Distinct B-cell populations are present in hepatic and intestinal *Schistosoma mansoni* granulomas

W. Jacobs*,**, J. Bogers*, E. Van Marck*

* Department of Pathology, University of Antwerp (U.I.A.), ** Research Assistant of the Fonds voor Wetenschappelijk Onderzoek — Vlaanderen.

Abstract

Although it is generally accepted that schistosomal granuloma formation results from a T-cell dependent host response towards the parasite egg, attention has recently been focused on the involvement of B-cells in the induction of schistosome-induced pathology. In this study we investigated the involvement of two functionally different B-cell populations in the formation of the *Schistosoma mansoni* granuloma : naive and antigen-stimulated B-cells. In liver granulomas two distinct B-cell populations were found, namely unstimulated B-cells at the periphery of the granuloma and antigen-stimulated, syndecan-1 positive B-cells in the inner part near the deposited egg. Intestinal granulomas differed by their relative lack of unstimulated B-cells at the granuloma periphery, but like hepatic granulomas intestinal granulomas also have syndecan-1 positive B-cells. From our results, we conclude that B-cells are important constituents of the *S. mansoni* granuloma. (Acta gastroenterol. belg., **1999**, 62, **178-181**).

Key words : schistosomiasis, granuloma, B-cells, CD40, syndecan-1.

Introduction

Schistosomiasis is a helminthic disease caused by pathogenic blood dwelling (mesenteric and bladder veins) trematodes of the genus *Schistosoma*. It is estimated that 600 million people are at risk for the disease and 200 million are actually infected with an annual death rate of 500,000 - 800,000 (1). In the case of infection with *Schistosoma mansoni* or *S. japonicum*, the disease results from a delayed hypersensitivity response generated against parasite eggs deposited in the target organs (liver, intestine) leading to the development of granulomatous inflammation (2). Clinically, the disease ranges from the relatively mild intestinal to the severe hepatosplenic form. The latter is seen in a small subpopulation (< 10%) of infected patients and is characterized by the formation of extensive liver fibrosis (Symmers' fibrosis) resulting in portal hypertension, oesophageal varices, and haematemesis.

Cellular adhesion molecules are important immunological mediators in the formation of the schistosomal granuloma (3,4). One cellular adhesion molecule belonging to the group of heparan sulphate proteoglycans is syndecan-1 (CD138). In murine tissues, syndecan-1 immunoreactivity is observed at the surface of cells from epithelial origin and on plasma cells (5). Sanderson *et al.* (6) demonstrated that syndecan-1 is a stage specific B-cell marker. Expression of syndecan-1 is only seen on immature B-cells present in the bone marrow or on antigen-driven B-cells in the connective tissue. It is suggested that syndecan-1 is expressed only when and where B-cells associate with extracellular matrix (e.g. in bone marrow and in connective tissue) and thus that changes in syndecan-1 expression on B-cells are a mechanism for controlling B-cell localization within specific microenvironments (7).

The aim of this morphologic study was to investigate the involvement of ontogenically different B-cells (naive CD40 positive B-cells and antigen-stimulated, syndecan-1 positive B-cells) in the formation of *S. mansoni* induced granulomas in the liver and intestine.

Materials and methods

Male OF1 mice (Iffa Crédo, St. Germain sur L'Arbresle), 6 weeks of age, were infected with 70 cercariae of the Puerto Rican strain of *S. mansoni* via the transcutaneous route. The animals were sacrificed at 8 and 13 weeks post-infection (respectively acute and chronic stage of infection, n = 6 for each group). Liver, proximal colon, distal ileum, and dorsal skin were snap frozen in liquid nitrogen and stored at -80° C.

