Immunological System and *Schistosoma mansoni*: Co-evolutionary Immunobiology. What is the Eosinophil Role in Parasite-host Relationship?

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Schistosomes, ancestors and recent species, have pervaded many hosts and several phylogenetic levels of immunity, causing an evolutionary pressure to eosinophil lineage expression and response. Schistosoma mansoni *adult worms have capitalized on the apparent adversity of living within the mesenteric veins, using the dispersion of eggs and antigens to other tissues besides intestines to set a systemic activation of several haematopoietic lineages, specially eosinophils and monocytes/macrophages. This activation occurs in bone marrow, spleen, liver, lymph nodes, omental and mesenteric milky spots (activation of the old or primordial and recent or new lymphomyeloid tissue), increasing and making easy the migration of eosinophils, monocytes and other cells to the intestinal periovular granulomas. The exudative perigranulomatous stage of the periovular reaction, which present hystolitic characteristics, is then exploited by the parasites, to release the eggs into the intestinal lumen. The authors hypothesize here that eosinophils, which have a long phylogenic story, could participate in the parasite - host co-evolution, specially with* S. mansoni*, operating together with monocytes/ macrophages, upon parasite transmission.*

Key words: eosinophils - phylogeny - *Schistosoma mansoni* - egg release - granuloma - co-evolution

The immune system and *Schistosoma mansoni* co-evolved for millions of years. Therefore, there has been plenty of time for the development of complex and intimate interactions between the two. Most recent research, either in paleontology or in molecular biology, tends to demonstrate that not only hominids but also modern humans originated from Africa. This means that the contact between humans and schistosomes started long ago in Africa, perhaps in the early emergence of the human lineage. In Asia, this contact could only have occurred later, either during the migration of *Homo erectus* (around 1.2 millions years ago?), or during that of *Homo sapiens sapiens* (around 80.000 years ago?) (Combes 1990a).

HISTORY OF *SCHISTOSOMA MANSONI* (PHYLOGENY)

Ancestors of schistosomes were probably hermaphroditic trematodes that lived in the blood vessels of cold-blooded vertebrates. Members of the family Sanguinicolidae parasitized fish, and the family Spirochidae inhabit the circulatory system of freshwater and marine turtles worldwide (Platt 1992), and they are generally considered similar to the ancestors of schistosomes. It seems likely that the transition from hermaphroditism to dioeciousness (male and female genitals do not occur in the same individual) in blood flukes accompanied the acquisition of homeothermy by their host, such as the mammals and birds (Basch 1990). These homeothermic vertebrates have probably evolved from cold-blooded reptilian ancestors. Evolutionary selection toward the schistosome pattern was probably driven by profound physiological adaptations in dinosaurs or in derivative transitional forms to birds and mammals, as these animals radiated rapidly and broadly during the *Mesozoic era* (Basch 1990). Schistosoma species of humans and larger mammals have separated each other fairly recently, while *Schistosomatium* of rodents represents a more ancient divergence (Basch 1990). It is improbable that a lineage of schistosomes evolved before in our primate ancestors. Rather, species of schistosomes that parasitize man are most likely the result of lateral transfers from non-primate hosts in a very recent event on an evolutionary scale (Combes 1990a). Prob-

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ably, the rodents were important in this lateral transference. This hypothesis is reinforced by the kinship, attested by the use of the same vector species and by the success of experimental hybridization between *S. mansoni* and *S. rhodaini* from Kenya (Combes 1990b) (Fig. 1).

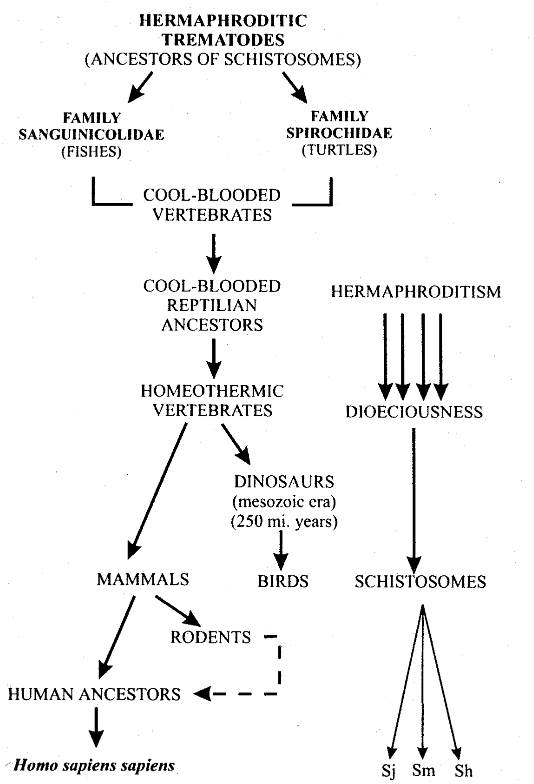

Fig. 1: schematic view of the history of the schistosomes and the switch of their host during the evolutional process (Sj = *Schistosoma japonicum*; Sm = *S. mansoni*; Sh = *S. haematobium*).

