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Letters to the Editor

SUPPRESSION OF LYMPH NODE LYMPHOPROLIFERATION TO VIABLE *MYCOBACTERIUM LEPRAE* BY PERIPHERAL BLOOD-DERIVED MONOCYTES

Sir,

The presence of *Micobacterium leprae* reactive lymphocytes within the lymph node (LN) of lepromatous patients was convincingly demonstrated in our earlier study[1] without the use of any exogenous addition of lymphokines[2,3] or resorting to any *in vitro* manipulations, such as the depletion of suppressor cell populations[4] and/or using modified antigenic preparations of *Micobacterium leprae*.[5,6] It appears, therefore, that although antigen-specific cells are generated in the lymph node the inability to detect them in circulation[7] may either be due to a selective sequestration within the lymph node or due to differential handling of *M. leprae* within the two tissue compartments, namely peripheral blood (PB) and the lymph node.

The experiments designed to probe this aspect are comprised of the following: (a) depletion of monocytes by carbonyl iron (cFe) from blood and LN mononuclear cells (MNC); (b) determining the ability of LN lymphocytes to be suppressed by lepromatous PB-derived macrophage lysate; and (c) lymphoproliferation to viable *M. leprae* in a co-culture of PB monocytes with autologous LN lymphocytes.

The present communication demonstrates through co-culture experiments the exclusive suppressive function of PB monocytes as compared to lymph node (LN) adherent cells when presenting viable *M. leprae* to lymphocytes.

Twenty millilitres of heparinized PB and one inguinal LN (removed under local anaesthesia) were collected from each patient. Five lepromatous patients were included in the study. The LN of two of these patients were bacteriologicially positive (3+ and 5+) which the remaining 3 were negative.

The inguinal LN was divided into two, one part was fixed for histopathology while the other piece was dissected free of fat and gently rubbed on a fine wire mesh to obtain a single-cell suspension. The cells thus obtained were used in a lymphoproliferation assay.

Depletion of monoctyes by cFe (5 mg/ml) treatment was achieved by adding sterilized cFe to the mononuclear cell population and incubating the cells at 37°C for 1 hr with intermittent agitation. After incubation, they were again layered on lymphoprep. The cells which had ingested cFe settled to the bottom and were discarded, while those at the interphase were collected and used in the lymphoproliferation assay. Co-culture of PB-derived monocytes with autologous lymph node lymphocytes was undertaken as follows:

1×10^4 monocytes enriched on a Nycodenz–Monocyte gradient (Nyegaard & Co., Norway) were added per well of a flat-bottomed 96-well microtitre plate. Viable *M. leprae* (3×10^6 organisms/ml) or PPD (4 μg/ml) was added to the monocyte cultures. After 24 hr the cultures were washed and the nonadherent lymphocyte population was added at a concentration of 1×10^6 cells/100 μl. Cultures were maintained in 5% CO_2 atmosphere are 37°C for 5 days and assayed for lymphocyte proliferation.

Table 1. Effect of carbonyl iron (cFe) treatment on *M. leprae*/PPD stimulation of LN mononuclear cells from lepromatous patients

	CPM ± SE		LN	
Stimulation of cells	P2(BI-ve)	P3 (BI 5+)	P4 (BI-ve)	P5 (BI-ve)
Control	1331 ± 292	1565 ± 135	1522 ± 240	1305 ± 95
a. *M. leprae*	2784 ± 658	2968 ± 1258	3778 ± 141	3624 ± 282
b. PPD	44230 ± 3270	9773 ± 3935	31616 ± 975	—
After cFe treatment				
Control	1258 ± 180	1179 ± 85	969 ± 249	803 ± 57
c. *M. leprae*	2956 ± 136	4258 ± 411	3148 ± 430	2071 ± 144
d. PPD	3992 ± 456	4213 ± 555	8730 ± 65	—
a : c	N.S.	$P < \cdot 05$	N.S.	N.S.
b : d	$P < \cdot 01$	$P < \cdot 05$	$P < \cdot 02$	—

Lymphoproliferative response to M. leprae *in lepromatous leprosy patients*: An absence of a response to *M. leprae* was noted in the mononuclear cells derived from PB of lepromatous patients confirming earlier reports.[7] In contrast, mononuclear cells from LNs or these patients showed a significant response to viable *M. leprae* as reported earlier.[1] The stimulation index ranged from 2 to 4. No difference was observed in the extent of *M. leprae* reactivity in bacteriologically negative (BI-ve) patients (P_2, P_4, P_5) as compared to the bacteriologically positive (BI + ve) lepromatous cases (P_1, P_3).

Effect of cFe treatment on M. leprae/*PPD induced stimulation of LN mononuclear cells* (Table 1): To compare the role of macrophages versus other APC's in the LN, mononuclear cultures from LN were depleted of phagocytic cells by treatment with cFe and subsequently stimulated with *M. leprae*/PPD.

