Virchows Archiv A Pathol Anat (1990) 416:527–534

Virchows Archiv A
Pathological Anatomy and Histopathology

The immunopathology of *Schistosoma mansoni* granulomas in human colonic schistosomiasis

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Received July 24, 1989 / Received after revision November 16, 1989
Accepted November 17, 1989

Summary. The immunopathology of *Schistosoma mansoni* infection was studied in colonic biopsies obtained from 14 patients with established schistosomiasis. The characteristic lesions of this parasitic infection are mainly induced by the presence of living eggs in the tissue. Different types of lesions can be present simultaneously. The earliest lesions contain T-lymphocytes as well as accessory cells around living eggs. They transform into granulomas composed of eosinophils, T-lymphocytes, a few B-lymphocytes and large mononuclear cells expressing major histocompatibility (MHC) class II antigens. These cells are also Mac 387 positive. This means that they are monocytes/macrophages freshly recruited from the blood. In other, probably older, granulomas, MHC class II positive cells tend to disappear and the centrally located multinucleated giant cells are negative for antibodies directed against MHC class II antigens. It appears thus that the composition of the granulomas in schistosomiasis is variable. The lesions may have characteristics of cell-mediated immunity and/or of a foreign-body reaction. Contrary to what is often seen in Crohn's disease or intestinal tuberculosis no major hyperplasia of the lymphoid tissue is observed in the colon in association with *S. mansoni* infection.

Key words: *Schistosoma mansoni* – Immunopathology – Colon – Granuloma

Introduction

The differential diagnosis of granulomatous ileocolitis includes idiopathic conditions such as Crohn's disease as well as a variety of infectious diseases of bacterial, mycotic or parasitic origin and also foreign-body reactions. Crohn's disease is probably the most common cause of granulomatous ileocolitis in Europe and the United States. Tuberculosis and parasitic infections are only sporadically seen in this part of the world. But in Africa and Asia, infectious causes of granulomatous ileocolitis are much more common. Ileocaecal tuberculosis is currently seen in India and Iraq (Tandon and Prakash 1972; Al-Bahrani and Al-Saleem 1982) and schistosomiasis is a common cause of ileocolonic granulomatous disease in Egypt (Higashi and Aboul-Enein 1981; Nash et al. 1982).

Schistosomiasis is an endemic helminthic disease caused by either *S. mansoni* or *S. haematobium*. The former is the agent which is mostly responsible for the intestinal lesions. The formation of granulomas around the parasite eggs in the tissue is the characteristic morphological lesion. Experimental evidence suggests that the granulomatous reaction is the result of an immunological process involving humoral and cell-mediated egg specific immune responses. The lesions are formed as the consequence of a complex series of events that follows an initial immunospecific recognition of (surface?) antigens by T-lymphocytes. The role of B- and T-lymphocytes and of monocyte/macrophage cells in the formation of these granulomas has already been studied exhaustively in experimental conditions (Stadecker and Wright 1984; Doughty and Phillips 1982). The immunopathology of the schistosomal granulomas in biopsies from human patients has not yet been adequately studied, however. The purpose of this study was to examine the inflammatory and cellular immune responses elicited by *S. mansoni* in the human colon by means of immunohistochemistry. It was performed on colorectal biopsies since these are easy to obtain and are representative of the lesion.

Materials and methods

Biopsies were obtained from 14 patients with established active schistosomiasis and from 12 controls. The schistosomiasis group was composed of 8 male and 6 female patients with a mean age of 36 years (range 18–59 years). All 14 patients were diagnosed and followed in the gastroenterology unit of Mansoura University

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Hospital, Egypt. The diagnosis of schistosomiasis was established by means of stool and serological examinations and confirmed by the biopsies. The 12 control patients (6 male and 6 female, mean age 26 years, range 18–29 years) were examined and followed at the Department of Gastroenterology of the University Hospital of Leuven, Belgium because of abdominal pain and/or diarrhoea.

