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MILKY SPOTS REACTION TO SCHISTOSOMAL MANSONI INFECTION

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Milky spots (MS), considered by the authors as a Coelomatic Lympho-myelopoietic Organ (CLMO), present a strong reactivity during experimental schistosomal mansoni infection, characterized by an increase of lymphocytes, macrophages, plasmocytes, mast cells, neutrophils and expression of eosinophil metaplasia. Intraperitoneal injection of purified Schistosoma mansoni *(Sm) eggs provoked a rise in the number and size of MS, which developed the sessile marginal and pedunculated types. The authors conclude that egg antigens are, at least partially, responsible for MS reactivity during Sm infection.*

Key words: milky spot – *Schistosoma mansoni* – eosinophil – peritoneum

In previous work we have shown that during murine schistosomal mansoni infection there is a strong milky spot reactivity characterized by polypoid transformation and local plasmocytogenesis (Weinberg et al., 1992). Milky spots (MS) were first described by Recklinghausen, in 1863, as whitish spots in the omentum and in the serous layers of the thoracic cavity of young rabbits. They have also been observed in human omentum (Seifert, 1921; Kampmeier, 1928; Mixter, 1941) and in various experimental animals: rat, mouse, ground squirrel (*Citellus tridicemlineatus*), gray squirrel (*Sciurus carolinensis*), rabbits, cat, dog, bat (*Eptesicus fuscus*), mole (*Scalopus aquaticus*) (Mixter, 1941), guinea pig, cattle, chicken, frog (Hamazaki, 1925), pig (Trebichasvski et al., 1981), sheep, goat (Brand & Schnorr, 1983), *Nectomys squamipes* and *Calomys callosus* (personal observation). Few reports in the literature have stressed their structural and functional characteristics and the sequential changes in them after stimulation by organic or inorganic material (Kanazawa et al., 1979; Beelen et al., 1980; Mandache et al., 1987), and only rarely were they studied in the context of parasitic diseases (Weinberg et al., 1992).

In this paper we report the sequential reactivity patterns of MS during experimental schistosomal infection and after intraperitoneal (i.p.) *Schistosoma mansoni* (Sm) egg injection.

One hundred and two Swiss Webster mice of both sexes were studied at 5-day intervals from 15 to 60 days, and 10-day intervals from 60 to 120 days, and at 160 days after infection. The animals were infected when they were five days old by percutaneous exposure to 70 cercariae of the Belo Horizonte strain of Sm. Six animals were sacrificed each day, together with the same number of matched controls. Samples were taken from mesentery and omentum, fixed in Millonig formalin, and embedded in paraffin. Section were stained with hematoxylin and eosin, Lennert's Giemsa, Gomori's reticulum and Picrosirius (plus polarization microscopy).

To test the egg capability to induce MS reactivity, Sm eggs were purified from 8 weeks infected mice gut, based on serial filtration through sieves of 300, 180, 150, 106 and 45 meshes, followed by sedimentation and centrifugation. Forty eight Swiss mice, 50 days old, were injected i.p. with 2000 eggs/0.5 ml sterile isotonic saline solution/animal and sacrificed on 4, 8, 12, 24, 36, 48 hr and 5 and 15 days after injection. The same number of matched controls received only 0.5 ml of sterile saline i.p./animal. For MS tridimentional observation, the mesenteries were distended by an intraintestinal spiral copper wire and fixed in Millonig formalin. After 24 hr, they were

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stained with Giemsa-Glycine for 5 min, dehydrated in alcoholic series, clarified in xylene and mounted in Canada balsam.

During the Sm infection, MS reactivity began to appear after 20 days, showing increase of mononuclear cells, presence of immunoblastic cells (plasmoblast?) and few eosinophils. Eosinophil metaplasia was identified from 40 or 45 days after infection, and remained until the end of the experiment (Fig. 1.A-C). The MS were covered by hypertrophic mesothelial cells and presented, in different intensities (depending on the time of infection), lymphocytes, plasmocytes, mast cells, monocytic-macrophage cells, mature and immature eosinophils, neutrophils, immunoblastic and lymphoblastoid cells, and, occasionally, megakaryocytes. From 70-80 days after infection, coinciding with the increase in the number of mono-lymphoblastoid cells in the peritoneal cavity, the MS exhibited an increase in the number of lymphocytic cells, forming perivascular aggregates or sheaths (Fig. 1.E,F). Presence of plasmocytes was frequent and intense, sometimes forming Russell bodies, surrounding the periphery of lymphocytic infiltration with immunoblastic cells (Fig. 1.D). The MS were rich in blood vessels, stromal cells, and presented a framework of reticular fibers, with few collagen fibers.

