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HUMAN FASCIOLIASIS : T CELL SUBSETS IN LIVER BEFORE AND AFTER BITHIONOL TREATMENT

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ABSTRACT

Liver biopsies from 5 patients with established fascioliasis, before and after bithionol treatment were studied by immunoalkaline phosphatase technique for relative distribution of T cells and their subpopulations. T cell and its subsets are defined for $OKT3^+$ (pan T), $OKT4^+$ (helper/inducer) and $OKT8^+$ (suppressor/cytotoxic) cells by using mouse monoclonal antibodies. Before bithionol treatment, lymphocytic infiltration in all hepatic lesions were predominantly of $OKT3^+$ (pan T) lymphocytes. The distribution of $OKT8^+$ cells was moderate to severe in comparison to the few $OKT4^+$ cells presentation. After bithionol a noticable regression of the OKT3 lymphocytic in all liver sections. The majority of the lymphocytic infiltration was of the $OKT8^+$ cells, in comparison to the absence of the $OKT4^+$ ones. This may indicate that suppressor/cytotoxic lymphocytes may have a role in the immune regulation of the disease and the mode of action of bithionol is by the accentuation of this immunoregulartory effect.

INTRODUCTION

Human fascioliasis in Egypt has become a problem in the last decade (Ali et al., 1974; Ragab and Farag, 1974; Farag et al., 1979). Literature concerning the pathology of such disease in humans is very

few (Acosta Ferreira et al. 1979). Most of the information about the pathology are derived from animal experiments (Lang, 1966, 1967; Massake et al, 1978; Massba, 1981). On analysis a previous work we described the histopathology of human fascioliasis before and after bithionol treatment (Abou Busha et al., in press). Fasciolasis was manifested pathologically as hepatic degeneration, lymphocytic infiltration between liver cells, portal tract fibrosis and bile duct proliferation. There was remarkable regression of lymphocytic infiltration as well as regeneration of the liver cells after bithionol treatment. The current study is directed primarily on the immunohistochemical delineation of various T-cell subsets population within liver biopsy samples of the same Fasciola patients befor and after bithionol treatment.

MATERIAL AND METHODS

Ten liver biopsies were taken from 5 patients with established fascioliasis before and after treatment. The diagnosis was verified by the presence of ova in stools (Martin & Beaver 1967-. Bithionol was given in a dose of 30 mg/kg body weight every other day for 10 days.

Liver biopsies were quick frozen in dry ice in an acetone bath 4 um sections were cut on a cryostat. Human tonsils were used as a positive control. Some hepatic sections were stained with H & E for histopathological examination; the rest were fixed at 4 C for 10 minutes in a mixture of chloroform and acetone 1 : 1 for immunohistochemical study (Lwin et al 1985). After 2 washes in phosphate buffered saline (PBS) at pH 7.4; all sections were incubated with monoclonal antibodies induced in mouse OKT3, OKT4 and OKT8 (Behringwerk, Germany) for 1 hour at room temperature. After 3 washes in PBS, the sections were incubated with the relevant second antiserum directly conjugated to alkaline phosphatase (Sigma, St Louis), 1 : 20 dilution for 1 hour at room temperature. Following 3 washes with PBS the red colour was developed by incubation with naphthol As-bi phosphoric acid (Na salt), dimethyl formamide and fast red in veronal acetate in phosphate buffer for 45 minutes. Levamisole was included in the reaction media to block endogenous alkaline phosphatase. The slides were then washed 3 more times in PBS and mounted in glycerin buffer 9 : 1. The extent of the red colour around the lymphocytic cells were calculated as mean percentage/10 low power fields. It was ex-

pressed as few (less than 25%), moderate (25-70%) and severe (more than 70%). Serial sections of both H & E and immunohistochemically stained were compared for the relative distribution of $OKT8^+$ and $OKT4^+$ cells in the liver tissue.

RESULTS

Before bithionol treatment : Two cases showed marked liver degeneration up to necrosis, with fibrous bands and lymphocytic infiltration in between the liver cells (Figure 1a), In other case there was a granulomatous lesion made up of central necrotic material surrounded by circumferencial fibrosis then eosinophilic and lymphocytic infiltration (Figure 2a). The other 2 cases showed patchy areas of necrotic cells with foci of perivascular lymphocytic infiltration and bile canalicular hyperplasia. Immunohistochemistry : Most of the lymphocytic infiltration present in the above pathological lesions was T lymphocytes defined as $OKT3^+$ cells. $OKT8^+$ cells showed moderate to severe degree of positivity as compared with the few degree of positivity expressed by $OKT4^+$ cells. This picture was present in the different pathological lesions, namely : around the fibrous bands, degenerated liver cells (Figure 1b, c), in the granulomatous lesions, in the portal tracts and the perivascular lymphocytic areas (Fig. 2b & c).