The material was composed of endoscopic ($n=24$) and surgical ($n=2$) colorectal specimens. The endoscopic biopsies were taken with forceps during rigid rectosigmoidoscopy and contained mucosa and submucosa. They were obtained from 13 patients with schistosomiasis and from 11 control patients. In addition one operative specimen from a patient with schistosomiasis was compared with a surgical specimen from a patient operated for diverticulosis coli. After obtaining the specimens, the biopsies were immediately fixed for 2 h in B5, a fixative composed of mercuric chloride and formaldehyde, and afterwards kept in methanol until further processing. After paraffin embedding, semi-serial sections were cut and used for routine H & E staining or immunohistochemistry. The following monoclonal antibodies were used: MB2, LN1 (Biotest Seralo, Brussels, Belgium) defining B-lymphocytes (Hall et al. 1987); MT1 (Biotest Seralo) recognizing partially neuraminidase sensitive membrane antigens expressed on T-cells, histiocytes, monocytes, interdigitating reticulum cells, myeloid cells and erythrocyte precursors (West et al. 1986; Poppema et al. 1987); anti HLA-DR (Becton Dickinson, Mechelen, Belgium) reacting with the common framework of human major histocompatibility (MHC) class II antigens and Tal 1B5 (Dr. WF Bodmer, Imperial Cancer Research Fund, London, UK) directed against the MHC class II alpha chain; Mac 387 (Dakopatts, Copenhagen, Denmark) directed against macrophages and infiltrating histiocytes (Brandtzaeg et al. 1987, 1988; Flavell et al. 1987) and KP1 (Nuffield Department of Pathology, Oxford, UK) which has been shown to label the cytoplasm of most cells of mononuclear phagocytic origin and also occasional granulocytes as well as plasmacytoid T-cells (Pulford et al. 1989).

Results

Histopathology

Granulomas were present in the biopsies from 13 patients with schistosomiasis. In 5 of them, free ova were also present. In 1 patient free ova alone were found in mucosa and submucosa (Table 1). Most of the schistosomal granulomas were composed of centrally located ova or remnants of ova, surrounded by eosinophils, lymphocytes, multinucleated giant cells (in 4 cases) and/or epithelioid cells. Eosinophils outnumbered the lymphocytes in small lesions. The lymphocytes were present in small numbers and diffusely distributed within the granuloma. In 3 cases lymphocytes were seen forming a rim at the periphery of some granulomas.

In 2 of the 13 cases granulomas were found in close association with colonic lymphoglandular complexes, although not within the follicle area. The free living eggs (containing a nucleated miracidium) in the lamina propria were usually surrounded by a cellular infiltrate composed of lymphocytes and larger mononuclear cells. In 2 cases a very dense cellular infiltrate surrounding a high number of eggs resulted in the formation of "schistosomal polyps".

In the submucosa the cellular infiltrate surrounding the ova was usually more limited. Dead ova were not associated with a cellular infiltrate. Hyperplasia of the lymphoglandular complexes or an increase in solitary lymphoid aggregates were never observed.

Table 1. Findings observed in 14 patients with established schistosomiasis

Granulomas	13/14
with ova	$n=9$
without ova	$n=2$
both with and without ova	$n=2$
Granulomas and free ova	5/14
Free ova alone	1/14
Adult worms	1/14

Immunopathology

Biopsies from all patients with schistosomiasis and all controls were stained and examined. In the biopsies from patients with schistosomiasis MHC class II positive (HLA-DR and Tal 1B5 positive) cells are present in the centre and at the periphery of the granulomas containing ova or remnants of ova. These cells are absent from these granulomas in which no remnants of ova can be identified (Fig. 1). When present, centrally located multinucleated giant cells are negative for HLA-DR and Tal 1B5 (Fig. 2, Table 2).

KP1 positive Mac 387 negative cells are both found in the centre of the granulomas, mostly in association with the ova, while KP1 positive, Mac 387 positive cells are more frequent in an intermediate area and at the periphery of the granuloma. Small numbers of MT1 positive lymphocytes and of MB2 positive, LN1 positive lymphocytes are diffusely present within all the granulomas. The MB2 positive, LN1 positive lymphocytes show a tendency towards the formation of a small peripheral rim in some of the granulomas observed in 3 cases. In the 2 cases in which the granulomas were associated with colonic lymphoglandular complexes, the surrounding cells were MT1 positive cells.