Four hours after i.p. Sm egg injection, MS increased in number and size along the edges of fatty sheath that surround the mesenteric vessels (Sessile marginal type) (Fig. 2.A,B). From 12 hr on, with more intensity and frequency at 15 days after egg injection, the MS presented a papilary pattern, characterized by long stalks (pedunculated type). Sometimes, they showed mast cells and could reach a size around 430 x 280 μm of diameter (Fig. 2.C-G).

The present study on the mouse omentum and mesentery clearly showed that MS can be activated during different times of Sm infection and by i.p. injection of purified eggs. The various reactivity patterns described indicated the versatility of these small coelomic structures, which can behave like a lympho-monocytic or a myelocytic organ, with eosinopoeitic activity. This activity seems to be stimulated by factors present diffusely in the peritoneal cavity and in associated tissues, including liver (Borojevic et al., 1981; Lenzi et al., 1987), and is dependent, at least partially, upon stimulated macrophages (El-Cheikh & Borojevic, 1990). Intraperitoneal egg injection, although induced peritoneal eosinophilia, stimulated the appearance of sessile marginal and pedunculated MS types, composed predominantly by lymphocytes with or without mast cells. These two types of MS were described by Kanazawa (1979) after subcutaneous or intravenous injection of asbestos in CBA/lac female mice, and they were also observed in Sm infected mice (Weinberg et al., 1992).

The fact that histyocytes can be generated *in vitro*, from omental fragments (Aronson & Shahar, 1965); some proportion of the free peritoneal macrophages originate from MS macrophages (De Barker et al., 1985), and erythropoiesis (Kampmeier, 1928), and eosinopoiesis (data shown) can occur within them, indicate that MS present pluripotent cells capable of differentiating into a wide variety of cell types. The occasional presence of megakarocytes and the more frequent presence of mast cells suggest that MS provide also a favorable environment to other cell population (Fig. 2.D-E). Yong et al. (1975, 1977) found in rats the greatest concentration of mast cells per unit area in the omental milky spots.

The MS have a stroma composed by sparse fibers of collagens type I and III, delicate mesh of reticulars fibers, small amount of acidic proteoglycans. There is no elastic fibers and most of the extracellular matrix components predominates in the submesothelial region. These aspects correspond to an organ, which performs expansive-contractile activities, as is usually observed in lympho-myelopoietic organs. Beelen et al. (1980) has observed, in the MS stroma, the presence of reticulum cells, which have long and slender cell processes connected, sometimes, with neighboring cells to form a cellular network or adhered to adjoining fine collagenous fibers with semi-desmosome-like structures.

Although there was a large number of plasmocytes, which stained intensely with anti-IgG or anti-IgM antibodies (personal observation), germinal centers were never made up. Probably, the B-cell maturation into secreting plasmocytes in schistosome-infected mice MS is germinal center independent. Only Mandache et al. (1987), in the literature, observed lymphatic follicle-like structures with germinal centers in stimulated omental MS. Portis, in 1924, was one of the first to outline the role

