Organized Nerve Culture. II. DNA Synthesis in Schwann Cells in the Presence of *M. leprae*[1]

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In the companion article ([6]), we reported that once *M. leprae* invade Schwann cells, they are rendered incapable of effectively associating with nerve fibers and hence synthesizing myelin in organized nerve culture. In the present study, we suggest that this interference in the effective Schwann cell-axon interaction as a result of *M. leprae* infection may perhaps be due to inhibition of Schwann cell proliferation. This is indicated by the lack of DNA synthesis in those cells which contain *M. leprae*. Like axon-association, it has been shown that mitosis is also one of the important events that takes place before Schwann cells begin to secrete myelin.

MATERIALS AND METHODS

Schwann cells were cultured *in vitro* as a component of organized nerve culture or as relatively pure cells in isolation for periods of 2 to 4 weeks by the methods described ([6]). Two week old cultures were exposed to 5–8 million *Mycobacterium leprae* for 96 hr after which the cultures were washed with Eagle's Minimal Essential Medium and then pulsed with ^{3}H-thymidine (2 μCi/ml, Specific Activity 45 Ci/mM, Isotope Division, BARC, Bombay) for 24 hr. The cultures were then washed with Hanks' Balanced Salt Solution (BSS), fixed in formal-saline for 24 hr, and the coverslips were then processed for liquid scintillation counting. The total amount of radioactivity incorporated was determined in a liquid scintillation counter (Electronic Corporation of India). Xylene-based scintillation fluid was used. After counting, the coverslips were rinsed several times in fresh xylene and processed for autoradiography. Ilford K5 emulsion diluted 1:1 in distilled water was used for coating the coverslips in total darkness. The emulsion was air dried, and the coverslips were then stored in light proof boxes at 4°C. They were developed after 10 or 12 days and fixed and stained with Ziehl-Neelsen for acid-fast bacilli and with methylene blue for visualizing cellular morphology. Thus each coverslip preparation yielded data on the presence of acid-fast bacilli, radioactivity, and cell morphology.

The liquid scintillation counting data correlated well with the findings on autoradiography in the sense that the total radiography present in the culture was proportional to the total number of cells labelled. The autoradiography data were considered to be more relevant, however, and are used for drawing conclusions.

Background silver grains in the autoradiographs were minimal in comparison to the highly labelled nuclei (The Figure). For this reason the silver grains per nucleus were not counted. The labelling index was determined by microscopically counting 200–300 Schwann cell nuclei using a ×100 oil immersion objective.

RESULTS

Schwann cells were identified in these cultures by the criteria already described ([6]). Schwann cells migrate out of the explant, and as they move to the periphery, they associate with nerve fibers and undergo mitosis as indicated by their nuclear uptake of ^{3}H-thymidine (The Figure) ([6]). Mitosis in these cells peaks in the second week of culture and declines sharply thereafter. Two week old cultures were inoculated with *M. leprae* and then observed for thymidine incorporation. The labelling index of these cells dropped significantly, suggesting that fewer of the Schwann cells were undergoing division. On scanning these cultures under the light microscope,