The living eggs, lying free in the lamina propria mucosae are surrounded by small clusters of HLA-DR, Tal 1B5, Mac 387, and KP1 positive cells and MT1 positive lymphocytes with an excess of non-lymphocytic mononuclear cells (Fig. 3). Occasionally only single cells of each phenotype are found in the vicinity of the eggs. In the submucosa similar findings can be observed, but more often we find free ova without apparent associated cellular infiltrate. In schistosomal polyps caused by the presence of multiple eggs numerous KP1 and Mac 387 positive cells are found. They are seen as a diffuse infiltrate of the lesions as well as in clusters around the ova. In the latter position they express also HLA-DR and Tal 1B5 (Fig. 4). Dead eggs are usually not surrounded by any cellular infiltrate recognizable with the antibodies used in this study. The surface epithelial cells and the epithelial cells lining the crypts of the colonic mucosa are negative for HLA-DR and Tal 1B5, but in all biopsies a band like infiltrate composed of irregular HLA-DR positive cells can be observed in a subepithelial position in the lamina propria. These HLA-DR positive cells are negative for Mac 387 (Table 3).

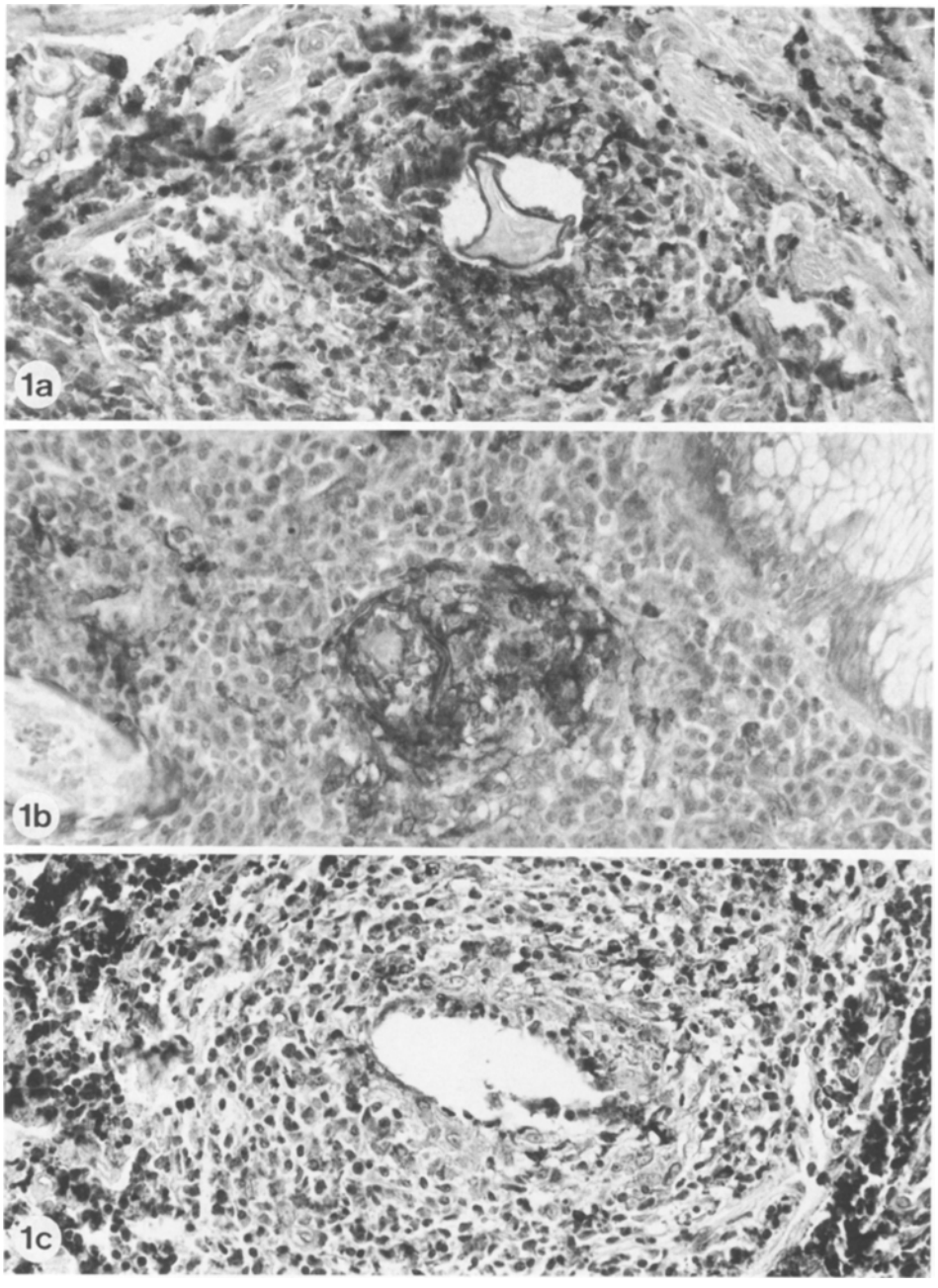

Fig. 1a–c. Human colonic biopsy with schistosoma granulomas: immunohistochemistry for MHC class II antigens. Positively staining cells can be observed in the centre of a granuloma surrounding remnants of an egg (**a**), or even in granulomas without elements of an egg (**b**) recognisable on the section. MHC class II positive cells (**c**) are absent from other granulomas (where an empty space points towards the earlier presence of material). ×125