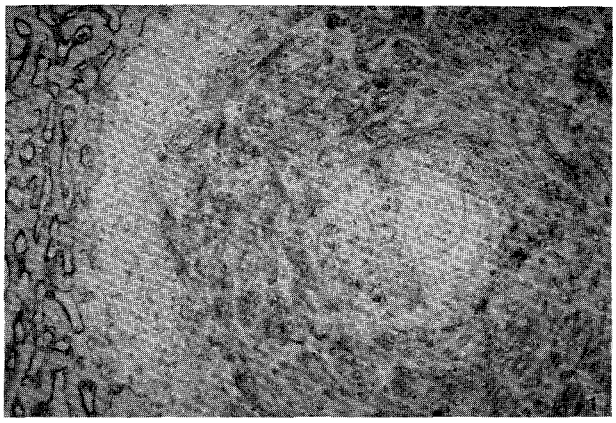
Immunohistochemical testing on 5 µm thick frozen sections was performed using an indirect immunoperoxidase or alkaline phosphatase/anti-alkaline phosphatase (APAAP) technique according to the method described previously (3-4). Antibodies directed against naive B-cells (rat anti-mouse CD40 IgG, Serotec, Oxford, UK [dilution 1 :50]), antigen-stimulated, syndecan-1 positive B-cells (rat anti-mouse syndecan-1 $IgG_{2a,k}$, Pharmingen, San Diego, USA [dilution 1:320]), or dendritic cells (rat anti-mouse NLDC-145 IgG [dilution 1:50], BMA, Augst, Switzerland) were tested. A mouse-anti-human α-neurofilament IgG antibody (Dako) in a similar protein concentration as the antibody under investigation was used as a negative control.

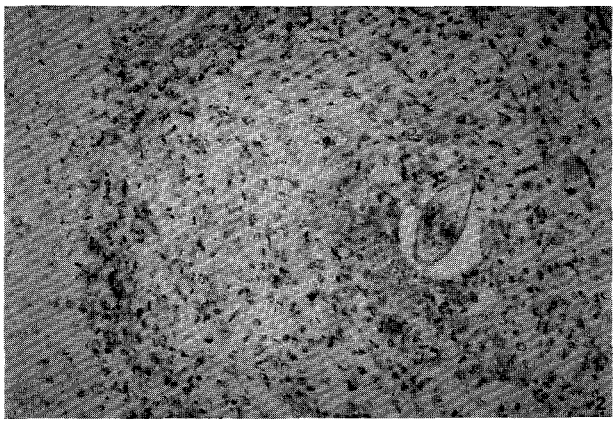
Address of correspondence : Prof. dr. E. Van Marck, Dept. of Pathology, University of Antwerp (U.I.A.), Universiteitsplein 1. B-2610 Wilrijk.

Paper presented in part at the 'Belgian Week of Gastroenterology', Knokke (20/02/1997). JACOBS W., KUMAR-SINGH S., BOGERS J., DEELDER A., VAN MARCK E. Proteoglycan expression in experimental, hepatic schistosomiasis mansoni. *Acta Gastro-Enterol. Bel.*, 1997, 60, A16.

Results

In the liver, basal syndecan-1 expression was observed in sinusoidal lining cells and bile duct epithelium. Absence of syndecan-1 was noticed on endothelial cells of portal tract vessels. In the intestine, syndecan-1 staining was sometimes observed on enterocytes, mainly at the basolateral side of these cells and on rare, isolated cells in the lamina propria. Cellular up-regulation of syndecan-1 was seen in hepatic (Fig. 1), ileal and colonic (Fig. 3) schistosomal granulomas. A typical rimming pattern of immunoreactivity was seen in the inner part of the granuloma close to deposited schistosome eggs. A band of CD40 positive cells was located at the periphery of the hepatic granulomas (Fig. 2). CD40 positive cells were sparsely present in the inner part of the granuloma. Few CD40 positive B-cells were seen in colonic granulomas and virtually no CD40 positive cells were present in the ileum. To distinguish B-lymphocytes from dendritic cells in the CD40 positive cell population, immunohistochemical staining for NLDC-145 was performed. No immunoreactivity in the granulomas was observed for NLDC-145 in contrast to the murine skin (epidermis) where numerous NLDC-145 immunoreactive dendritic cells were seen. These results and the staining pattern in the various anatomical parts of the liver and intestine are summarized in Table I.





