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LETTER TO THE EDITOR

COMMERCIALLY AVAILABLE ANTI-S-100 PROTEIN SERUM STAINS M. LEPRAE IN LEPROSY TISSUES BY IMMUNOHISTOCHEMICAL PROCEDURES.

Demonstration of bacilli and consequently of their antigenic products is easily feasible in multibacillary (LL, BL, BB) forms of leprosy. However, the presence of a non specific chronic inflammatory infiltrate, as seen in indeterminate leprosy, or the persistence of a granuloma in the paucibaccilary (BT, TT) forms of leprosy in the absence of demostrable bacilli, may indicate that free antigenic products are initiating the apparently non-specific inflammation and/or perpetuating the granuloma (9).

Immunohistochemistry proved to be useful to demonstrate infectious organisms and/or their antigens in tissues. Antigenic analysis indicates that there are common antigenic sites among mycobacterial species. On this basis rabbit anti-BCG serum has been widely used as the primary antibody to demonstrate both the bacilli and their antigens in leprosy tissues (5,6). Recently monoclonal antibodies against **M. leprae** have been produced which recognise specific antigens on cell surfaces of leprosy lesions (8).

A phenolic glycolipid with a structure related to mycoside A of **Mycobacterium kansasii** was found in **M. leprae** preparation and had its structure elucidated by HUNTER & BRENNAN (3) and HUNTER, FUJIWARA & BRENNAN (4). A highly specific trisaccharide for serodiagnosis of leprosy was synthesized and proved to be highly sensitive in ELISA (1). This synthetic trisaccharide (ST) is antigenic and anti-serum against it was raised in rabbits by a standard procedure, using incomplete Freund's adjuvant, and was used as primary antibody in an avidin-biotin peroxidase immunohistochemical reaction by us. In multibacillary leprosy, bacilli and/or their antigens were heavily stained in essentially similar manner by anti-BCG and anti-ST sera. In paucibacillary leprosy isolated macrophages in the granuloma were stained by both anti-sera, probably indicating antigenic products which might be relevant in the perpetuation of the granulomatous inflammation.

S-100 is an acidic calcium binding protein so-named because of its solubility in 100% ammonium sulphate solution at neutral pH; it is distributed in the brain of a wide variety of species and is regarded as species non-specific (7). The finding of S-100 antigen in non-nervous tissues and, particularly in antigen-presenting cells of the skin in normal conditions, suggests that S-100 should no longer be considered strictly as a nervous system specific protein. In paucibacillary leprosy S-100 antigen detection was used as a marker to cutaneous nerve branches, since dermal nerves impairment by inflammatory reaction permits the differential diagnosis between paucibacillary leprosy and other skin granulomatosis (2).

A positive staining of **Mycobacterium leprae** and/or its antigens with commercially available (DAKO, Denmark) polyclonal anti S-100 rabbit serum was detected by us in multibacillary leprosy. Essentially similar antigenic sites were demostrated by S-100, anti BCG and anti ST sera. Lepromin absorbed anti S-100 serum failed to stain bacilli but maintened its staining properties as far as antigen presenting cells and dermal nervous branches were concerned. Therefore, the use of non-specifically absorbed commercial anti S-100 protein polyclonal serum in paucibacillary leprosy stains structures known to be usually stained by this anti-serum together with bacillary antigens. The staining properties of **M. leprae** by commercially available (DAKO) polyclonal anti S-100 protein serum is really an artefact. This anti-serum is raised in rabbits using complete Freund's adjuvant, which contain mycobacteria. Consequently different antibodies are present in the anti-serum, some recognizing **M. leprae** and others recognizing S-100 protein.

Therefore, care should be taken when using in immunohistochemical procedures commercially available anti-serum in infectious diseases, chiefly in countries where tuberculosis and leprosy are endemic.

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"Instituto Adolfo Lutz", S. Paulo Health Service and Institute of Tropical Medicine, USP.

S.A.C. Siqueira
A. Wakamatsu
V.A.F. Alves
T. De Brito

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