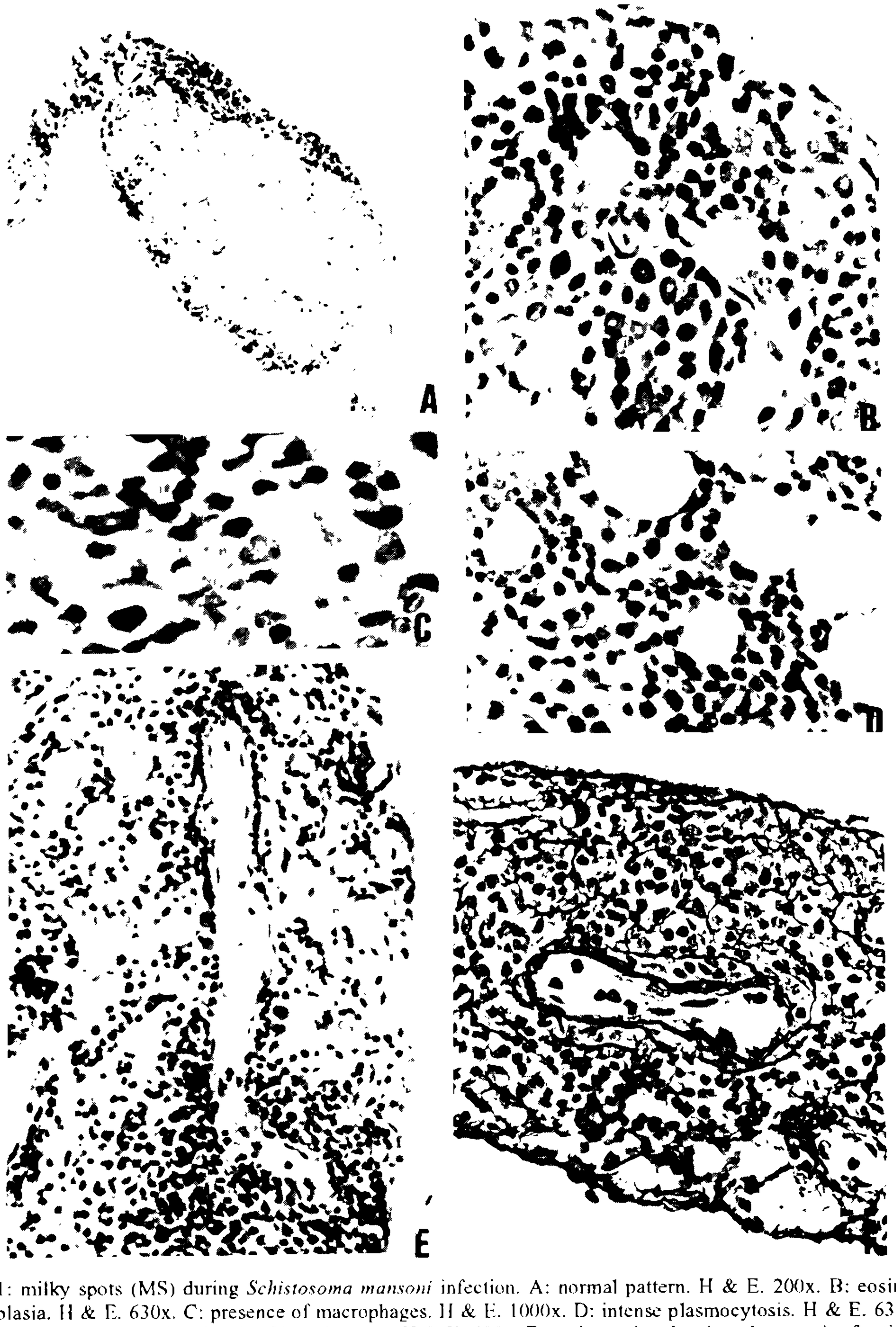

Fig. 1: milky spots (MS) during *Schistosoma mansoni* infection. A: normal pattern. H & E. 200x. B: eosinophil metaplasia. H & E. 630x. C: presence of macrophages. H & E. 1000x. D: intense plasmocytosis. H & E. 630x. E: aggregate and perivascular sheath of lymphocytes. H & E. 400x. F: perivascular sheath and network of reticulum fibers. Gomori's reticulum. 630x.

of the omentum in antibody production. This role was supported later on by the works of Oakley (1954), Roberts (1955), Walker et al. (1960/61), Walker & Rogers (1961) and Walker (1963).

The MS were called by different names: "macrophagal foci" (Mixter, 1941), Omental Lymphoid Organ (OLO) (Dux et al., 1977), Dense Lymphatic Areas (DLA) (Trebichavski et al., 1981), "Lymphoreticular organ" (Carr,