HISTORY OF THE HAEMATOLYMPHOPOIETIC SYSTEM (PHYLOGENY OF THE IMMUNE SYSTEM)

Recent results gathered while sequencing genes from sponges (Porifera) indicate that the kingdom of Animalia is a monophyletic group of multicellular organisms (arising or descendent from a single cell type) (Morris 1993, Müller et al. 1994).

Embryological data suggest that sponges, together with protostomes (Protostomia = a series of the Eucoelomata, in all of which the mouth arises from the blastopore), deuterostomes (Deuterostoma = a series of Eucoelomata, in all of which the site of the blastopore is posterior, far from the mouth, which forms a new structure unrelated to blastopore) and cnidarians derived from the same protist lineage (Borojevic 1966, Salvini-Plawen 1978). Transplantation studies revealed that both marine and freshwater sponges have acquired the structural prerequisites for an immune system, necessary for the establishment of individuality (Van de Vyver 1970, Hildeman et al. 1979, Müller et al. 1979). It is estimated that the oldest multicellular animals, the sponges, diverged from the other animals approximately 800 millions years ago (Müller et al. 1994). Studies indicate that morphoregulatory molecules, cell adhesion molecules, as well as homeodomain proteins have developed during the transition period in the evolution from the Protist to the Animalia (Müller et al. 1994).

Multicellular animals above the sponge level can be conveniently divided into two groups: *Diploblastic* and *Triploblastic*. The diploblastic animals comprise the phylum Cnidara, or Coelenterata (hydras, jellyfish, sea anemones and corals). In contrast to sponges, cnidarians possess a gut cavity lined by endoderm, as do most other animals (= coelenteron, or gastrovascular cavity). Their two cell layers are the ectoderm (epidermis) and the endoderm (gastrodermis) lining the gastrovascular cavity. Between these two layers there is an extracellular layer called mesoglea. The mesoglea ranges from a thin, non-cellular basal lamina, as in hydras and many other hydrozoans, to a thick, fibrous, jelly-like, connective tissue with or without mesenchymal cells (Ruppert & Barnes 1994).

The triploblastic animals have a third cellular layer, the mesoderm, between the ectoderm and endoderm. The triploblastic are bilateral animals and have well-developed, mesodermally derived tissues and organs, which create *regulated extracellular compartments* (evolution of compartmentation). The coelenteron in the cnidarians could not evolve into a regionally specialized gut. In large bilateral animals, the multifunctional coelenteron lining was replaced by two new epithelia that delineate a total of three new compartments: the *gut* cavity and its specialized lining, which function primarily in digestion and absorption; the *coelom* and its lining mesothelium for hydrostatic support, circulation, reproduction and excretion; and a specialization of the connective tissue called the blood-vascular system, which is important in circulation (Ruppert & Barnes 1994). Some bilaterians have an unpartitioned coelom that is continuous throughout the body, in which the coelomic fluid reaches all tissues and is the sole circulatory system. In most bilateral animals, however, the coelom is divided by septa and mesenteries, and because of them, the coelomic fluid can only circulate locally. For whole-body transport, these animals have evolved a blood-vascular system, which consists of fluid-filled channels in the connective tissue (blood vessels) (Ruppert &

Barnes 1994). In the great majority of multicellular animals the following fluids are present: tissue fluid, coelomic fluid and blood. Lymph is derived from blood plasma modified in its passage through the tissues.

Certain major sequential steps in the phylogeny of immunologic reactivity have been suggested by Tam et al. (1976). Cell or species-specific aggregation (level 1) could be observed in plants, sponges and protozoans. Specific immunorecognition/ immunoimcompatibility (level 2) was first evident at the invertebrate level of coelenterates, while cell-mediated immunity with at least short-term memory (level 3) has been detected initially in advanced invertebrates, notably annelids, echinoderms and possibly, mollusks. Immunoincompatibility as regularly shown by allogenic contact reactions appears primitive and has surely persisted as an effective surveillance mechanism throughout metazoan phylogeny. Progressively more differentiated leucocytic cells probably assumed the second-level function as adaptative specialization continued during phylogeny, and were detected in cnidarians (coelenterates), tunicates (adult urochordates) and vertebrates. Primordial cell-mediated immunity with memory was regarded as a third-level associated with cooperation of granulocytes, macrophages and T lymphocytes in allograft-type reactions. This function became well developed in primitive fishes associated increasingly with longer-lived memory. Integrated cell and humoral antibody immunity (level 4) may have first evolved in advanced bony fishes. At this vertebrate level, helper T cells and B cells capable of producing two or more molecular classes of antibodies were demonstrable. If the thymus is indeed the source of both T and B lymphocytes in fishes and amphibians, evolution of the bursa of Fabricius as a separate source of B cells may be merely a special adaptation of the reptilian-avian branch of the phylogenic tree (Tam et al. 1976). Complex immunoregulation in birds and mammals, involving multiple classes and subclasses of immunoglobulins and heterogeneous T and B repertoires, constituted the level 5 of immunologic organization (Fig. 3).