In addition a variable number of HLA-DR and Mac 387 positive cells and HLA-DR positive Mac 387 negative cells are found in the remaining part of the lamina propria. The HLA-DR and Mac 387 positive cells show a focal or sometimes patchy distribution within one biopsy and within several biopsies coming from the same patient and the same area. They can be observed as single cells or in small groups. KP1 positive cells show a similar distribution and are mostly the same cells. Mac 387 positive cells show a more focal distribution. Some areas or even whole biopsies can be negative for Mac 387 positive cells and still contain KP1 positive cells while other biopsies are heavily infiltrated by Mac 387 positive cells. They are sometimes seen between epithelial cells lining the crypts. The expression of Mac 387 by the cells is variable. Positively stained cells located in the granulomas often show a reduced staining whereas Mac 387 positive cells infiltrating the lamina propria are usually smaller and more intensely stained. They seem to reach the lamina propria especially in areas where lymphoglandular complexes are located (Fig. 5). In addition we find numerous Mac 387 positive cells within the lumen of the capillaries, lining the endothelial wall. This phenomenon is most apparent in areas containing ova. No accumulation of HLA-DR and Mac 387 positive cells is observed around submucosal or myenteric ganglia.

In the normal colon, surface and crypt epithelial cells are both negative for HLA-DR; HLA-DR positive histiocytic cells are also present in a zonal distribution