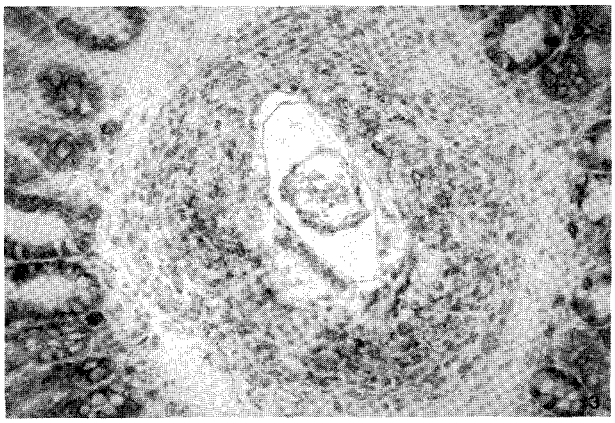


Fig. 1. — Antigen-stimulated B-cells expressing the syndecan-1 surface antigen are present in the inner part of a *S. mansoni* liver granuloma (8 weeks post-infection) in the vicinity of a deposited egg (figure 1, peroxidase stain, X145). CD40 positive, naive B-cells (figure 2) are located mainly at the periphery of the hepatic granuloma (APAAP stain, X145, 8 weeks post-infection). This photograph was a serial section from the same liver as in figure 1. Colonic granuloma (13 weeks post-infection) with central immunoreactivity for stimulated B-cells (syndecan-1 positive) (figure 3). Enterocytes also demonstate expression of the syndecan-1 antigen (peroxidase stain, X180).

Table I. — **Expression pattern of the antibodies under investigation in the various organs**

	liver	colon	ileum	skin
syndecan-1	granuloma ++ (IP) sinusoidal lining cells bile ducts	granuloma ++ (IP) enterocytes	granuloma ++ (IP) enterocytes	—
CD40	granuloma ++ (OP)	granuloma + (OP)	granuloma -/+ (OP)	epidermis +
NLDC-145	—	—	—	epidermis ++

++ strong immunoreactivity ; + limited immunoreactivity ; — no immunoreactivity.
IP : inner part of the granuloma ; OP : outer part of the granuloma.

Discussion

During schistosome infection, the host's immunological response switches from a Th1-type cell response during prepatent infection towards a Th2 cell response after egg deposition (8-9). Th2 cytokines (e.g. IL-4 and IL-10) are pivotal in the genesis of the schistosome granuloma (10-11). Several authors have pointed out the importance of humoral immunity in the pathogenesis of human schistosomiasis (12-14). Recent experimental observations clearly demonstrate that — like T-cells — B-cells are also involved in the genesis and modulation (15-16) of the *S. mansoni* granuloma since B-cell deficient mice develop augmented tissue pathology and fail to downmodulate the granulomas during chronic infection (16). Furthermore, B-cells promote Th2-type cell responses (16).

In this study, we could demonstrate the existence in liver granulomas of 2 distinct functional populations of B-cells, located at 2 distinct sites in the schistosome granuloma. Naive, unstimulated B-cells were located mainly at the periphery of the granuloma whereas stimulated B-cells expressing the stage-specific marker syndecan-1 were located in the inner part of the granuloma near deposited schistosome eggs. Earlier work by our group (17) demonstrated that egg antigen laden macrophages were present in a similar location. It is therefore an attractive hypothesis to assume that naive B-cells reside at the periphery of the granuloma from where they can migrate towards the parasite egg and become antigenically stimulated by T-lymphocytes activated by egg antigen presenting macrophages (18-19) (Figure 4). Although syndecan-1 positive B-cells are clearly present in intestinal granulomas, colonic and ileal granulomas often lack an outer lymphocytic halo, which in liver granulomas is composed of numerous B-cells (20). In intestinal granulomas, B-cells are likely to be recruited from the surrounding connective tissue where inflammatory cells are abundantly present during schistosome-induced tissue inflammation.