The divergence of ancestral protostomes and deuterostomes occurred 500 to 600 million years ago, a time in which all of the extant variants of animal body forms appeared. The "Big Bang" of gene duplication occurred after tunicates (from tunicates to chordates). In the deuterostomes, elasmobranchs were the most primitive species in which genes specifying MHC products have already been identified (Bartl et al. 1994, Bartl & Weissman 1994).

Immunoglobulins and recombination activating genes were also detected for the first time in elasmobranchs and in lower deuterostomes. It has recently been hypothesized that the B cells of sharks most probably resemble the CD5+ cells of man, which produce polyspecific IgM often showing autoantibody activity and that their T cells resemble γ/δ T cells rather than a/b TCR-bearing helper cells (Marchalonis & Schluter 1994, Bartl et al. 1994, Horton & Ratcliffe 1996).

WHAT KIND OF IMMUNE SYSTEM DID THE SCHISTOSOMES MEET DURING THEIR PHYLOGENY?

The schistosomes and their ancestors probably presented the following sequential animals as co-evolutive hosts: 1st.: fishes and turtles; 2nd.: dinosaurs and derivative forms transitional to birds; 3rd.: birds and rodents; 4th.: hominids. Therefore, the schistosomes cohabited with animals originated since the Devonian period of the Paleozoic era, occurring from 350 to 400 millions years ago and characterized by the dominance of fishes and the advent of amphibians and ammonites. Later on, the schistosomes lived with animals from the Permian, Triassic, Jurassic and Cretaceous eras. Rodentia and hominids appeared in Paleogene (Eocene epoch) and Quaternary Periods, respectively (Fig. 2).

FISHES AND TURTLES
(DEVONIAN PERIOD-PALEOZOIC ERA)
(350-400 mi. years)

↓

DINOSAURS AND TRANSITIONAL FORMS TO BIRDS
(MESOZOIC ERA)
(250 mi. years)

↓

BIRDS AND RODENTS
(PALEOGENE and EOCENE EPOCHS)
(67 and 58 mi. years)

↓

HOMINIDS
(QUATERNARY PERIOD)
(1.6 mi. years)

↓

Homo sapiens sapiens

Fig. 2: schistosomes and co-evolutive hosts according to geological scale of time.

As was seen before, schistosomes, therefore, have experienced or cohabitated with animals with differentiated leucocytic cells and primordial cell-mediated immunity; animals with integrated cell and humoral antibody immunity and, finally, animals with complex immunoregulation: multiple molecular classes and subclasses of immunoglobulins and heterogeneous B and T repertoires (Fig. 3).

DIFFERENTIATED LEUCOCYTIC CELLS
(INCLUDING EOSINOPHILS)
(Level 2)

↓

PRIMORDIAL CELL-MEDIATED IMMUNITY WITH MEMORY
(Level 3)

↓

INTEGRATED CELL AND HUMORAL ANTIBODY IMMUNITY
(Level 4)

↓

COMPLEX IMMUNOREGULATIONS
(MULTIPLE MOLECULAR CLASSES AND SUBCLASSES OF IMMUNOGLOBULINS AND HETEROGENEOUS (DIVERSIFIED) T AND B REPERTOIRES)
(Level 5)

Fig. 3: major steps in phylogeny of immunologic reactivity experienced by schistosomes (see Tam et al. 1976).

Paleoparasitology should try to answer the question if the schistosomes have enjoyed a long relationship with their hosts extending into the early Mesozoic prior to the breakup of Pangaea (continental drift). Using Brooks and McLennan (1993) analysis on parasite evolutionary biology, we can say that, at the moment, we have very little information about the difference, if any, in the relationship between the degree of pathology, the age of the parasite-host relationship and the type of host switch. For example, the assertion that high pathogenicity is an evidence of recent and still imperfect parasite-host relationships (Hegner 1926) needs to be confirmed.

