Immunofluorescence as an Adjunct to the Histopathologic Diagnosis of Chagas’ Disease

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Chagas’ disease (American trypanosomiasis) is a zoonotic infection caused by the protozoan Trypanosoma cruzi (1, 2, 5). The disease, which is usually transmitted by blood-sucking triatomid bugs, is endemic in Central and South America but occurs rarely in the United States (10, 11, 13). When patients die of the acute form of Chagas’ disease, trypanosomes can be found in almost any organ but are more easily detected in the heart, where amastigotes 3 to 5 μm in diameter multiply in the myocardial fibers. Rupture of parasitized cells causes a severe inflammatory reaction with microabscess formation and phagocytosis of amastigotes by histiocytes and neutrophils. Experience in our laboratories has shown that the phagocytosed amastigotes can be mistaken for other cardiopathic microorganisms of similar size and shape, particularly Histoplasma capsulatum var. capsulatum and Toxoplasma gondii. Identification is especially difficult when the diagnostic intracytoplasmic, bar-shaped kinetoplast of T. cruzi cannot be detected. Amastigotes of Leishmania donovani, although morphologically similar to those of T. cruzi, are slightly smaller and do not ordinarily parasitize myocardial fibers.

Difficulty with the histopathologic differential diagnosis of Chagas’ disease prompted us to investigate the possibility of detecting and identifying T. cruzi amastigotes in Formalin-fixed, paraffin-embedded tissue sections by indirect immunofluorescence (IF). Because several investigators have reported enhanced IF of viruses and reduction of nonspecific background fluorescence when sections of Formalin-fixed, paraffin-embedded tissue are first digested in a weak trypsin solution (7, 8, 12), we adapted this technique with T. cruzi after initial studies without enzymatic digestion were unsatisfactory.

Tissues selected for IF studies included myocardia from two humans who had died of acute chagasic myocarditis and from four mice experimentally infected with T. cruzi and killed 1 month later. All tissues were taken at necropsy, fixed in 10% neutral buffered Formalin, embedded in paraffin, and stored in the files of the Centers for Disease Control for 6 to 23 years before IF examination. In each case, histopathologic examination of the heart revealed acute chagasic myocarditis with typical amastigotes of T. cruzi in myocardial fibers and phagocytes. The pathologic features of chagasic myocarditis have been reported elsewhere in detail (5, 6, 9, 10).

For IF examination, all tissues were sectioned at 5-μm thickness, mounted on alcohol-cleaned glass microscope slides coated with poly-d-lysine, deparaffinized by two 5-min immersions in xylol, and rehydrated through graded concentrations of alcohol to phosphate-buffered saline free of Ca2+ and Mg2+. To enhance IF staining of T. cruzi, the mounted tissue sections were then gently immersed in a solution of 0.25% trypsin in Hanks balanced salt solution free of Ca2+ and Mg2+ (GIBCO Laboratories, Grand Island, N.Y.) for 1 h at room temperature (7, 8, 12). Before enzymatic digestion, the trypsin solution had been removed from a 4°C refrigerator, slowly brought to room temperature by immersion in a 60°C water bath, and adjusted to pH 7.6 with a 7% solution of sodium bicarbonate. After trypsin digestion, the tissue sections were gently rinsed in phosphate-buffered saline free of Ca2+ and Mg2+ for 5 min. Rabbit antiserum raised against culture-derived and sonicated T. cruzi epimastigotes (lot no. 202, Tulahuen strain), prepared in the Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, and diluted 1:10 was then applied to each tissue section. To control specificity, the rabbit antiserum to T. cruzi was also applied to other tissue sections that contained H. capsulatum var. capsulatum, Toxoplasma gondii, or L. donovani, and normal rabbit serum diluted 1:10 was applied to the tissue sections infected with T. cruzi. All sections were then placed in a moist chamber at room temperature for 30 min. Afterwards, sections were rinsed by two 5-min immersions in phosphate-buffered saline free of Ca2+ and Mg2+ and fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (Dako Corp., Santa Barbara, Calif.) was applied to the tissue sections. After sitting in a moist chamber at room temperature for another 30 min, the sections were washed twice in phosphate-buffered saline, and a drop of buffered glycerol saline, pH 7.8, was placed on each tissue section before a cover slip was applied. All specimens were examined with an Orthoplan Ploem...
binocular fluorescence microscope (Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.) illuminated by a 100-W halogen lamp and equipped with an H2 filter block and a K480 barrier filter. Sections of Formalin-fixed, paraffin-embedded normal human and murine myocardia that were processed identically to the test sections served as negative tissue controls.

Enzymatic treatment of Formalin-fixed tissue sections is thought to unmask immunoreactive sites by freeing cross-linked antigen molecules (7). In tissue sections infected with T. cruzi and treated with trypsin before IF staining, amastigote cell membranes were intensely and uniformly fluorescent (3+ to 4+) on a scale of 0 to 4+) each time slides were processed (Fig. 1): the nuclei, kinetoplasts, and cytoplasm were not stained. Nonspecific fluorescence was minimal, and background tissues appeared faint yellowish brown. In contrast, in nontrypsinized tissue sections, amastigote cell membranes were weakly fluorescent (1+ to 2+) and poorly defined; nonspecific, faint-green, background fluorescence was usually present. One hour of digestion in a 0.25% trypsin solution was optimal for achieving discrete, bright-green IF of T. cruzi amastigotes. Further trypsinization usually resulted in lifting and fragmentation of tissue sections and did not improve the intensity of fluorescence. Sections of normal human or mouse myocardium treated with trypsin and stained by IF for T. cruzi showed little or no background fluorescence and appeared faint yellowish brown. No staining occurred in any of the specificity controls.

Because of its immunologic specificity, IF was particularly helpful in revealing intracellular, amorphous antigenic material from T. cruzi that could not be seen with conventional histologic stains (Fig. 2) and in identifying intact amastigotes that could not be differentiated morphologically from small yeastlike fungi or Toxoplasma gondii (Fig. 2, inset). IF was also more sensitive than any current histologic method, since more intact fluorescent amastigotes were seen in tissue sections stained by IF than in corresponding sections stained with hematoxylin and eosin, Woldbch Giemsa, or Wilder reticulum stains (5, 9). Electron microscopy can also help identify tissue forms of T. cruzi, but in our experience, it is more time-consuming, less specific, and very restrictive for sampling compared with IF.

There is mounting evidence that subcellular components of T. cruzi share common antigenic determinants with certain somatic cells and that the pathogenesis of Chagas' disease, especially the chronic form, involves autoimmune mechanisms (2–4). However, in the present study of acute chagasic myocarditis, rabbit antiserum to culture-derived T. cruzi epimastigotes did not show any cross-reactivity with myocardial fibers, fibroblasts, endothelial cells, or other tissue components.

The rapid and specific IF identification of T. cruzi amastigotes in Formalin-fixed, paraffin-embedded tissue sections should prove to be a useful diagnostic tool in anatomic pathology. However, until antiserum to T. cruzi is widely available, specimens must be referred to specialty laboratories for immunohistologic confirmation of Chagas' disease. Because fixation and prolonged storage of paraffin-embedded tissues do not appear to affect the antigenicity of T. cruzi, retrospective studies are possible, and unstained sections, tissue blocks, or “wet tissues” can be shipped long distances.

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LITERATURE CITED