DEVELOPMENT OF THE MEGAKARYOCYTE IN THE LUNG AND LIVER OF THE DEFINITE HOST MOUSE DURING SCHISTOSOMIASIS

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ABSTRACT

In livers of mice with *Schistosoma mansoni* and *S. margrebowiei* infections, megakaryocytes were found in the hepatic sinusoids, around granulomas and infiltration of the cells after 35, 42 and 49 days post-infections (p.i.). Some of these cells were found in *S. mansoni*, few in *S. margrebowiei* and hydrocortisone treated space of the lung infected with *S. mansoni* after 10 days p.i.

INTRODUCTION

The name megakaryocyte given to the large cell with a lobate nucleus found in the bone marrow and in the blood forming organs of embryos. In the bone marrow of normal mice megakaryocytes are scattered at random, while in the spleen they seem to be concentrated in the subscapular and peritrabecular red pulp, although individual cells are present in other portions of the red pulp. Occasionally they are found in the medullary cords of normal lymph nodes (Potter and Ward, 1940). Morphologically recognizable megakaryocyte can be identified with light microscopy by one of two characteristics: their large size or their characteristics cytoplasmic granulation and staining properties. The granulation is acquired with maturity, so pathologically small megakaryocytes may not be recognized when immature unless examined by electron microscopy. The most immature cell that is recognizable by light microscopy has clear, homogenous basophilic cytoplasm. The nucleus which appears to be single but often is irregularly shaped at this early stage of development is clearly lobulated when viewed by electron microscopy of sections of megakaryocyte (Paulus, 1970; MacPherson, 1971). In some pathological conditions there is evidence of a series within a single periportal lesion in a liver section of the adult mouse the large megakaryocyte has a polymorphous nucleus with many lobulation all connected to form one irregular mass which, in countered Feulgen preparations, shows varying numbers of plasmasome nucleoli (Ebbe, 1976). The different maturation stages of megakaryocyte development from the beginning of differentiation to the shedding of platelet at maturity (Levine, 1980). Mitotic figures were regularly found in the stage I megakaryocytes, but generally looked like prophase; anaphase to telophase forms (Alberts *et al.*, 1989). The function of this cell is not well known. It is assumed by many authors that the platelet of circulating blood originate through budding of the cytoplasm of megakaryocytes (Wright, 1910). The sole function of megakaryocytes appears to be to produce blood platelets. They are formed during the gestation within the megakaryocytic cytoplasm (Ebbe, 1976). Experiment have shown that between 42 and 47 days after infection with *S. mansoni* infected mice had showed from 43-52% fewer platelets than uninfected matched controls. Both the adult worms and egg stages of the infection appeared to contribute to the stages of the thrombocytopenia (Ngiaza and Doenhoff, 1987).

The cell populations of the granulomas differ according to the immune status of the host and the duration of infection. They consist mainly of eosinophils, lymphocytes, large mononuclear cells including macrophages, epithelioid cells, fibroblasts and a few neutrophils (Epstein *et al.*, 1979; Moore *et al.*, 1977). Other cells, such as basophilic mononuclear cells, plasma cells, multinucleated giant cells and mast cells were rarely seen (Moore *et al.*, 1977). Eosinophils constitute up to 50% of the granuloma cells and are responsible for destroying the retained schistosome eggs (Moore *et al.*, 1977; Olds and Mahmoud, 1980). A significant increase in eosinophil counts both in bone marrow and peripheral blood is an outstanding feature in schistosomiasis (Mahmoud, 1982). From above review it is clear that majority of the cell population are present in the schistosome infections but not any body has reported megakaryocytes in the lungs and liver of the definitive host mouse.

MATERIALS AND METHODS

Age-matched Bantim and Kingmann Tylers original (BKTO) female mice were infected with 200 cercariae of *Schistosoma mansoni* (Puerto Rican strain maintained in albino *Biomphalaria glabrata* snails and random-bred To mice by the method of Taylor *et al.*, 1969) and *Schistosoma margrebowiei* (originally obtained from Lochinar National Park, Zambia) and maintained in *Bulinus natalensis* intermediate host snails (the original stock was obtained from the Experimental Taxonomy Unit, British Museum of Natural History, London). In the first experiment mice were killed on day 10. In the second experiment mice were killed on day 35 and 49. In third experiment these were killed on day 42 p.i.. In this experiment from 24 animals, half of the animals were immunosuppressed, the others were normal infected. Immunosuppression was achieved with hydrocortisone acetate (BDH) (5mg/0.1 ml/mouse). All mice of this experiment were killed when one of the hydrocortisone treated animal died. Tissues include lungs and liver were fixed in Heidenhain's Susa fixative, washed, dehydrated in ethanol, infiltrated and embedded in historesin (Soomro, 1996). Randomly and longitudinal 4 μm thick sections were cut with glass knives on LKB Historange microtome, stained in 1% toluidine blue in 1% borax and Ehrlich's haematoxylin and eosin methods for general histology and analyzed on Ernst Leitz Wetzlar (Model No. 786554) light microscope using oil immersion.