INTERACTION BETWEEN SCHISTOSOMES AND RECENT HOSTS

Migratory pathways - Human and experimental infections with *S. mansoni* are characterized by a permanent presence of adult worms in the venous mesenteric system. To reach this final habitat, the parasites travel through complex migratory pathways. After the penetration through the skin, schistosomula are launched by right ventricle to the pulmonary circulation and return to heart (left camaras), being carried out to the systemic circulation, including: muscle and skeleton (31.7%), intestines (31.6%), kidney (12.8%), brain (7.9%), heart (6.4%), lung (4% - brachial artery?), reproductive organs (3%), skin (1.7%) and liver (0.9%) (Wilson & Coulson 1986). Individual parasites may make multiple circuits around the pulmonary-systemic vasculature with a probability of entering an artery leading to the portal vein of approximately 0.3 on each circuit (Wilson 1987).

The parasites accumulate slow and asynchronously in hepatic vessels, where they can be found from day 4 to day 23, as young and/ or adult specimens (Faust et al. 1934, Coelho 1970). The accumulation of parasites in mouse liver appears to be complete by 21 days post-infection (Wilson 1987). Wilson and Coulson (1986) estimated that 97 % of those schistosomula which reach the liver do so via the splanchnic capillaries and hepatic portal system. Most of them fail to negotiate the sinusoids to reach the hepatic vein, instead they transform into blood-feeding worms and begin to grow, ultimately pairing and migrating upstream to the mesenteric venules for oviposition (Wilson 1990). Trapping of schistosomula in the liver is not completely efficient, and in a previously uninfected mouse, an estimated 14 - 30% may return to the lungs (Wilson et al. 1986).

In mice, intrahepatic paired adult worms appear for the first time, on day 20 of infection (Lenzi 1991), the schistosomae begin to migrate to mesenteric vessels from the third week onward (Pinto & Almeida 1948) and the occurrence of eggs laying is detected between 30 and 34 days after infection (Brener 1956, Prata 1957).

The initiation of parasite growth after arrival in the liver may be under endogenous control by ecdysteroids (Nirde et al. 1983) and these hormones may in addition moderate the process of tegument membrane turnover (Torpier et al. 1982).

Once schistosomula have transformed to adult worms, they tend to live inside mesenteric venous vessels, although some of them, specially the females, may undertake further intravascular migration, as we have often observed in Swiss webster mice, from 40 to 160 days after infection (Lenzi 1991).

Advantages of the final habitat and egg dispersion - The habitat of most adult *S. mansoni* within the mesenteric veins of the host caused three biological consequences: (1) long and cozy life within the nutrient-rich mesenteric veins, producing a prodigious total number of eggs (Bash 1990); (2) easy access of the eggs to the intestinal vessels and (3) dispersal of the eggs and parasitic soluble products to extraintestinal organs (mainly to the liver), provoking systemic reactions (Lenzi et al. 1987, 1995).

Extraintestinal consequences - Schistosomiasis is essentially an intravascular infection, and schistosomes live in direct contact with the mesenteric or portal vein endothelium. The adult worms produce large numbers of intravascular eggs daily and continuously secrete and excrete numerous inert, toxic or antigenic substances that can interfere directly with the contiguous endothelium surface (Lenzi et al. 1988) or can provoke simultaneous and parallel activation of eosinophil metaplasia in the liver and eosinopoiesis in the bone marrow and stimulation of extramedullary hematopoiesis in lymph nodes and omental and mesenteric milky spots (Byram et al. 1978, Borojevic et al. 1981, Weinberg et al. 1992, Lenzi et al. 1987b, 1990, 1995).

Activation of the old or primordial (immune system) lymphomyeloid tissue - Coelom was a key evolutionary innovation that has occurred in the body plan of the major animal phyla (Raven & Johnson 1996, Erwin et al. 1997). A major advantage of the coelomate body plan was that it allowed contact between mesoderm and endoderm, facilitating the occurrence of primary induction processes during development (Raven & Johnson 1996). For example, contact between mesoderm and endoderm permitted localized portions of the digestive tract to develop into complex, highly specialized regions like the stomach. The presence of a coelom also allowed the digestive tract, by its coiling or folding within the coelom, to be longer than the animal itself. Such an arrangement limited the animal's exposure to predators due to the capacity to hide large amount of food during the digestive process (Raven & Johnson 1996).