We identified naive B-cells by their expression of the CD40 marker on their cell membrane (21). Recent observations have demonstrated that CD40 is not exclusively B-cell specific but is also expressed on antigen presenting dendritic cells (22). The presence of antigen presenting dendritic cells was described in the portal tract of the liver in auto-immune liver disease and during chronic hepatitis (23). We have therefore immunohistochemically tested hepatic and intestinal granulomas for the presence of dendritic cells using the

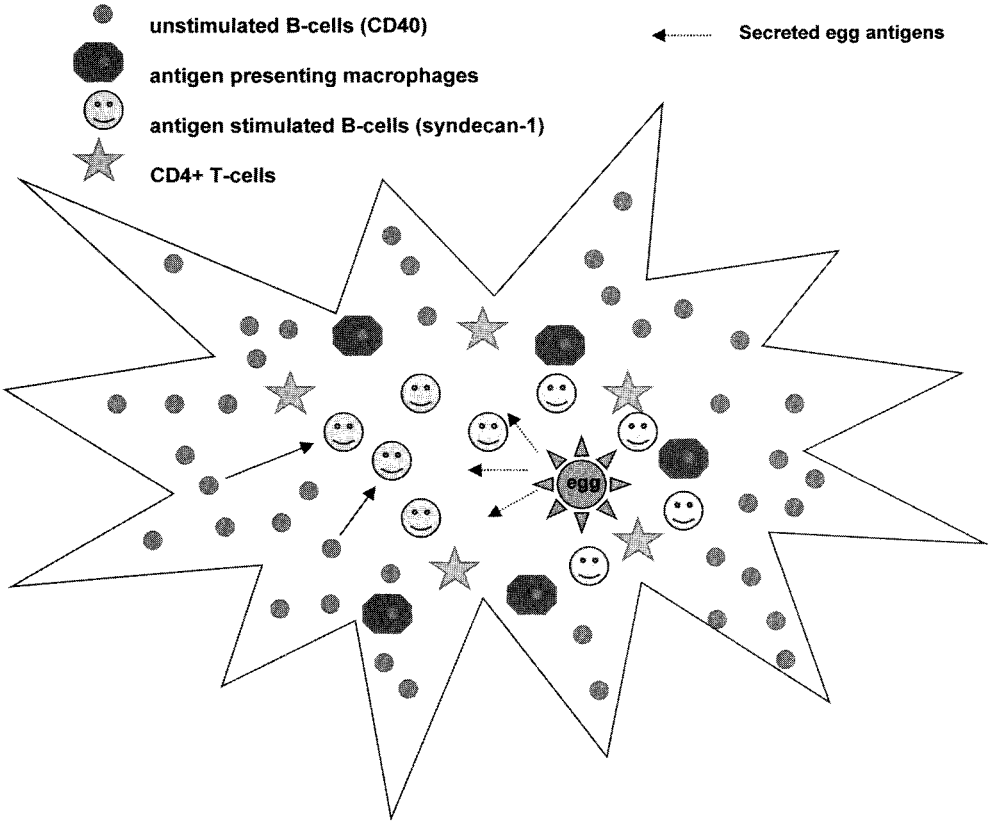


Fig. 4. — Schematic representation of the morphological distribution of inflammatory cells in the hepatic *S. mansoni* granuloma. Deposited schistosome eggs secreted soluble egg antigens (dashed arrow) that are presented by antigen-presenting macrophages (APC's). These APC's stimulate egg antigen specific T-cell clones that can stimulate through specific cytokine pathways naive B-cells, located at the periphery of the granuloma. When these B-cells become antigen-stimulated, they migrate (full arrow) towards the egg and express the syndecan-1 antigen. B-cells — naive or antigen-stimulated — can modulate the host's immunological response generated against the parasite egg antigens.

monoclonal antibody NLDC-145 which specifically recognizes dendritic cells (24). Since immunoreactivity was seen in murine epidermis where dendritic cells are commonly present, but not in schistosomal granulomas, we can safely conclude that CD40 positive cells in the granuloma are indeed of B-cell origin.