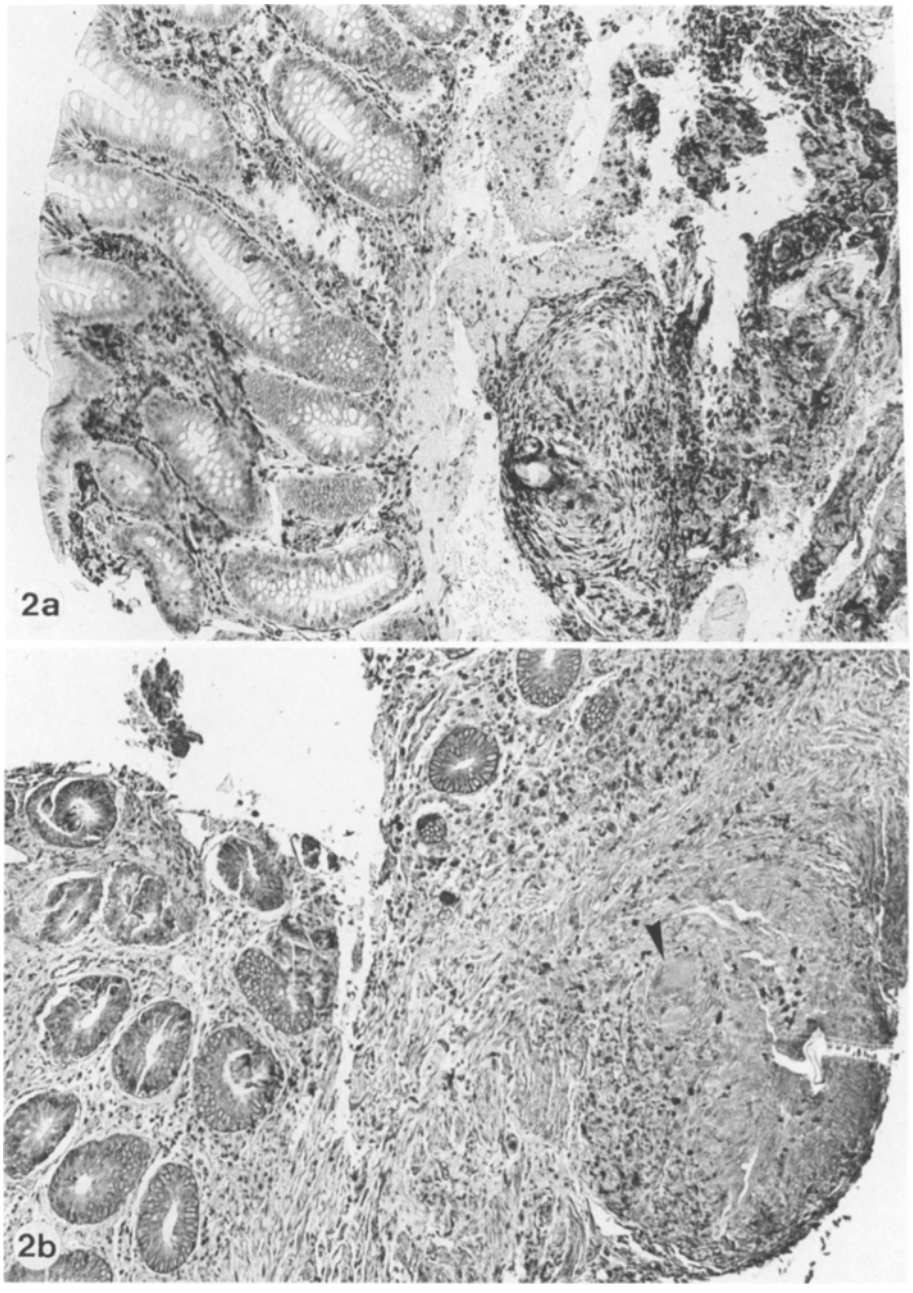

Fig. 2. Human schistosomiasis: immunohistochemistry for MHC Class II antigens showing positive cells in a granuloma (**a**) and in the overlying mucosa, and loss of positive cells as well as negative giant cells (*arrowhead*) in another granuloma (**b**). ×40

just underneath the enterocytes. In addition a few scattered positive cells can be found in small clusters in the deeper layers of the lamina propria. T-lymphocytes are intermingled with HLA-DR positive cells and KP1 positive cells are regularly present, whereas Mac 387 positive cells are only rarely observed.

Discussion

Our data, from routinely stained sections and immunohistochemistry, confirm the presence of different types of lesions within the same biopsy from patients with schistosomiasis. A similar observation has been made in experimental infections. The variability of the lesions depends on the degree of destruction of the egg shell

Table 2. Tissue MHC class II expression

	Human colonic schistosomiasis	Controls
Surface epithelial cells	−	−
Crypt epithelial cells	−	−
Lamina propria cells mononuclear cells in	+	+
– subepithelial bandlike position		
– deeper layers	Focally ++ Clusters ++	Focally +
Granulomas		
with ova	++	
without ova	+/−	
Free ova	Clusters ++	