The intraembryonic coelom in humans and vertebrate animals is divided into pericardial, pleural and peritoneal cavities. In these three cavities, specially in the omentum of the peritoneal cavity and in pericardiodiaphragmatic membrane, there are the milky spots (MS) which were described by Recklinghausen in 1863 in young rabbits (Recklinghausen 1863). We proposed that milky spots (individual structures), considered as a whole, constitute an organized coelom-associated lymphomyelopoietic tissue (CALT), which represents the main immune tissue in the peritoneal cavity (Lenzi et al. 1996). Like the thymus, CALT appears to be a mixed lymphoid organ, with secondary (Dux et al. 1986, Mandache et al. 1987) and/ or primary lymphoid organ functions, being an important site of monocyte/ macrophage (Beelen et al. 1980, Wisffels et al. 1992) and B1 cell (CD5+/ IgM+) (Solvason et al. 1992) generation, and of plasma cell maturation, not dependent on germinal center (Weinberg et al. 1992, Lenzi et al. 1996). The peritoneum (probably the milky spots) might either serve as a preferential site for extrathymic T cell differentiation - perhaps a non-conventional T cell lineage - or might provide a microenvironment that favors the expansion and/ or accumulation of a particular T cell repertoire (Kroemer et al. 1993).

As the schistosome adult worms live inside the mesenteric veins, which are connected with MS vasculature, their products can easily reach the milky spots. In fact, when activated by schistosomal infection, the milky spots showed, in mice, pronounced lymphocytosis, plasmocytogenesis (IgM > IgG > IgA > IgG_{2a} > IgG_1) and myelomonocytosis. The lymphocytes were mainly of the B1 type (double positive CD5/ IgM), with smaller number of T cells [TCR αβ (+), TCR γδ (+), CD3 (+), CD5 (+)] and conventional B2 cells [B220 (+), CD23 (+)]. The infection also caused an increase of myeloid/ erythroid proliferation and differentiation, mainly at 50 and 90 days after infection, with expression of monocyte/ macrophage, eosinophil, neutrophil, megakaryocyte and erythroid lineages. Some milky spots were rich in mucosal and connective tissue mast cells (Lenzi et al. 1992, 1996).

Milky spots, during *S. mansoni* infection, presented different histologic patterns that were modulated, turning from lymphomonocytic to lymphoplasmocytic, showing an intermediate stage rich in eosinophils.

CALT system is probably equivalent to very primitive lymphomyeloid tissues that develop during evolution of vertebrates (Horton & Ratcliffe 1996). It is an appropriate environment for myelomonocytic and lymphoid lineages, specially B1 cells (CD5 B cells). These cells are highly connected, multispecific and over-represent 3' VH gene families (Mitchison 1992).

Activation of recent or new lymphomyeloid tissue - Our observations, together with data from the literature, point to the following conclusive results: During the murine schistosomal infection there are three distinct evolutional phases: (a) low or non-productive (before 30-35 days of infection); (b) acute-productive (between 35 and 70-90 days) and (c) chronic-productive (after 70-90 days of infection).

The lymphohematopoietic changes that occur in the acute-productive phase (eosinopoiesis and monocytopoiesis intra and extramedullar, mastocytosis in intestine, mesenterium and in granulomas) are more indicative of a preferential skew of T cell cytokine producers towards a TH_2 phenotype (Grzych et al. 1991). Otherwise, the cellular changes observed in the productive-chronic phase (early or late chronic subphases) do not display restricted cytokine profiles of the TH_1 or TH_2 types (Grzych et al. 1991, Henderson et al. 1992, Chensue et al. 1992). Interferon-γ, IL-4 and IL-10 exert cross-regulatory effect on TH_1-TH_2 balance as IL-4 drives the reaction toward TH_2, IFN-γ toward TH_1 and IL-10 may inhibit either trend depending on the circunstances (Chensue et al. 1994, Jankovic & Sher 1996). However, Borojevic (1992) regards the chronic phase of murine schistosomiasis as predominantly TH_1-mediated, largely on the basis of increasing ratio of IgG_{2a} to IgG_1, decreasing eosinophil and IgE levels, and enhanced macrophage function in chronically infected mice.

Only a sequential study during different phases of infection, documenting the cytokine production *in situ* (immunocytochemical and PCR/ *in situ* hybridization, using tissue sections) in the responding lymphoid organs and in the granulomas, could yield new insights of profound importance on TH_1 or TH_2 responses in schistosomiasis. For a better criticism on this subject we recommend the papers of Kelso (1995) and Zhang and Tarleton (1996).

Exploitation of the host responses by S. mansoni to continue its life cycle - S. mansoni have capitalized the primordial and recent systemic responses of the host caused by the dispersal of eggs and their products, using the new formed cells to compose the periovular reaction, and consequently to continue the life cycle. This phenomenon was qualified by Damian (1987) as an example of immune exploitation by the parasite, making possible its propagation to new host. Torres and Pinto (1945), analyzing the lesions in male armadillo (*Euphractus sexcinctus*), experimentally infected by *S. mansoni*, suggested for the first time in the literature that the egg release to the feces is due to the inflammatory process surrounding the eggs. They pointed out that the main factors affecting the liberation of *S. mansoni* eggs, as observed in the armadillo model, are the following: (1) the extrusion of eggs from the capillaries and their transient fixation in the mucous coat; (2) the formation of a cellular infiltrate around the extruded ova; (3) the histolysis of this cellular infiltrate as well as of the surrounding tissue; (4) disintegration of the walls of the adjoining Lieberkühn's glands as the histolysis increases, and consecutive transfer of the eggs to the Lieberkühn's crypt and (5) their further elimination in conjunction with the intestinal juice secreted by the glands.