On cFe treatment the response to PPD was significantly reduced. However the response to *M. leprae* remained unaffected in 3 of the 4 patients studied.

Effect of L + -lysate on M. leprae *induced stimulation of lepromatous LN mononuclear cells* (Table 2): Earlier studies by Salgame *et al.*[9] had reported the presence of suppressor factors

Table 2. Effect of L + -lysate on *M. leprae* stimulation of LN mononuclear cells from lepromatous patient

	Mean cpm ± SE		
Stimulation of cells	P3 (BI 5+)	P4	P5
a. Control	1565 ± 135	1522 ± 240	1305 ± 94
b. *M. leprae*	2968 ± 1258	3778 ± 141	3642 ± 282
c. *M. leprae* + L + -lysate	1100 ± 157	2256 ± 683	1185 ± 377
P value b : c	N.S.	$P < 0{\cdot}05$	$P < 0{\cdot}001$
% suppression	63	41	68
After cFe treatment			
d. Control	1179 ± 85	969 ± 249	803 ± 57
e. *M. leprae*	4258 ± 411	3148 ± 430	2071 ± 144
f. *M. leprae* + L + -lysate	N.D.	637 ± 38	807 ± 74
p = value e : f		$P < 0{\cdot}05$	$P < 0{\cdot}001$
% suppresion		80	62

Table 3. Co-culture in lepromatous patients of peripheral blood-raleted monocytes (PB-Mo) with autologous LN lymphocytes in the presence of *M. leprae* / PPD.

	cpm + SE											
	PB-lymphocytes + PB-Mo			LN Lymphocytes + LN-Mo			LN lymphocytes			Ln lymphocytes + PB-Mo		
Patients	C	+ ML	PPD	C	+ ML	PPD	C	+ ML	PPD	C	+ ML	PPD
P1 (BI 3+)	1203 ±174	1419 ±216	N.D.	1182 ±282	4140 ±166	N.D.	1422 ±81	489 ±113	N.D.	849 ±179	800 ±85	N.D.
P2 (BI-ve)	1835 ±223	1459 ±202	16300 ±1300	1331 ±292	2784 ±658	44230 ±3270	626 ±125	1478 ±68	1646 ±228	1430 ±170	1612 ±171	4880 ±839
P4 (BI-ve)	1699 ±184	2182 ±512	89878 ±10466	1522 ±240	3778 ±141	31616 ±975	969 ±249	3148 ±438	8703 ±65	780 ±40	644 ±60	2474 ±164

(L + -lysate) from macrophages of lepromatous patients which significantly inhibited lymphoproliferative responses to *M. leprae*. To determine whether LN cells were sensitive to modulation by the suppressor factor, 200 μl L + -lysate (protein concentration: 200 μg/ml) was added to LN mononuclear cells stimulated with *M. leprae*. Substantial suppression of lymphoproliferation was observed to the antigenic stimulus. This indicates that the LN cell population is sensitive to suppression mediated by PB-derived macrophages.

Co-culture of (PB-Mo) with autologous LN lymphocytes in the presence of M. leprae/*PPD in lepromatous patients* (Table 3): Though substantial lymphoproliferation was seen when the total LN mononuclear cells were used, in co-culture with PB monocytes an absence of lymphoproliferative response was noted with viable *M. leprae* in spite of antigen reactive T cells being present within the lymph node. In contrast, significant lymphoproliferation was observed with PPD using a similar protocol. This suggests that PB monocytes but not LN macrophages are suppressive in the overt presence of viable *M. leprae* though they may be capable of normal functions with related antigens such as PPD.

Substantial early evidence of the suppressive function of PB macrophages with respect to viable *M. leprae* has been documented.[8,9] What is not known is whether tissue macrophages as reflected in this study by LN adherent cells also share similar suppressive function. When the entire cell population from the LN was used in the lymphoproliferative assay in this study, one would expect the *M. leprae*-laden macrophages to suppress the proliferative response. Despite that, we have observed significant stimulation with exogenously added viable *M. leprae*. In addition, even after depletion of the LN macrophages by cFe treatment the response to viable *M. leprae* was unaffected. Though this may imply non-participation of LN-derived adherent cells in the LN lymphoproliferative response, it also demonstrates that unlike the PB macrophages, they do not exert a suppressive function in the presence of viable *M. leprae*. The fact that the PB-derived macrophage lysate was suppressive indicates the sensitivity of the lymphoproliferation induced by cells of the B cell and dendritic lineage to suppression by peripheral blood-derived monocytes.

The central concept of immunosuppression in lepromatous leprosy therefore appears to involve the PB monocyte; since the tissue macrophages do not appear to be suppressive in the overt presence of viable *M. leprae*. Any attempt to restore immune function must take this fact into cognizance.

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