Rev. Inst. Med. trop. São Paulo
36 (1): 95-96, janeiro-fevereiro, 1994

CORRESPONDENCE

ON LEISHMANIAL ANTIGEN DETECTION IN TISSUE SECTIONS OF MONTENEGRO'S REACTION.

Sir,

Although histological changes induced by administration of Montenegro's antigen has been recently described [6] very few is known about the behavior of parasite antigens inoculated in dermal tissues. Using rabbit polyclonal antibodies and the peroxidase-antiperoxidase (PAP) method for leishmanial antigen detection in formaldehyde fixed tissue FESTA NETO et al. [9] described the presence of intracellular parasite forms and presence of mononuclear cells with diffuse cytoplasmic leishmanial antigen immunoreactivity in the site of Montenegro's reaction. Immunoperoxidase method for detection of *Leishmania* amastigotes and its antigens in routinely fixed tissue sections using polyclonal antibodies was described by SELLS & BURTON [8]. Afterwards LIVMI et al. [4], SOTTO et al. [9] and BARBOSA et al. [2] also reported on the use of immunoperoxidase methods and anti-*Leishmania* polyclonal antibodies for *Leishmania* demonstration in tissue section.

Searching for leishmanial antigen immunoreactivity in positive Montenegro's reaction we applied the PAP method using antibodies raised in rabbits against *L. m. amazonensis* and *L. b. guyanensis* as previously described [2]. The subjects studied were those from the groups I, III and IV from a previous work on histology of Montenegro's reaction [6]: group I (n = 9), individuals with American cutaneous leishmaniasis; Group III (n=9) the 18-yr-old army conscripts presenting positive Montenegro's reaction after vaccination against leishmaniasis (they were negative by Montenegro's test before vaccination) and Group IV (n = 4, two of them receiving placebo instead specific antigens), subjects presenting Montenegro's reaction negative before and after vaccination. Individuals from the groups I and III presented positive Montenegro's reactions 48 hours after the test. Skin fragments were then collected, fixed in 4% formaldehyde, embedded in paraffin and sectioned at 5 µm. Hlistological examination of these tissue samples showed just discrete, small foci of mononuclear inflammatory cells. Necrosis, giant cells, granuloma-like structures, and epidermal changes were not observed. We could not find any *Leishmanial* antigen-like immunoreactivity.

Several interpretations could explain the differences between the results of FESTA NETO et al. [3] and ours findings. Concerning histology of Montenegro's reaction, these authors reported the presence of a relatively intense inflammatory reaction and/or presence of necrosis in most of their cases, 48 - 72 hours following the test. The discrepancy between this more striking inflammatory reaction plus the presence of patent leishmanial antigen immunoreactivity in the site of Montenegro's reaction in comparison to ours negative results could be due to differences in Montenegro's antigen preparation. FESTA NETO et al. [3] used 100 µl of Montenegro's antigen with 2 -3 x 10^6 promastigotes/ml prepared in Salles Gomes medium. On the other hand, MAYRINK et al. [5] used Montenegro's antigen containing sonicated promastigotes. After sonication parasites were disrupted and just small antigenic particles probably were present in the inoculum. Afterwards, the formaldehyde fixation of tissue samples could destroy the remaining epitopes recognized by anti-*Leishmania* antibodies. Differently, the immunoreactivity of Montenegro's antigen containing entire microorganisms could be better preserved. The remaining few immunoreactive sites preserved after formaldehyde fixation of tissue samples could give a positive immunocytochemical reaction by the PAP method. In addition, it should be emphasized that ours polyclonal anti-*Leishmania* antibodies are not very efficient in detecting *Leishmania* in tissue sections and so we have employed relatively low titres (from 60 up to 160) of our antisera in PAP technique while FESTA NETO et al. [3] used anti-*Leishmania* serum at titre 320. These low titres of rabbit antisera usually give undesirable non-specific background staining. In formalin fixed tissues most of this background staining is not very strong because most non-specific immunoreactive sites are destroyed. Omission of the first

antibody could not be enough as unique negative control once the use of rabbit normal serum or pre-immune serum at the same low dilutions of the primary antibody may give the same non-specific reaction. High dilutions of the primary antiserum, and also other negative controls, as adsorption tests, are consequently recommended for application in the "amplified" immunoperoxidase methods (as PAP and ABC) when polyclonal antibodies are used.

These high dilutions has not been reached by rabbit and mouse polyclonal anti-*Leishmania* antibodies, as far as we known [2, 3, 4, 5, 8, 9]. Monoclonal antibodies, for instance, has been used for more accurate detection of leishmanial antigens [5, 7]. In our experience formalin fixation is not adequate for immunocytochemical identification of small parasite antigens particles diffusely distributed in tissue components [1]. Therefore, other fixation procedures as well as the use of frozen tissue sections and monoclonal antibodies, may give better results in studies on leishmanial antigens in tissue sections.

A. J. A. BARBOSA
P. RASO
O. GENARO
C. A. da COSTA
W. MAYRINK

Departments of Pathology and Parasitology, Federal University of Minas Gerais, Av. Alfredo Balena 190, 30130-100 Belo Horizonte/MG, Brazil.

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Recebido para publicação em 03/08/1993.
Aceito para publicação em 04/11/1993.