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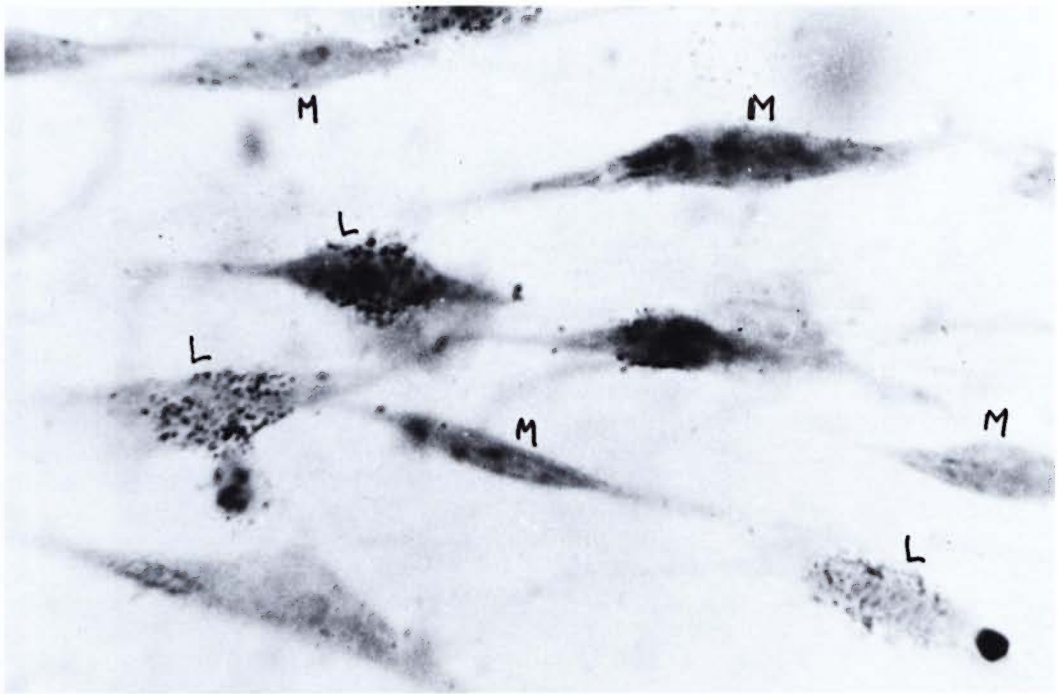

THE FIGURE. Schwann cells showing labelling with ^{3}H-thymidine and *M. leprae*. Cells containing *M. leprae* (M) do not show radioactive labelling whereas others (L) show heavy labelling.

it was observed that the ^{3}H-thymidine label was present in cells that had not engulfed *M. leprae* while most of the cells which contained *M. leprae* did not contain any label. The Figure illustrates such observations, which were consistently made in 18 cultures infected with *M. leprae*. Tables 1 and 2 present data tabulated from these cultures. It is to be noted that only 2.34% of the Schwann cells containing bacilli incorporated the label while the label was taken up readily by neighboring cells that had not phagocytosed the bacilli. This suggests that once *M. leprae* enter the Schwann cell, the bacilli somehow inhibit their DNA synthesis as exhibited by the lack of incorporation of ^{3}H-thymidine. In contrast, a large number of other cells in the culture, which had engulfed *M. leprae* but which were not Schwann cells, incorporated ^{3}H-thymidine without any discrimination.

Three Schwann cell cultures were inoculated with heat killed *M. leprae*, ICRC-C44, and cultivable acid-fast strains obtained from Professor L. Kato (Laval, Canada). These cultures were maintained in the same manner as those exposed to viable *M. leprae* and then labelled with ^{3}H-thymidine. These bacilli did not induce the effect seen with fresh *M. leprae*. The label was uniformly present both in the cells with or without bacilli, suggesting therefore that this phenomenon of DNA synthesis inhibition is unique to *M. leprae* freshly obtained from human biopsies.

Six other cultures were labelled with ^{3}H-leucine (2 μCi/ml, Isotope Division, BARC, Bombay) and processed autoradiographically to look at their protein synthesis. In these cultures, uptake of ^{3}H-leucine by Schwann cells containing *M. leprae* was not noticeably different from that by cells without the bacilli, i.e., all were uniformly labelled.

DISCUSSION

In vitro, Schwann cells migrate along with nerve fibers, align on the longitudinal axis, and undergo mitosis, as indicated by their uptake of ^{3}H-thymidine autoradiographically, perhaps due to the mitogenic effect of the associated nerve ([8]). These cells then interact, associate with axons, and secrete myelin, which becomes evident

TABLE 1. *Percentage of cells containing bacilli which incorporated ^{3}H-thymidine.*

Experiment number[a]	Bipolar cells (Schwann cells)			Flat cells (Neurofibroblasts)		
	No. labelled with ^{3}H-thymidine	Total no. counted	% labelled	No. labelled with ^{3}H-thymidine	Total no. counted	% labelled
1	8	76	10.5	6	8	75.0
2	0	88	0.0	8	8	100.0
3	2	201	1.0	4	21	19.0
4	2	155	1.3	10	71	12.7
5	2	58	3.4	14	34	41.2
6	3	147	2.0	2	3	66.7
Total	17	725	2.3	44	145	42.2