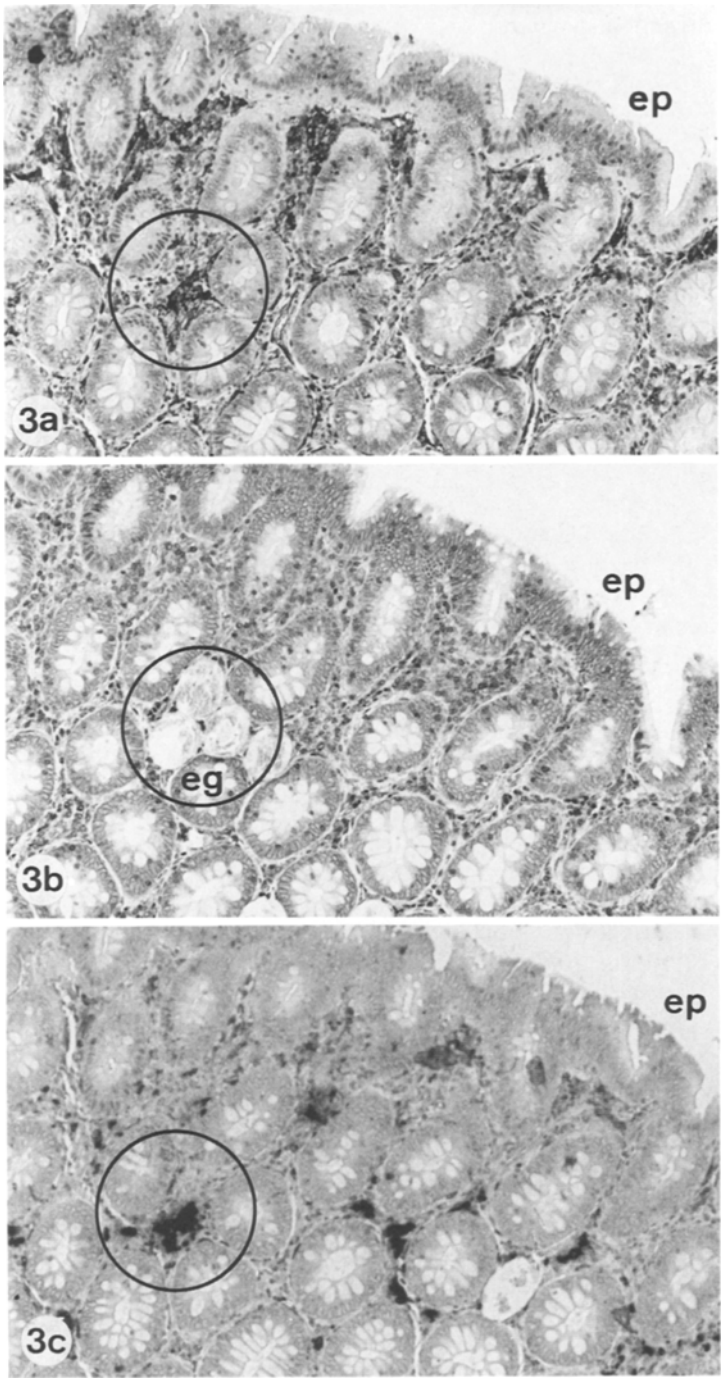

Fig. 3. Human schistosomiasis. Semiserial sections from a biopsy stained immunohistochemically for MHC class II (**a**), MT1 (**b**) and Mac 387 (**c**). × 50. MHC class II positive cells can be observed in a subepithelial position (*ep*, epithelium) as well as in the area of the eggs (*eg*). Whereas the Mac 387 positive cells are mainly concentrated around the eggs, MT1 positive cells can be observed in the vicinity of the eggs but are also diffusely present in the lamina propria

(which might be related to the presence of eosinophils) and the subsequent variability in amount and location of antigens (Deelder et al. 1985; El-Dosoky et al. 1984). No accumulation of inflammatory cells is observed around dead eggs. This can probably be explained by the need for soluble substances produced by the eggs in order to elicit an inflammatory (immunological) reaction, although the absence of a foreign-body type reaction is strange.

The earliest lesions induced by schistosomiasis are probably those composed of a single, well-formed egg surrounded by a few cells. These early lesions can be observed in the submucosa as well as in the lamina propria. Immunopathology shows that the single, well-formed living ova are surrounded by MT1 positive T-lymphocytes, and by larger cells expressing MHC class II antigens, as well as KP1 and Mac 387. These are accessory cells with antigen-presentation properties. This finding is in agreement with the previously described macrophage heterogeneity of the colonic mucosa (Allison et al. 1988). It is also in agreement with experimental data indicating that the granuloma formation is initiated by T-cells and T-cell recognition of egg antigens (Doughty and Phillips 1982; Chensue et al. 1980) and that monocytes/macrophages function as antigen-presenting cells (Schook et al. 1983). In addition the expression of Mac 387 is an argument in favour of the myelomonocytic origin of these cells. Such an origin is in agreement with other experiments showing that peripheral blood mononuclear cells from patients with *S. mansoni* infection can be stimulated by living schistosomula (Vieira et al. 1987). These findings also explain the patchy distribution of the Mac 387 positive cells accumulating in the vessels along the endothelial wall in inflamed areas. The composition of the well-formed granulomas in the human tissue is comparable with the description of experimentally induced granulomas (Kunkel et al. 1984; Abdul-Aal and Attallah 1987; Attalah et al. 1987). Each granuloma is composed of several cell types including T-lymphocytes, B-lymphocytes, eosinophils and macrophages or monocytes. In experimentally induced granulomas eosinophils outnumber B-lymphocytes, T-lymphocytes outnumber B-lymphocytes and monocytes outnumber lymphocytes (Stadecker and Wright 1984). In our cases numerous eosinophils were only observed in small lesions whereas monocytes outnumbered lymphocytes in all lesions. The distribution of the MHC class II positive accessory cells within the granulomas is variable. In most of the granulomas these cells are present in the centre of the lesions, surrounding the ova or remnants of ova. Yet some experimental studies report the presence of Ia positive cells (Ia is the murine analogue of HLA-DR or MHC class II antigens) only in the peripheral areas of the granulomas (Stadecker and Wright 1984). These authors suggest that the expression of the MHC class II antigens occurs only during a limited period of time and that young macrophages may later convert into MHC class II negative cells. Such an evolution might explain why the multinucleated giant cells in the centre of the granulomas in our cases do not express any of the markers used. They could act as foreign-body giant cells and this is in agreement with the fact that some of these cells are seen engulfing the egg shell. This also explains the different distribution of KP1 positive, Mac 387 positive and KP1 positive Mac 387 negative cells. The latter are probably older macrophages.