Doenhoff et al. (1978, 1981) and Dunne et al. (1983) provided the first evidence that the passage of *S. mansoni* eggs through the intestinal wall of experimentally infected mice, until they are excreted in the feces, depends on immunological mechanisms. Damian (1987), like Torres and Pinto (1945) also proposed that the granuloma is the agent of egg translocation to the intestinal lumen, due to the mobility of T and B lymphocytes, plasma cells, eosinophils, neutrophils, fibroblasts, macrophages and multinucleated giant cells. In fact, Lenzi et al. (1987a, 1991), analyzing intestinal serial sections of mice, observed that the eggs released to the feces were always wrapped in inflammatory cells, specially eosinophils and monocytes. This observation was confirmed in three different models: Swiss webster (Rodentia, Muridae), *Calomys callosus* (Rodentia, Cricetidae) and *Nectomys squamipes* (Rodentia, Cricetidae), and allow us to conclude that the egg excretion is dependent of the *exudative-pre-granulomatous stage* to schistosome eggs. This stage has a lytic character, which prepares the space by destruction of the parenchyma to the establishment and organization of the granulomatous stage. In the intestine, this stage destroy, focally, the usual intestinal extracellular matrix (ECM) components in the chorion, form a cellular wave in front of the eggs (unidirectional preovular wave), which corrodes the epithelia basement membrane, causing destruction or detachment of the superposed epithelial cells, thereby opening channels for the passage of the eggs to the intestinal lumen (Lenzi et al. 1987a, 1991). The histolytic effects are probably derived from eosinophils and/or monocytes collagenases, elastase and non-specific proteases. When the eosinophils are blocked, for instance by anti-IL-5 (Sher et al. 1990), the local histolysis can be done by monocytes alone or by monocytes and neutrophils. The eosinophils and monocytes appear to be chemically attracted by some intraluminal or epithelial factor(s) and the cells of the preovular waves are highly CD11a (+), CD11b (+), CD18(+), CD44(+) and ICAM (-), and apparently move by haptotaxy on a gradient of laminin and tenascin in the upper part of villosities. ICAM-1 was detected only in lamina propria mucosae and not in the preovular wave and in the epithelial cells (Lenzi, unpublished data). When we treated *S. mansoni*-infected Swiss webster mice with Dexametasone (0.75 mg/ kg I.M., 72/ 72 hr) from the day 16 to 45, 55 and 70 after infection, the ratio between eggs in the feces/ eggs in the tissues decreased up to 60% due to reduction in the excretion and increase in egg retention in the tissues by drug effects on periovular reaction (Lenzi 1991).

A comparison of neutral protease (collagenase, elastase) levels within the granulomas and granuloma secretion showed that the large granulomas (or pre-granulomas ?) of acutely infected mice contained and secreted more enzymes than their smaller immunomodulated counterparts (Truden & Boros 1985).

More recently, Ngaiza and Doenhoff (1990) and Ngaiza et al. (1993) have shown that, at least in some models, the anchoring of egg to endothelial cells may be mediated by platelets or their release products, since egg excretion is markedly suppressed when *S. mansoni*-infected mice are rendered thrombocytopenic by treatment with antiplatelet serum. Then, during the exudative stage of the periovular reaction (it is not a granuloma, but a pre-granulomatous stage), collagenase, elastase and non-specific protease production by the cell [monocytes/ macrophages, eosinophils, neutrophils and platelets (?)] destroy fibers of the extracellular matrix of the intestinal chorion and epithelial basal membrane, creating an afibrillar, easily penetrable environment for the eggs, thus allowing them to be passively and mechanically ejected to the feces by intestinal peristalsis. The intestinal mastocytosis that occur during the *S. mansoni* infection (Lenzi et al. 1987b) probably increase the intestinal smooth muscle contractility, amplifying the peristalsis (Finkelman et al. 1997). The chance of the eggs to be excreted is entirely probabilistic and it depends on the confluence of momentary factors: embolization to the mucosal layer and exudative pre-granulomatous reaction (focal histolysis). Afterward, the periovular reaction evolves to granulomatous stage, with fiber production and internal cohesive organization that retain the eggs in the tissue (Lenzi et al. 1991). Interestingly, Santos et al. (1992) have shown that schistosomal periovular granulomas in the intestines are smaller and contain less collagen than those in the liver. Due to saturation of the mucosal vessels during the infection, occurs a gradative deepening of the granulomas in the intestinal wall, decreasing the chance of the eggs to be excreted to the feces, favoring their dropping into the peritoneal cavity (Melro & Mariano 1987).