Marked differences between hepatic and intestinal *S. mansoni* granulomas exist. Intestinal granulomas are smaller in size compared to liver granulomas (19,25-26) and are only mildly fibrogenic (26-27). Differences in the cellular composition of hepatic, ileal, or colonic granulomas also exist (20). Ileal granulomas fail to downmodulate during chronic infection in contrast to hepatic and colonic granulomas (19,25). The exact mechanisms underlying schistosomal granuloma formation in the various organs are poorly understood, but organ-dependent differences (19) are likely to be important. Since B-lymphocytes are potential modulators of schistosome-induced granuloma formation and fibrosis (15-16), organ-related differences in the cellular composition of the evoked granulomas may be a contributing element in the differential pathogenesis.

From our observations, we infer that in hepatic *S. mansoni* granulomas 2 distinct populations of functionally different B-cells are present, located at two different morphological sites in the granuloma. In contrast, in ileal colonic granulomas, only one distinct B-cell population is present. Colonic granulomas display an intermediate morphology. B-cells may exert important pathophysiological functions and differences observed may be a contributing factor for the differential genesis of the schistosome granulomas in the liver, colon, and ileum.

References

1. JACOBS W. *Schistosoma mansoni*-induced hepatic and intestinal damage : a morphological study of granulomogenesis, fibrogenesis, and immune modulation. PhD thesis, Universiteit Antwerpen, 1998.
2. BOROS D.L. Immunopathology of *Schistosoma mansoni* infection. *Clin. Microbiol. Rev.*, 1989, **2** : 250-269.
3. JACOBS W., BOGERS J., DEELDER A., VAN MARCK E. Expression of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 in experimental *Schistosoma mansoni* infection and in synchronous periparticular hepatic granulomas in mice : immunohistochemistry, confocal laser scanning microscopy and immunoelectron microscopy. *Parasitol. Res.*, 1997, **83** : 405-412.
4. JACOBS W., BOGERS J., DEELDER A., WERY M., VAN MARCK E. Adult *Schistosoma mansoni* worms positively modulate soluble egg antigen-induced inflammatory hepatic granuloma formation *in vivo*. Stereological analysis and immunophenotyping of extracellular matrix proteins, adhesion molecules, and chemokines. *Am. J. Pathol.*, 1997, **150** : 2033-2045.
5. HAYASHI K., HAYASHI M., JALKANEN M., FIRESTONE J.H., TRELSTAD R.L., BERNFIELD M. Immunohistochemistry of cell surface heparan sulfate proteoglycan in mouse tissues. A light and electron microscopic study. *J. Histochem. Cytochem.*, 1987, **35** : 1079-1088.
6. SANDERSON R.D., LALOR P., BERNFIELD M. B lymphocytes express and lose syndecan at specific stages of differentiation. *Cell Regul.*, 1989, **1** : 27-35.
7. SANDERSON R.D., SNEED T.B., YOUNG L.A., SULLIVAN G.L., LANDER A.D. Adhesion of the B lymphoid (MCP-11) cells to type I collagen is mediated by the integral membrane proteoglycan, syndecan1. *J. Immunol.*, 1992, **148** : 3902-3911.
8. GRZYCH J.M., PEARCE E., CHEEVER A., CAULADA Z.A., CASPAR P., HEINY S., LEWIS F., SHER A. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J. Immunol.*, 1991, **146** : 1322-1327.
9. PEARCE E.J., CASPAR P., GRZYCH J.M., LEWIS F.A., SHER A. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.*, 1991, **173** : 159-166.
10. LUKACS N.W., BOROS D.L. Lymphokine regulation of granuloma formation in murine schistosomiasis mansoni. *Clin. Immunol. Immunopathol.*, 1993, **68** : 57-63.