After bithionol treatment : The overall histopathology of the liver in the 5 cases showed marked regression in the intensity of lymphocytic infiltration, areas of hepatic necrosis were diminished and substituted by regenerating liver cells (Fig. 3a). Immunohistochemical staining of the above serial sections showed that most of the remaining lymphocytes were of the T type as detected by $OKT3^+$ cells. The intensity of $OKT8^+$ cells ranged from few to moderate (Fig. 3b), while $OKT4^+$ were absent in all liver sections.

DISCUSSION

The present study is an extension of previous work on the ultrastructural study on the liver before and after bithionol treatment (Abou Basha et al., 1990). There was a marked regression of the lymphocytic infiltration of the liver after treatment. In this study the immunohistochemical staining showed wide spread lymphocytic in-

filtration of the OKT3$^+$ type, concentrated in the perivascular areas, inside the granulomatous lesions as well as surrounding degenerated liver cells. These findings support the view that pathological change in fascioliasis is a cell mediated immune response and the damage is caused by a delayed hypersensitivity reaction of T lymphocytes (Lang, 1967; Massaba, 1981).

In the present study the expression of helper/inducer cells was few before treatment in contrast to the relative increase to the suppressor/cytotoxic cells in the liver sections. This may indicate that helper/inducer cells may be responsible for the damage occuring in the liver in the invasive stage. The suppressor/cytotoxic cells may have a role in the immunoregulation of the reaction in the established infection. This is supported by the presence of allergic phenomena in some patients and high titre of specific antibodies in prematuration phase and the decline of antibody titre in the mature stage (Capron 1968). Similar results were obtained in the compensated stage of schistosomal hepatic fibrosis (Helal et al, in press), chronic active hepatitis, alcoholic biliary cirrhosis and acute hepatitis (Husby et al., 1982) where there is marked increment of OKT8 more than OKT4 in the liver tissue.

Bithionol is the treatment of choice for the mature fluke (Dawes 1966, Abou Basha & Farag 1982). Results in the present work are in support with this view where the picture of the liver pathology reggressed to a near normal picture showing areas of regeneration with residual lymphocytic infiltration. These findings were in accordance with the experimental work done by many authors (Lang 1966, 1967; Mask et al 1978; Massaba 1981). Moreover this was approved by our ultrastructural previous study before and after bithionol (Abou Basha et al. 1990).

Immunohistochemically after bithionol, marked regression of T lymphocytes expressed as OKT3$^+$ cells was detected in the liver of established cases. A relative increase of suppressor/cytotoxic cells over the helper/inducer which were nearly absent. This supports our previous suggestion that the suppressor/cytotoxic cells have a great role in the immunoregulation of the reaction induced by fascioliasis. Moreover the curative mechanism of bithionol therapy may be through the

accentuation of this immunoregulatory effect. Gaber et al (1988) reporteed that bithionol had a curative effect on *Schistosoma mansoni* infected mice which was manifested by improvement of the liver cells and decreasing the granuloma cellularity and size. Further immunological studies on lymphocytes both in the blood and tissue, are needed to clarify the pathogenesis of fascioliasis.

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EXPLANATION OF FIGURES

Figure 1. a) Frozen liver section showing degenerated hepatocytes, crossed by a thick band of fibrosis. (H & E, X100).

b) Immunohistochemical staining with OKT8 reagent, suppressor/cytotoxic T cell of the above serials sections showing moderate to severe staining around the degenerated liver cells. X100.

c) Immunohistochemical staining with OKT4 reagent showing few helper/inducer T cells around the degenerated liver cells. X100.