PHYLOGENY OF EOSINOPHILS

The long evolutionary story of *Schistosoma* ancestors, dealing with several different species of animals, raises an important question: Why do the schistosome hosts usually present eosinophilia during the infection? Is the evolutionary pressure to eosinophil lineage expression a favorable or unfavorable condition to the parasite? Unfortunately, the "military paradigm" applied to the interpretation of the eosinophil function (cytotoxic/killer cell) has blurred and obstructed a deeper understanding of the eosinophil physiology.

Ancestral cell of the lymphohematopoietic system existed among primitive invertebrates. This cell probably recognized foreign material or antigens, and responded by phagocytosis. Phagocytic cells have been conserved throughout the phylogenetic scale as macrophages and granulocytes; probably mast cells are also related to this ancestral cell. Thus, the phagocytic cell and its function is common to and pervades all the phylogenetic levels of immunity (= first evolutionary step) (Cooper 1982). When coelomate invertebrates appeared, diverse leukocyte types developed, some of which are considered to be precursors of lymphocytes (Wright & Cooper 1976) (Fig. 4).

Patterson and Landolt (1979) have reported neutrophilic as well as eosinophilic granules in amebocytes of the sea anemone *Anthopleura elegantissima* (Coelenterata). There have been ultrastructural investigations of the blood cells of nemerteans, which are less primitive acoelomates than platyhelminths. Light microscopy observations (Ohuye 1942, Vernet & Gontchanoff 1975, 1976), however, have revealed four or five types of leukocytes, including eosinophilic granulocytes with reniform eccentric nuclei.

In the japanese horseshoe crab *Tachypleus tridentatus* (artropoda, phylum Chelicerata), the granular amebocytes have been subdivided by Shishikura and Sekiguchi (1979) into two morphologically distinct types, based on the structure of the granular inclusions. The first type, "Type A", accounts for approximately 10 % of the cell population, while "Type B" accounts for 90 % of the cells and enclose large, faintly and smaller, eosi-

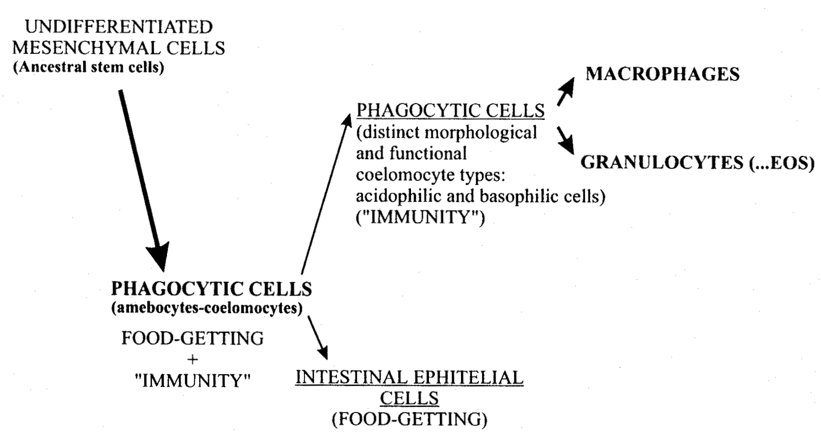

Fig. 4: evolutionary pressure led to the modification of ancestral cells. The phagocytic cell, represented by monocytes or tissue and organ macrophages of vertebrates, is probably the only blood cell that has persisted since the protozoan and as a unique unit, functioning in phagocytosis since the primitive metazoan. When food getting and immunity became separate functions, cells resembling granulocytes, including eosinophil cells, evolved (see Cooper 1982).

nophilic granules. Deevey (1941) found that the hyaline leukocytes (equivalent to plasmatocytes?) and eosinophils (granular cells?) of Haitian tarantula, *Phormictopus cancerides* (arachnid), ingest a variety of dyes.