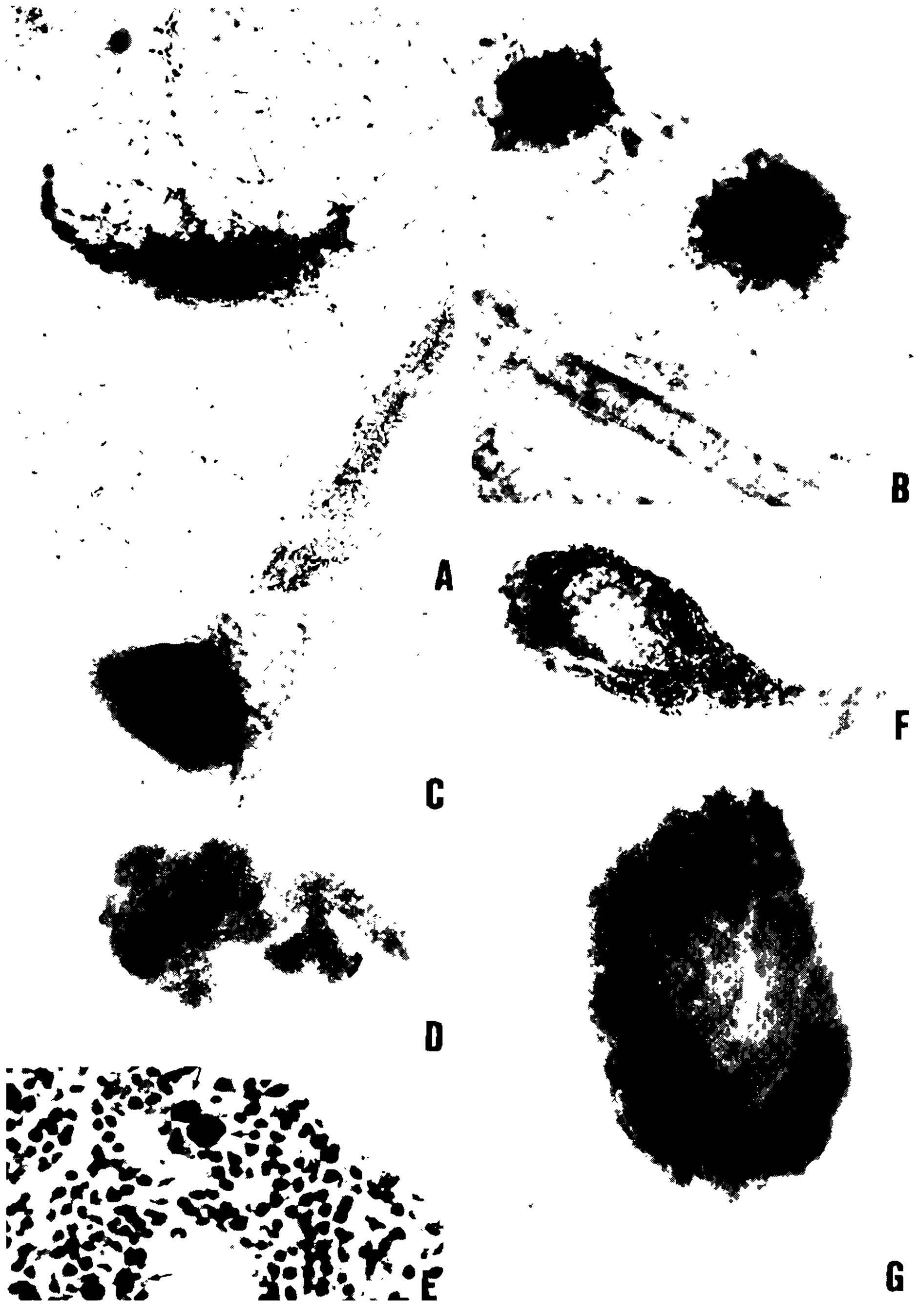

Fig. 2: milky spots induced by intraperitoneal injection of purified *Schistosoma mansoni* eggs. A: normal pattern, 200x. B: two milky spots of sessile marginal type (4 hr after injection) 200x. C: initial pedunculated type (12 hr after injection) 200x. D: pedunculated type with mast cells (48 hr after injection) 200x. E: mast cells in milky spot (arrows) Lennert's Giemsa. 630x. F: pedunculated type (36 hr after injection) 200x. G: pedunculated type showing the capillary network (15 days after injection) 200x. (Giemsa-Glycine, except # E).

1967), Immune Factory of the abdomen (Fisher & Malchow, 1969) and were considered as a highly reactive structures (Beelen et al., 1980) or as subsidiary foci of secondary lymphoid organ (Mandache et al., 1987; Dux et al., 1986). We propose to call them Coelomic Lympho-myelopoietic Organ (CLMO). Due to their rich vascularization they can react to local (i.p.), abdominal or systemic exposure to any foreign substance either inert or with antigenic prop-

erties (Kanazawa et al., 1979; Mandache et al., 1987). It has been shown that MS can be the sites of plasma cell development and antibody production following intraperitoneal administration of antigen or Sm infection (Weinberg et al., 1992). They are considered to be involved also in both production of macrophages and lymphocytes into the peritoneal and pleural cavities (Aronson & Shahar, 1965), and finally, in homing selectively and, accumulating antigen-specific B cells (Dux et al., 1986). Lang (1962) suggested that they compose a sewer system to drain off unwanted materials from the serosal cavities. The twisted course of their capillary network is particularly favourable to the trapping of foreign bodies in the blood. It has been demonstrated that MS take up materials injected either intravenously or subcutaneously, and that proliferative changes subsequently occur in them (Cappell, 1930).

Experimental schistosomiasis appears to be an excelent model to study these structures because mesenteric and portal venous system are permanently exposed to aggressions by worms and their products, and they are continously the sites of inflammatory reactions to the host's response to parasitic antigens (Weinberg et al., 1992). In this paper we showed that the egg antigens are, at least partially, responsable by MS reactivity during Sm infection.

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