RESULTS

Megakaryocytes were appeared in the lungs and liver of mice during acute *Schistosoma mansoni* and *S. margrebowiei* infections. The distribution of these cells in the organs of mice infected with *S. mansoni* and *S. margrebowiei* and hydrocortisone treated are summarized in Table 1.

From Table 1 it was clear that a single megakaryocyte was observed in the blood space of the lung with the presence of the *S. mansoni*. Schistosomulum in the capillary was attached to the alveoli and cellular reaction around some of the blood vessels after 10 days p.i. The diameter of the cells was 25.70 μm. However, the length of the cell and nucleus was 30.46 and 17.13 μm, respectively.

Variable number, size (small, medium or large) and shape (spherical, irregular or oval) of the megakaryocytes were apparently randomly distributed in the histological material with the presence of adult worms and granulomas with eggs in both parasite infections. These cells were located mainly found in the granulomas with single egg (Fig. 1), hepatic sinusoids (Fig. 2, 4, 5, 6) and infiltration of cells around the blood vessels in the liver of mice (Fig. 3). Megakaryocytes were characterized by the compact lobulated nucleus, homogenous cytoplasm and cell membrane stained well. The nuclear membrane (nuclear envelope) appear as a dark blue-purple line due to deposit of granular chromatin scattered along the inner surface of the nucleus. In few section, cells were surrounded by lymphocytes and enlarged endothelial cells. The mean diameter of the cell in *S. mansoni* and *S. margrebowiei* was 30.46 μm and while 26.65 μm after 35, 42 and 49 days p.i. (Fig. 1-4). In hydrocortisone treated liver, the megakaryocytes appeared large in *S. mansoni* and whereas small cells in *S. margrebowiei* infections (Fig. 5-6).

DISCUSSION

In the present study megakaryocytes were observed in variable number, size and shape in the lungs and liver of adult female mice during *S. mansoni* and *S. margrebowiei* infections. These cells were defined as their large size of the nuclei and homogenous cytoplasm. Potter and Ward (1940) have reported the development of the megakaryocytes in the leukaemia more particularly in the spontaneous types. Megakaryocyte has been observed in greater or smaller numbers in infiltration areas in lungs, liver, kidney, bone marrow, spleen, lymph nodes, thymus, muscle and in the circulation. Additionally, the medical correlation was most intensively studied in human disorders in which megakaryocytic involvement is prominent and could be classified as myeloproliferative: preleukaemia, acute non lymphocytic leukaemia, chronic granulocytic leukaemia, polycythemia vera, and primary thrombocythemia (McClure *et al.*, 1966; Spaet *et al.*, 1969; Cowan and Haut, 1972; Berger *et al.*, 1973; Adams *et al.*, 1974). A preleukemic panmyelopathy is associated with the presence of abnormally small mature megakaryocytes that they have only one or two nuclei (Saarni and Linman, 1971; Brecher, 1974). The same type of megakaryocyte are produced in chronic granulocytic leukaemia (Lagerlof and Franzen, 1972; Lagerlof, 1972; Undritz and Nusselt-Bohaumilitzky, 1971) and their presence has been interpreted as indicating a stem cell disorder with which leukaemia or leukaemic transformation is frequently associated (Brecher, 1974).