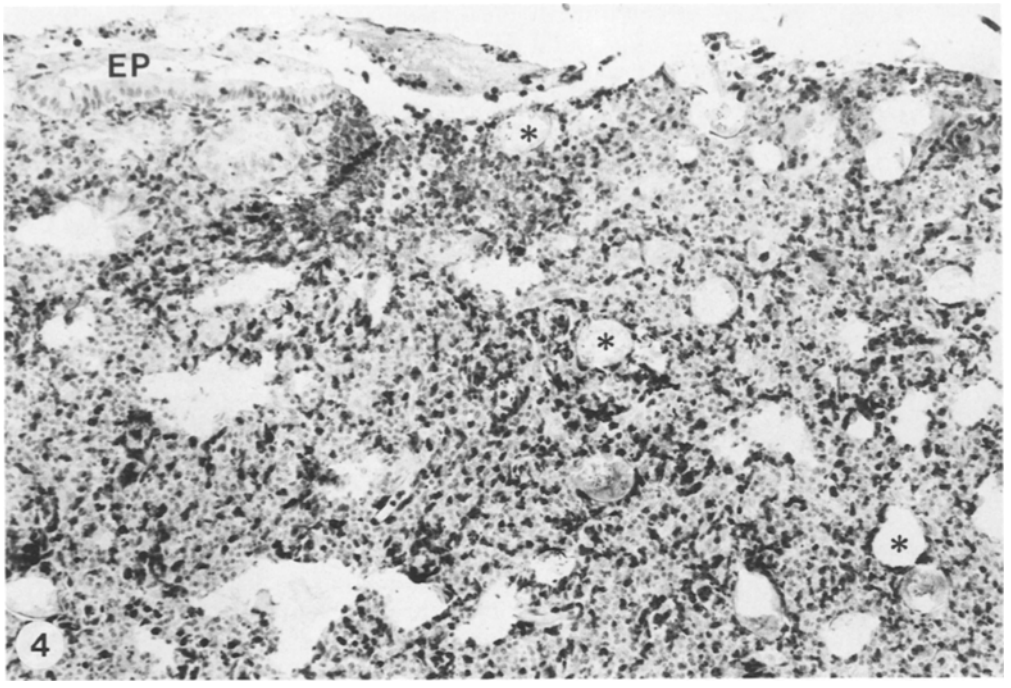

Fig. 4. Human schistosomiasis: endoscopic biopsy from a schistosomal polyp stained for Mac 387. Numerous eggs can be observed (*asterisk*) and the tissue underneath the epithelium (*ep*) is diffusely infiltrated by Mac 387 positive cells. × 50

This would mean that in young granulomas antigen-presenting cells are present in the centre of the lesion and that these cells are later replaced by or convert into phagocytic cells. The granulomas in schistosomiasis therefore have characteristics of hypersensitivity (immune) granulomas as well as of foreign-body reactions. They are the expression of a strong cell-mediated immune response (MacDermott 1986) that ultimately kills the ova and clears the antigens and of a phagocytic clearance of the remnants.

