Direct immunofluorescence in the diagnosis of toxoplasmic lymphadenitis

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SUMMARY The diagnosis of toxoplasmic lymphadenitis was established through the demonstration, by direct immunofluorescence, of toxoplasma cysts and trophozoites in a cervical lymph node biopsy which also had a characteristic histopathology. The patient had cervical lymphadenopathy and increased specific fluorescent antitoxoplasma IgG and IgM antibodies.

The inclusion of Toxoplasma gondii infections among the syndromes responsible for human cervical lymphadenopathy has necessitated the use of specific procedures to differentiate this condition from a more serious illness (Putschar, 1973). In such patients the presence of a raised titre of antibodies, detected by the Sabin-Feldman dye test, has usually suggested recent exposure to the protozoon (World Health Organisation, 1969). The co-existence of either complement-fixing (CF) or IgM antibodies, obtained by indirect immunofluorescence (IF), has often been correlated with acute infections (Remington et al., 1968; Dorfman and Remington, 1973). The histopathology of lymph nodes, characterised by follicular hyperplasia, associated with clusters of epithelioid histiocytes, is considered to be characteristic of toxoplasmic lymphadenitis (Gray et al., 1972; Dorfman and Remington, 1973). T. gondii has also been isolated from lymph nodes injected into laboratory mice. However, individual Toxoplasma, or cysts containing clusters of trophozoites, have rarely been observed in fixed stained histological preparations from lymph nodes. This has often handicapped the pathologist in his interpretation of preparations where the histopathology is not typical.

We report the results of a study in a patient with cervical lymphadenopathy who had increased titres of specific fluorescent IgG and IgM antibodies to T. gondii, where a diagnosis was achieved by the characteristic histopathology of the lymph node and the demonstration by direct immunofluorescence of Toxoplasma cysts and trophozoites within the lymphoid tissues.

Case history

A 24-year-old Palestinian sailor was seen at the American University of Beirut with a history of a gradually increasing number of palpable nodules in the neck of one year's duration. Physical examination of the cervical area revealed enlarged lymph nodes of the occipital and submaxillary regions. These were firm, discrete, and non-adherent to the skin or deeper tissues. A lymph node biopsy was obtained from the occipital region and diagnosed as being suggestive of toxoplasmic lymphadenitis. Blood samples were subsequently sent for diagnostic serology.

PATHOLOGY

The specimen consisted of a single formalin fixed occipital lymph node (1.2 cm diameter) with no gross lesions. Histopathological examination revealed reactive follicular hyperplasia where the follicles exhibited unusual variation in size and shape. Within the enlarged germinal centres, macrophages showing active phagocytosis were present. In addition, there was the distinctive presence of focal clusters of pale staining epithelioid histiocytes, which were noted within the interfollicular lymphoid stroma encroaching upon the germinal centres. Occasionally, the sinuses appeared packed with smaller histiocytes. The lymph node stroma contained plasma cells and large immunoblasts (Fig. 1).

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Indirect fluorescent antibody (IFA) test

Circulating IgG and IgM antibodies were demon-
strated by using *T. gondii* freeze-dried trophozoite antigens and monospecific anti-human IgG and IgM conjugates\(^1\). The procedure employed has been previously described by Kane *et al.* (1971). The serum anti-toxoplasma IgG and IgM titres were 1/1000 and 1/100, respectively.

**Direct immunofluorescence**

Thin sections from the paraffin embedded lymph node were passed through xylene and fixed on slides with methanol. The area to be tested was flooded with a 1 in 10 dilution of the anti-toxoplasma conjugate\(^1\). The slides were incubated in a humid chamber for 30 minutes. They were then rinsed in phosphate buffered saline (PBS), pH 7.2, for 30 minutes. The slides were next dried and mounted with cover slips using 10% glycerol in carbonated phosphate buffer. They were examined by the conventional method for immunofluorescence (Carver and Goldman, 1959).

A diffuse fluorescence of the capsular structure of the lymph node was observed. The follicular areas were faintly fluorescent with patchy areas of more intense staining. Under higher magnification these were made up of clusters of what appeared to be trophozoite-like bodies, surrounded by a non-fluorescent membrane (Fig. 2). The lymph sinusoids were congested with fluorescent bodies, similar to those seen in the clusters. The cells filling the sinuses were engorged with fluorescent material. A large number of free-lying intensely staining, single, crescent-shaped bodies were also seen. When compared with a preparation of known *T. gondii* freeze-dried trophozoites, the fluorescent bodies seen in the biopsy specimen had an appearance consistent with *T. gondii*. Similar immunofluorescent studies on five lymph node biopsies from Hodgkin's lymphoma, tuberculous lymphadenitis, sarcoidosis, and non-specific lymphadenitis were all negative.

Histopathological observation of lymph node biopsies are considered highly suggestive if not characteristic of toxoplasma lymphadenitis (Gray *et al.*, 1972). The distinctive histopathological features are a triad: (1) Striking follicular hyper-

\(^1\)Obtained from Wellcome Reagents Ltd, England
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Fig. 2 Section of cervical lymph node, stained with anti-toxoplasma conjugate, demonstrating fluorescent trophozoite-like bodies (arrows) surrounded by a non-fluorescent membrane. × 750.

plasia; macrophages within the germinal centres exhibiting phagocytosis of nuclear particles, imparting a 'starry-sky' appearance to the enlarged germinal centres, and marked mitotic activity. (2) Distinctive aggregation of pale staining epithelioid histiocytes in irregular clusters (granulomatoid) scattered in the inter-follicular stroma, often encroaching upon the follicles. (3) Packing of the dilated sinusoids, both peripheral and central, with smaller monocytoid cells. Plasma cells and immunoblasts are present in the lymphoid stroma. However, the above classical pattern may represent a narrow range within a broader spectrum. At either end of the spectrum the specific histological picture may not obtain.

The direct staining of *T. gondii* cysts and free-lying trophozoites with fluorescein-labelled antibody has been achieved in sections of spleen, liver, lung, and brain of mice experimentally infected (Carver and Goldman, 1959). Fluorescent Toxoplasma have also been observed in the retina of laboratory infected rabbits (Tabbara et al., 1974). Archer et al. (1971) demonstrated the specificity and sensitivity of direct immunofluorescence in the diagnosis of ovine abortion. Our data indicate that crescent-shaped bodies, single or in clusters, forming cysts fluoresce with specific anti-toxoplasma conjugate in thin sections of lymph node obtained from formalin fixed and paraffin-embedded tissue. A similar observation was made by Tsunematsu et al. (1964) in two patients with toxoplasmic lymphadenopathy.

Toxoplasma may persist in lymph nodes, skeletal muscle, and other tissues for months or years after infection. Their presence may thus be unrelated to the active disease. However, the detection of the proliferative forms of *Toxoplasma* in miscellaneous tissues should be of help in establishing an active infection. Hence their presence in patients with definite lymphadenopathy may be sufficient evidence to make the diagnosis.

We suggest the use of direct immunofluorescence as a sensitive and specific test in the diagnosis of active toxoplasmic lymphadenitis. We present this communication, hoping that others will have a better opportunity further to assess the diagnostic value of this procedure.

We thank the Wellcome Research Laboratories for their free supply of Toxoplasma *gondii* freeze-dried antigen, anti-human IgG and IgM conjugate, as well as anti-toxoplasma conjugate.

References


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