

## Cell Subset Analysis of Cutaneous Infiltrate in Atypical Mycobacteria Ulcerations

### TO THE EDITOR:

There is a lot of immunological information on the different types of cells in the skin lesions of leprosy (1, 4, 6, 7), but nothing is known about the cell characteristics of cutaneous ulcerations due to atypical mycobacteria. In this study, skin specimens, taken during surgical excision of the lesions, were studied in three patients (two females and one male aged 58, 9, and 62 years, respectively, with no other sign of disease) from French Guiana, in collaboration with an experienced Department of Dermatology. Following clinical diagnosis, a Ziehl-Neelsen stain showed the presence of some acid-fast bacilli intra- and extracellularly but,

as frequently happens, the laboratory cultures could not confirm the species involved. In our experience in French Guiana, the few identifications made by the laboratory have been *Mycobacterium chelonae*, *M. fortuitum* and, less frequently, *M. ulcerans*.

Histologic analysis showed a chronic inflammatory reaction in the dermis, with epithelioid and giant cells (of the Langhans' type) and areas of coagulative necrosis. The dermal infiltrates, with some acid-fast bacilli, presented histologic features similar to tuberculoid leprosy. The absence of morphologic changes in the small nerves and of bacilli in this location were the main his-

THE TABLE. *Skin granulomas in cutaneous ulcers due to atypical mycobacteria (sample of three biopsies).*

Monoclonal antibody <sup>a</sup>	Cluster of differentiation (cell type)	Results <sup>b</sup>		
IOT 11	CD2 (T cells)	109.4	72.9	65.1
IOT 4	CD4 (T-cell subset)	31.2	23.4	78.1
IOT 8	CD8 (T-cell subset)	19.5	36.4	52.1
IOT 14	CD25 (IL-2 receptor)	0.0	4.1	25.5
IOT 2a	— (HLA-DR)	7.8	11.2	18.2
IOB 1	CD37 (B cells)	3.9	0.0	1.3
IOM 2	CD14 (Monocytes, granulocytes)	12.2	22.6	26.8
Leu-11b	CD16 (NK <sup>c</sup> cells, granulocytes)	10.4	9.6	20.8
Leu-19	— (NK cells)	11.7	10.4	15.9

<sup>a</sup> Leu monoclonal antibodies from Becton-Dickinson, Mountain View, California, U.S.A.; all others from Immunotech, Marseilles, France.

<sup>b</sup> Results are expressed, for each patient, as the mean number of positive cells per square millimeter of granuloma.

<sup>c</sup> NK = natural killer cells.

tologic disparities compared with a leprosy granuloma.

Skin biopsies placed in OCT medium (Tissue Tek; Miles Laboratories, Naperville, Illinois, U.S.A.) were frozen in liquid nitrogen to be used for immunoperoxidase staining with the avidin-biotin method derived from the original description (2). A commercial kit (ABC; Vector Labs, Inc., Burlingame, California, U.S.A.) was used with different monoclonal antibodies (The Table) and diaminobenzidine (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) treatment followed by a counterstain with hematoxylin.

The total number of positively stained cells was counted on five microscopic fields with a standard area. To normalize the results, data are expressed in the number of positive cells per square millimeter of granuloma.

As expected by analogy with what is known in other granulomas caused by mycobacteria (1, 4, 6, 7), T cells predominated in the lesion. A significant number of monocytes/macrophages was also noted. B cells were rare or absent. One granuloma displayed a large percentage of activated T cells, as identified by the expression of the interleukin-2 (IL-2) receptor. T-cell subset analysis revealed some heterogeneity among the patients: For two of them, CD4 T cells predominated, with a CD4/CD8 ratio around 1:5, as seen in granulomas from tuberculoid leprosy patients; whereas CD8 T cells were more frequent in the last patient (CD4/CD8 ratio = 0:6) as observed in lepromatous leprosy lesions (5, 7). With T cells and macrophages making up the bulk of the cellular infiltrate, we were surprised to observe some staining with two natural killer (NK) markers, the second one (Leu-19) more specific than the first (3). These results must be confirmed on a larger sample and with appropriate technical precautions. New monoclonal antibodies, particularly those which distinguish subpopulations of CD4+ and CD8+ T lymphocytes (8), and double colorations might be useful to analyze more precisely the cellular immune reaction occurring in the lesions due to atypical mycobacteria.

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## REFERENCES

1. BACH, M.-A., CHATENOU, L., WALLACH, D., PHAN, F. T. D. and COTTENOT, F. Studies on T cell subsets and functions in leprosy. *Clin. Exp. Immunol.* **44** (1981) 491–500.
2. HSU, S. M., RAINE, L. and FANGER, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29** (1981) 577–580.
3. LANIER, L. L., LE, A. M., CIVIN, C. I., LOKEN, M. R. and PHILLIPS, J. H. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* **136** (1986) 4480–4486.
4. MODLIN, R. L., GEBHARD, J. F., TAYLOR, C. R. and REA, T. H. *In situ* characterization of T lymphocyte subsets in the reactional states of leprosy. *Clin. Exp. Immunol.* **53** (1983) 17–24.
5. MODLIN, R. L., MELANCON-KAPLAN, J., YOUNG, S. M., PIRMIZ, C., KINO, H., CONVIT, J., REA, T. H. and BLOOM, B. R. Learning from lesions: patterns of tissue inflammation in leprosy. *Proc. Natl. Acad. Sci. U.S.A.* **85** (1988) 1213–1217.
6. VAN VOORHIS, W. C., KAPLAN, G., SARNO, E. N., HORWITZ, M. A., STEINMAN, R. M., LEVIS, W. R., NOGUEIRA, N., HAIR, L. S., GATASS, C. R., ARRICK, B. A. and COHN, Z. A. The cutaneous infiltrates of leprosy. *N. Engl. J. Med.* **307** (1982) 1593–1598.
7. WALLACH, D., FLAGEUL, B., BACH, M.-A. and COTTENOT, F. The cellular content of dermal leprosy granulomas: an immuno-histological approach. *Int. J. Lepr.* **52** (1984) 318–326.