Apoptosis in Leprosy Patients

TO THE EDITOR:

The protection-associated host response to *Mycobacterium leprae* infection is strongly dependent upon subsequent cellular immunity ([1]). Apoptosis appears as a physiological mechanism that leads to cell elimination without inducing inflammation or damage to contiguous cells ([3, 5]). In this study, we have tested the hypothesis that among the various factors potentially responsible for the lymphocyte alterations in leprosy, apoptosis could be implicated. Along this line, we evaluated the impact of leprosy infection on the level of spontaneous apoptosis and the type of cells ($CD4^+$, $CD8^+$, $CD19^+$) that were likely concerned.

After informed consent, 19 newly detected leprosy patients (11 males, 8 females) were included in the study before receiving any treatment. Nine of them had multibacillary (MB) leprosy and 10 had paucibacillary (PB) leprosy. Seven (5 males, 2 females) Senegalese healthy adult donors were enrolled as controls.

Peripheral blood mononuclear cells were isolated from heparinized whole blood by Histopaque®-1077 density gradient (Sigma Diagnosis, St. Louis, Missouri, U.S.A.) and cultured at 10^6 cells/ml in 24-well plates under unstimulated (medium alone) conditions. The plates were thereafter incubated for 2 days (determined after a preliminary kinetic study) at 37°C in a water-saturated atmosphere containing 5% CO_2. Quantification of apoptosis was performed by flow cytometry as already described ([8]) by staining the lymphocytes with 7 amino-actinomycinD (7AAD; Sigma) which discriminates live from early apoptotic cells. $CD4^+$, $CD8^+$ and $CD19^+$ cells were identified using monoclonal antibodies (Becton Dickinson Immunocytometry Systems, San Jose, California, U.S.A.). The nonparametric Kruskal-Wallis test was used to compare the data between the different groups; a p value of <0.05 was considered as significant.

The proportions of apoptotic lymphocytes were compared in leprosy patients and controls. A highly significant increase ($p = 0.01$) in the level of spontaneous apoptosis in leprosy patients was found as compared to controls, suggesting a notable impact of the *M. leprae* infection. Hence, apoptosis seems to be an active phenomenon in leprosy as previously found for several other intracellular infections ([7, 10]), including malaria ([9]). However, the percentage of 7AAD-positive cells was not significantly different between the two groups of PB and MB patients. Such observation has to be confirmed with a greater number of patients.

The relative distribution of the lymphocyte subset within apoptotic cells was studied (ratio of the percentage of the apoptotic subpopulation studied on the percentage of total apoptotic cells of the culture). We found that the distribution of $CD4^+$, $CD8^+$ and $CD19^+$ cells within apoptotic cells did not differ between patients and controls, suggesting that the infection simply induced an exaggeration of a naturally occurring mechanism.

Another type of analysis was performed in calculating the ratio of the percentage of the apoptotic subpopulation on the percentage of the population concerned. This allowed us to determine the number of apoptotic cells within each subset separately.

THE TABLE. *Percentage of apoptotic cells within each lymphocyte subset.*

	CD4 % ± S.D.	CD8 % ± S.D.	CD19 % ± S.D.
Controls	5.9 ± 1.9	6 ± 2.2	4.8 ± 3.7
MB	9.4 ± 4.4	12.4 ± 7.5	13.5 ± 13
PB	9.8 ± 4	14.6 ± 7	13 ± 10.3

This analysis showed that CD8+ and CD19+ cells presented a higher proportion ($p < 0.004$) of apoptotic cells than did the $CD4^+$ cells in leprosy patients in comparison with controls (The Table), suggesting a possible preferential lymphocyte subset target in *M. leprae*-induced apoptosis.

Taken together, our results show that apoptosis is an existing mechanism of cell destruction in leprosy. It might represent a strategy of the immune system to eliminate infected cells. However, implications of several different factors in the fine mechanism inducing lymphocyte apoptosis, such as lymphocyte activation, cytokines, effect of free oxygen radicals or the effects of superantigens already described for other intracellular infections ([2, 4, 6]), have to be studied to further delineate the possible role of apoptosis in the physiopathology of leprosy.

—Mbayame Ndiaye Niang, Pharm.D.
Aissatou Toure Balde, Pharm.D.
Ronald Perraut, Pharm.D.

Laboratoire d'Immunologie
Institut Pasteur de Dakar
36 Avenue Pasteur
BP 220
Dakar, Senegal

—Ibrahima Mane, M.D.
Jean Louis Cartel, M.D.

Institut de Leprologie
Appliquee de Dakar
Dakar, Senegal

Reprint requests to Dr. Niang at the above address or FAX 221-39-92-10.

Acknowledgment. The authors are very grateful to Ababacar Diouf and Babacar Diouf for their expert technical contributions and to all the workers of the Institut de Leprologie Appliquée de Dakar.

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