[a] Each experiment represents data from 3 cultures. Cells were counted from several fields of each culture under ×100 oil immersion objective.

after 3–4 weeks. Similar events have been reported in the process of myelination of nerve fibers *in vivo* ([2,5,7]). Schwann cells undergo mitosis prior to their intimate association with nerve fibers to obtain a 1:1 relation in large fibers and then synthesize myelin. These observations both *in vivo* and *in vitro* suggest that the migrated Schwann cell has to undergo mitosis for its progeny to acquire the myelin synthesizing property. The same events take place following injury in a regenerating nerve, i.e., Schwann cells are first stimulated to divide ([1,3,4]). In our study, we have as a rule observed proliferation of Schwann cells. This event is inhibited in the presence of *M. leprae*. The long term effects of such inhibition could be poor or ineffective Schwann cell association with the axons and abnormal or no secretion of myelin. In the companion paper ([6]) we reported that the majority of Schwann cells containing *M. leprae* fail to effectively associate with nerve fibers. This is perhaps because they are rendered incapable of undergoing further mitosis and thus are unable to undergo differentiation to acquire the ability to synthesize myelin.

Inhibition of DNA synthesis of Schwann cells was not produced by heat killed *M. leprae*, ICRC-C44, or other cultivable human derived acid-fast bacilli. This suggests that this phenomenon is unique to viable, freshly obtained (human) *M. leprae*. It should be noted that the viability of the *M. leprae* inside the *in vitro* Schwann cells can only be assumed since it was not directly proved. In spite of the large number of bacilli added to the culture, only a few bacilli are found inside the Schwann cells. This probably is due to the low Morphologic Index (MI) of the *M. leprae* added to the culture. With cultivable bacilli such as ICRC-C44, the viability (MI) is very high, and the number of bacilli taken up by the Schwann cells is much higher. Thus we assume that the bacilli that are inside the Schwann cells are viable and that viability is probably an essential feature necessary for the molecular interactions occurring in our experiments. The presence of *M. leprae* did not alter protein synthesis by Schwann cells to the extent that it could be detected autoradiographically. It may be that such an ef-

TABLE 2. *Relationship of ^{3}H-thymidine incorporation to presence of bacilli.*

Experiment number[a]	Bipolar cells (Schwann cells)			
	^{3}H-thymidine labelled cells		Unlabelled cells	
	Without bacilli	With bacilli	Without bacilli	With bacilli
1	74	8	110	68
2	114	0	165	88
3	60	2	112	199
4	68	2	157	153
5	91	2	135	56
6	66	3	177	144
Total	473	17	856	708

[a] Each experiment represents data from 3 cultures. Cells were counted from several fields of each culture under ×100 oil immersion objective.

fect would require a long term exposure of the cells to the bacilli.

Only the Schwann cells are affected by *M. leprae*. DNA synthesis by the other types of cells in the culture was not affected by *M. leprae*. This alteration in the functional status of Schwann cells would have an important implication as a reason for poor myelination or non-myelination in the leprosy infection.

SUMMARY

Schwann cells that contain *M. leprae* fail to incorporate DNA precursor, indicating blockage of DNA synthesis. Such a block could lead to no proliferation of Schwann cells, an essential requirement for successful association with axons and consequent myelination.

RESÚMEN

Las células de Schwann que contienen *M. leprae* son incapaces de incorporar precursores del DNA. Esto indica un bloqueo en la síntesis del DNA que podría impedir la proliferación de las células de Schwann. La proliferación celular es un requisito esencial para la asociación de estas células con axones y para su consecuente mielinización.

RÉSUMÉ

Des cellules de Schwann contenant *M. leprae* ne parviennent pas à incorporer de précurseurs du DNA, ce qui indique un bloquage de la synthèse de DNA. Un tel bloc pourrait mener à l'absence de prolifération des cellules de Schwann, cette prolifération constituant une condition essentielle pour que soit réussie l'association avec les axones et la myélinisation qui en résulte.

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