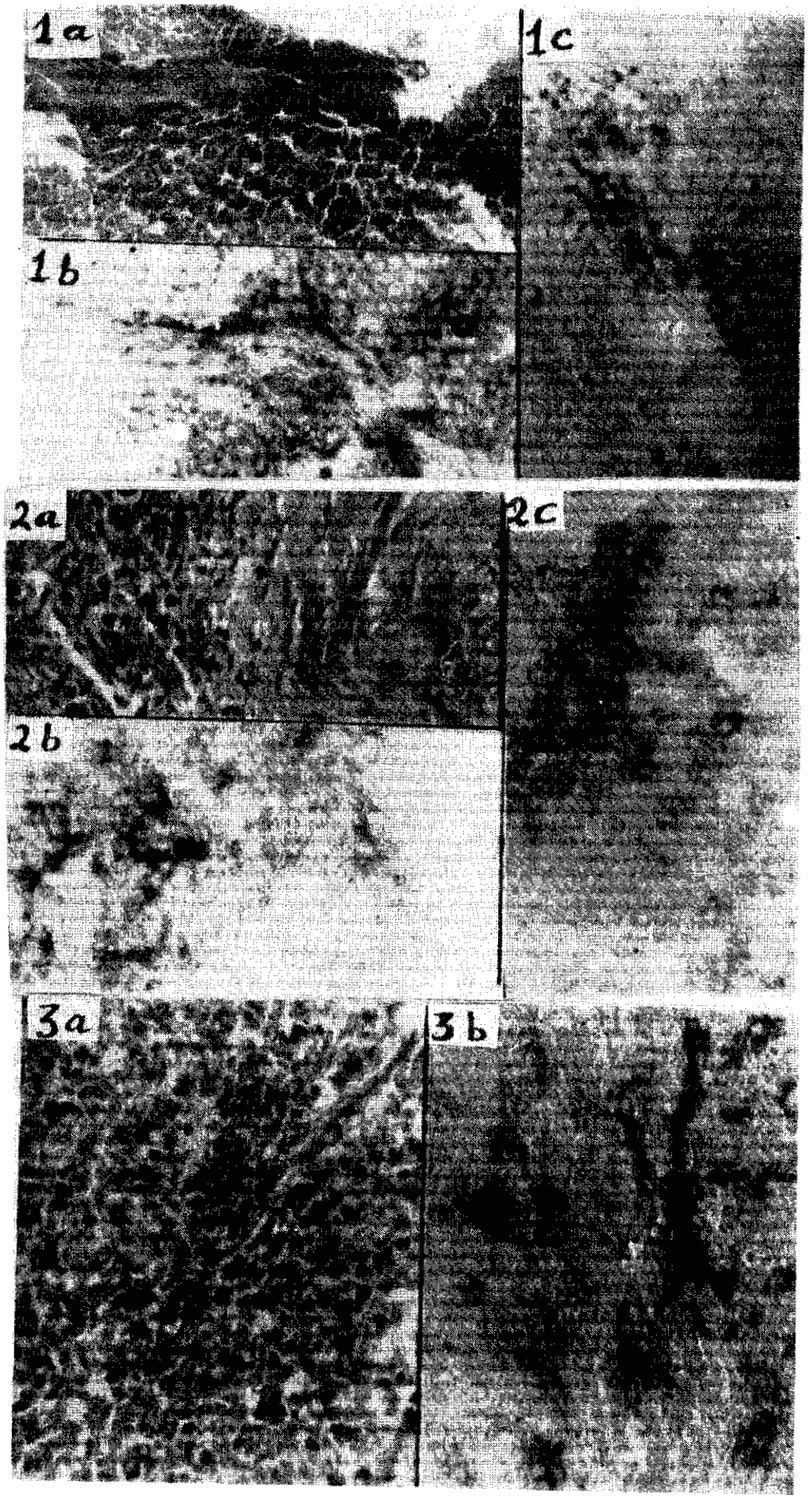
Figure 2. a) Frozen liver section showing a granuloma made up of fibrocytes (F), eosinophils (E) and lymphocytes (L) and surrounded by degenerated liver celels (D) H & E, X100

b) Immunohistochemical staining with OKT8 reagent showing moderate suppressor/cytotoxic T cells in the granulomatous lesion. X100

c) Immunohistochemical staining with OKT4 reagent showing few helper/inducer T cells in the granulomatous lesion, X100.

Figure 3. a) Frozen liver section after bithionol, showing regenerated liver cells with some lymphocytic infiltration. H&E X400

b) Immunohistochemical staining for OKT8 reagent showing the membranous perilymphocytic immunohistochemical staining for $OKT8^+$ T cells, X400.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