The negative staining of the multinucleated giant cells in the schistosomal granulomas is in contrast to what is described in tuberculosis and Crohn's disease. In these conditions the multinucleated giant cells may show or retain a positive staining for MHC class II antigens (Geboes et al. 1986). This difference may be authentic but on the other hand it may be due to technical factors such as the use of fixed or unfixed tissue, although in tuberculosis the staining was performed on fixed tissues (Gaffney et al. 1987). There are some other striking differences between schistosomiasis, tuberculosis and Crohn's disease. The granulomas in tuberculosis and Crohn's disease are often related to lymphoglandular complexes in the colon and the inflammation is characteristically associated with transmural lymphoid hyperplasia (Gaffney et al. 1987). In tuberculosis the increase in gut-associated lymphoid tissue has been explained as an increase in antigen-presenting tissue, elicited by antigens which are mainly coming from the lumen (Gaffney et al. 1987). In schistosomiasis the antigen or antigens are reaching the gut from the circulation or are released in the tissue itself and are not directly presented to the gut mucosa associated lymphoepithelial tissue. This may also explain why no expression of MHC class II antigens is observed on the surface epithelial cells of the colon in schistosomiasis in contrast to what is seen in Crohn's disease and ulcerative colitis (McDonald and Jewell 1987).

In summary, our data confirm several of the experimental descriptions of the immunopathology of schistosomal granulomas. Although the granulomatous reac-

Table 3

	MHC class II + cells	KP1$^+$ Mac 387$^-$	KP1$^+$ Mac 387$^+$	MT$^+$	MB2$^+$ LN$^+$
Granuloma					
with ova	Present – in centre and periphery	Present – in centre	Present – in intermediate area – in periphery	Diffuse	In periphery or diffuse
without ova	Absent	– in centre	– in periphery	Diffuse	Diffuse
free living ova	Present	–	Present	Present	Absent/present
dead ova	Absent	–	Absent	Absent	Absent

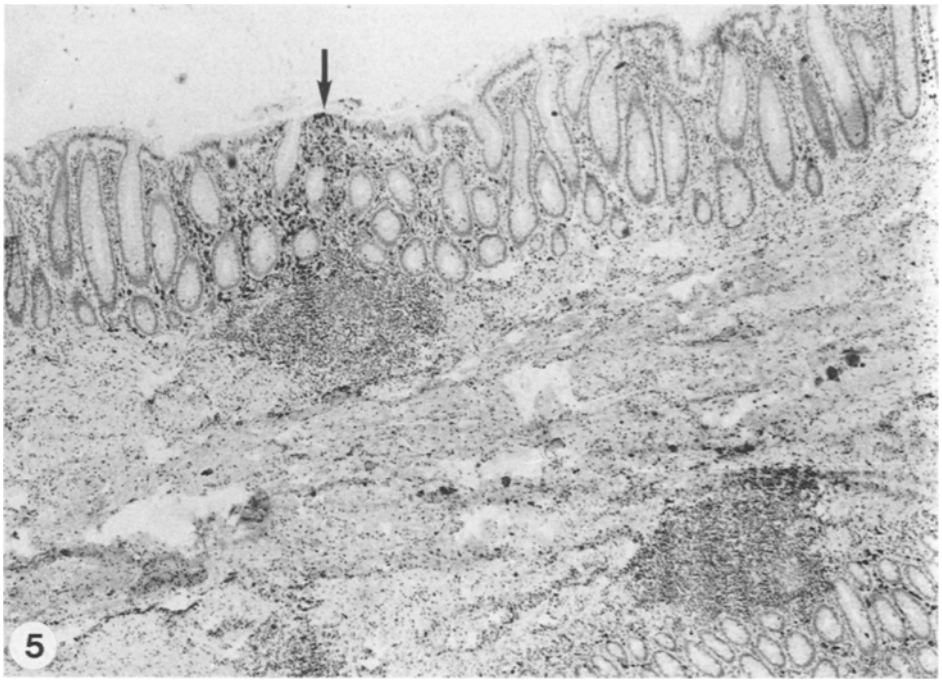

Fig. 5. Human colonic biopsy from a patient with schistosomiasis. Eggs and granulomas were present in semi-serial sections from this specimen. Immunohistochemistry for Mac 387. Positively staining cells infiltrating the lamina propria of the mucosa can be seen (*arrows*) near the lymphoglandular complex. ×25

tion of schistosomiasis is largely similar to what is seen in tuberculosis and Crohn's disease, there are some differences. These are mainly a less well pronounced reaction of the lymphoid aggregates associated with the epithelium or scattered in the mucosa and submucosa; the presence of foreign-body type cells in some granulomas; the absence of MHC class II expression by surface epithelial cells, and the absence of an important mucosal or submucosal cellular reaction.

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