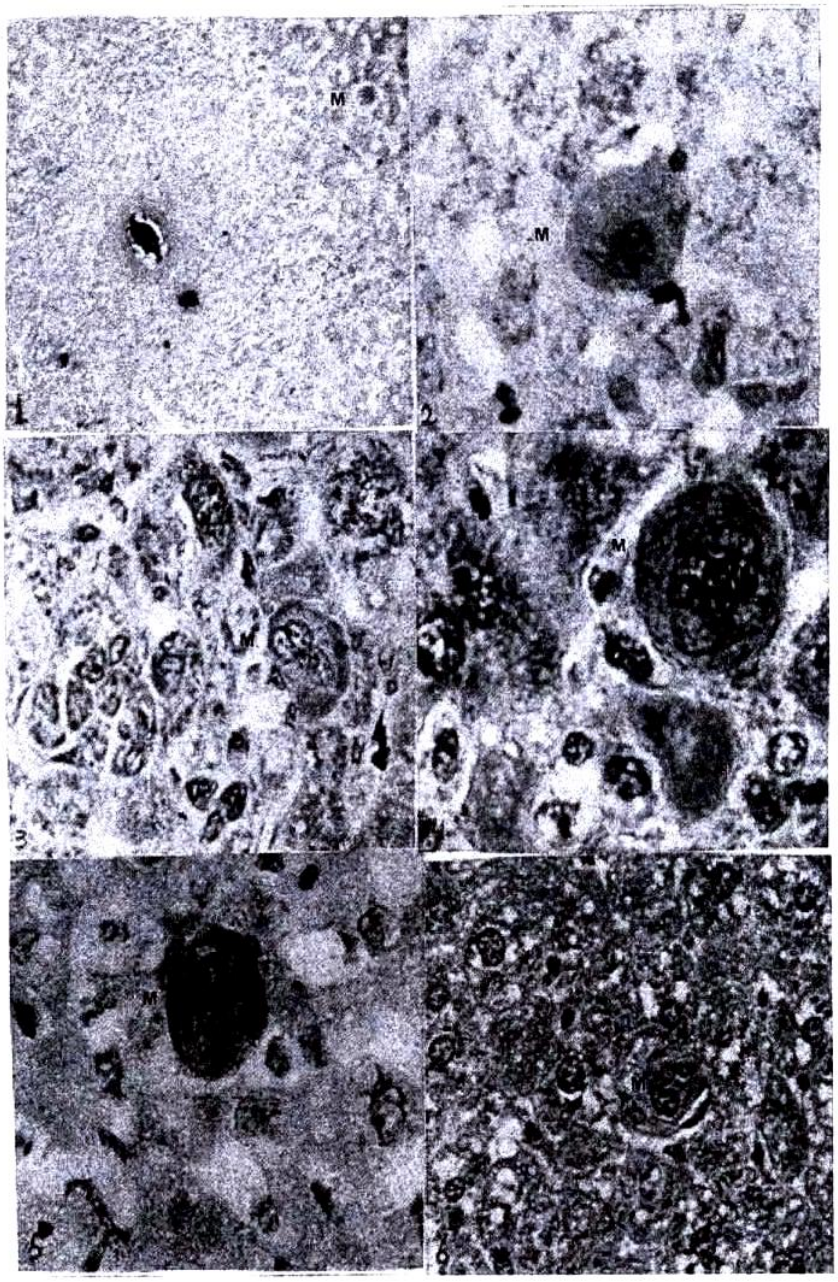

Fig. 1-6: Sections of liver showing megakaryocyte (M). 1) Megakaryocyte at the corner of the right side of the granuloma in *S. mansoni* (X 25), 2) Lymphocyte attached to the cell wall of megakaryocyte in *S. margrebowiei* (X 100), 3) Megakaryocyte close to the infiltration of cells in *S. mansoni* (X 100), 4) Round shape megakaryocyte in the hepatic sinusoid in *S. Margrebowiei* (X 250), 5) Oval shaped megakaryocyte in *S. Mansoni* infected and hydrocortisone treated liver (X 160) and 6) Irregular shape of megakaryocyte in *S. margrebowiei* infected and hydrocortisone treated liver (X 160).

Table 1: Determination of the megakaryocyte in the lungs and liver of mice.

dpi	Lungs		Liver			HC		
	Sm	Smg	Animal No	Sm	Smg	Animal No	Sm	Smg
10	1	-	2	-	-	-	-	-
35	-	-	2	4	4	-	-	-
42	-	-	1	1	-	7	3	-
			2	11	3	8	-	-
			3	-	1	9	1	1
			4	1	2	10	1	-
			5	6	-	11	-	-
			6	1	1	12	-	-
49			2	8	5	-	-	-

Abbrevations: Negative, dpi = Days post infection, HC = Hydrocortisone treated, Sm = *Schistosoma mansoni*, Smg = *Schistosoma margrebowiei*.

Megakaryocytes are short-lived and stages of degeneration are seen commonly. After the peripheral cytoplasm is shed as platelet, the megakaryocytes become shrunken and their nuclei fragment (Leeson and Leeson, 1976). The formation of the megakaryocytes have been found in the lungs of adult mouse; they do not originate there but are carried to the lungs from bone marrow (Kaufman *et al.*, 1965). deLeval and Paulus (1971) have discussed the mature megakaryocyte contains a lobulated nucleus rather than multiple separate nuclei, and the number of nuclear lobes bears no clear relationship to cell ploidy. Paulus (1971) has describe these megakaryocyte mature rapidly and enter the recognizable cellular compartment. Downey *et al.* (1930) presented evidence for derivation either directly from the reticular stellate cells of the liver sinusoids or from myeloblasts. Further the suggestive cases of this sort in their material but remained undecided about the phagocytic power of the megakaryocyte in humans.

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