11. WYNN T.A., ELTOUM I., CHEEVER A.W., LEWIS F.A., GAUSE W.C., SHER A. Analysis of cytokine mRNA expression during primary granuloma formation induced by eggs of *Schistosoma mansoni*. *J. Immunol.*, 1993, **151** : 1430-1440.
12. HAGAN P., BLUMENTHAL U.J., DUNN D., SIMPSON A.J., WILKINS H.A. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature*, 1991, **349** : 243-245.
13. DUNNE D.W., HAGAN P., ABATH F.G. Prospects for immunological control of schistosomiasis. *Lancet*, 1995, **345** : 1488-1491.
14. MUTAPI F., NDHLOVU P.D., HAGAN P., WOOLHOUSE M.E. A comparison of humoral responses to *Schistosoma haematobium* in areas with low and high levels of infection. *Parasite Immunol.*, 1997, **19** : 255-263.
15. JANKOVIC D., CHEEVER A.W., KULLBERG M.C., WYNN T.A., YAP G., CASPAR P., LEWIS F.A., CLYNES R., RAVETCH J.V., SHER A. CD4+ T cell-mediated granulomatous pathology in schistosomiasis is downregulated by a B cell-dependent mechanism requiring Fc receptor signaling. *J. Exp. Med.*, 1998, **187** : 619-629.
16. HERNANDEZ H.L., WANG Y., STADECKER M.J. In infection with *Schistosoma mansoni*, B cells are required for T helper type 2 cell responses, but not for granuloma formation. *J. Immunol.*, 1997, **158** : 4832-4837.
17. BOGERS J.J., NIBBELING H.A., DEELDER A.M., VAN MARCK E.A. Immunohistochemical and ultrastructural localization of *Schistosoma mansoni* soluble egg antigens processed by the infected host. *Parasitology*, 1996, **112** 537-543.
18. JACOBS W., KUMAR-SINGH S., BOGERS J., VAN DE VIJVER K., DEELDER A., VAN MARCK E. Transforming growth factor β, basement membrane components and heparan sulphate proteoglycans in experimental, hepatic schistosomiasis mansoni. *Cell Tis. Res.*, 1998, **292** : 101-106.
19. JACOBS W., BOGERS J., TIMMERMANS J.P., DEELDER A., VAN MARCK E. Adhesion molecules in intestinal schistosomiasis mansoni. *Parasitol. Res.*, 1998, **84** : 276-280.
20. WEINSTOCK J.V., BOROS D.L. Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with schistosomiasis mansoni. *J. Immunol.*, 1983, **130** : 418-422.
21. UCKUN F.M., GAJL-PECZALSKA K., MEYERS D.E., JASZCZ W., HAISSIG S., LEDBETTER J.A. Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B-lineage acute lymphoblastic leukemia as well as B-lineage non-Hodgkin's lymphoma cells. *Blood*, 1990, **76** : 2449-2456.
22. CELLA M., SCHEIDEGGER D., PALMER-LEHMANN K., LANE P., LANZAVECCHIA A., ALBER G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity : T-T help via APC activation. *J. Exp. Med.*, 1996, **184** : 747-752.
23. KAJI K., NAKANUMA Y., HARADA K., TSUNEYAMA K., KANEKO S., KOBAYASHI K. Dendritic cells in portal tracts in chronic hepatitis C and primary biliary cirrhosis with relevance to the bile duct. *Hepatology Research*, 1997, **8** : 1-12.
24. KRAAL G., BREEL M., JANSE M., BRUIN G. Langerhans' cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. *J. Exp. Med.*, 1986, **163** : 981-997.
25. WEINSTOCK J.V., BOROS D.L. Heterogeneity of the granulomatous response in the liver, colon, ileum, and ileal Peyer's patches due to schistosome eggs in murine schistosomiasis mansoni. *J. Immunol.*, 1981, **127** : 1906-1909.
26. DE OLIVEIRA SANTOS R., BARBOSA A.A. Jr., ANDRADE Z.A. Dynamics of fibrosis production and resorption in intestinal schistosomiasis of mice. *Mem. Inst. Oswaldo Cruz*, 1992, **87** : 25-31.
27. GRIMAUD J.A., BOROS D.L., TAKIYA C., MATHEW R.C., EMONARD H. Collagen isotypes, laminin, and fibronectin in granulomas of the liver and intestines of *Schistosoma mansoni*-infected mice. *Am. J. Trop. Med. Hyg.*, 1987, **37** : 335-344.