace et que les cellules de Schwann de souris restèrent réfractaires à l'infection par *Mycobacterium leprae* dans le milieu neural. L'atteinte nerveuse et l'infection des cellules de Schwann sont des événements indépendants chez la souris.

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