Ascidian (protochordata) blood cells are classified into several categories, as follows: (1) undifferentiated cells (hemoblasts or lymphocytes); (2) leukocytes (hyaline or granular); (3) vacuolated cells (signet ring, compartment, or morula); (4) various pigmented cells and (5) nephrocytes. The granules of granular leukocytes are homogeneous, strongly electron-dense, and with conventional blood stains, may be acidophilic, basophilic or neutrophilic (Wright & Ermak 1982). Eosinophils have been described in the blood and tissues of a number of different fishes, including lower vertebrates such as the elasmobranchs (nurse shark (*Gingilymostoma cirratum*) (Hyder et al. 1983), torpedoes (*Torpedo marmorata risso* and *Torpedo acellata rafinisque*) (Grimaldi et al. 1983), carps (Smith et al. 1970), stripped bass (*Morone saxatilis*) (Bodammer 1986), tench (Kelenyi & Nemeth 1969), loach, (*Misgurnus anguillicaudatus*) (Ishizeki et al. 1984) and some teleost species (Davies & Haynes 1975, Bielek 1981). However, eosinophils are absent in hagfishes (*Agnathan cyclostomes*) (Linthicum 1975). Curtis et al. (1979) describe the bone marrow of *Plethodon glutinosus* (amphibia) as containing large numbers of developing neutrophils and eosinophils, lymphocytes of various sizes, plasmablasts, and plasma cells. Eosinophils are also present in frogs (Kelenyi & Nemeth 1969, Meseguer et al. 1985).

On the evolutionary scale, reptiles are pivotal since they are the progenitors of both avian and mammalian classes. The most prominent and well developed reptilian lymphoid organs are the thymus and spleen. In the early phases of the splenic development, before the organ becomes lymphopoietic, it contains a large number of granulocytes. In the turtle, the eosinophils are restricted to the subcapsular region while basophils are scattered throughout the parenchyma. In later development, the subcapsular eosinophils disappear and the spleen becomes primarily lymphopoietic (Muthukkaruppan et al. 1982). Eosinophils of turtles and lizards do not contain a crystalloid internum (Kelenyi & Nemeth 1969). A primitive reptile, the tuatara *Splenedon punctatus*, has eosinophils without crystalloids (Desser & Weller 1979). The American alligator (*Alligator mississippiensis*) presents blood eosinophils positive for alkaline phosphatase and with some phagocytic capacity for bacteria *in vitro* (Mateo et al. 1984). Eosinophils are found in numerous mammals other than man, such as buffalos, camels, cattle, goats, sheep, swine (artiodactyla); cats, dogs, ferrets, mink, raccoons (carnivora); rabbits (lagomorpha); kangaroos and opossum (marsupials); horses (perissodactyla); primates and rodentia (familiae Muridae, Cricetidae and Caviidae) (Spry 1988).

The use of molecular probes for the unique constituents in eosinophils will be of great interest to determine the philogeny of eosinophils, mainly in invertebrates. We should also point out that there is considerable variation in the structure and biochemical composition of the eosinophils of different species (Spry 1988).

DISCUSSION AND CONCLUSION

Schistosomes, ancestors and recent species, have pervaded many hosts and several phylogenetic levels of immunity. In recent hosts, *S. mansoni* interacts with and activates the primordial lymphohematopoietic tissue (CALT) and the new immune system, reproducing a phylogenic "memory" (Lenzi et al. 1996). Taking advantages of the egg dispersion, it exploits the host cellular and immune responses to exteriorize its eggs (Damian 1987), maintaining the external concentration of eggs above the threshold level below which transmission cannot occur. We hypothesize here that the eosinophils could participate in the parasite host co-evolution, specially with *S. mansoni*, operating, together with monocytes/macrophages and platelets in parasite transmission.

S. mansoni infection uses two patterns of transmission: one relatively rapid with high number of eggs and more intense systemic and periovular reaction (eosinophils and monocytes) and other with slow transmission (chronic phase) and down-regulated granulomas and fall in the eosinophil and monocyte levels. *S. mansoni* adult worm have capitalized on the apparent adversity of living within the mesenteric vein, using the dispersion of eggs and antigens to other organs besides intestines to set a systemic activation of several hematopoietic lineages, specially eosinophils and monocytes/macrophages. This activation occurs in bone marrow, spleen, liver, lymph nodes, omental and mesenteric milky spots (CALT) (Lenzi et al. 1995), increasing the offer and making easy the migration of eosinophils, monocytes and other cells to the intestinal periovular reaction.

Therefore, our results (Lenzi et al. 1987, 1991), together with those of Torres and Pinto (1945) and Damian (1987) and the observations of Reis and Andrade (1987) do not support the previous belief that the eosinophils in the granulomas are the cells responsible for killing the miracidia. At least in the mouse model, eosinophils also do not kill mechanically alive transformed schistosomula, surrounding only dead ones (Andrade & Reis 1984). These observations exemplify some aspects of the intriguing complexity of the parasite-host relationship established during very long co-evolution (Fig. 5).

Bloch (1984) called attention to an apparent discrepancy in the extent of the attack on schistosomules by granulocytes between the *in vivo* observations and *in vitro* studies. The later experiments have repeatedly demonstrated that granulocytes and other cells involve a major number of schistosomules, adhering to them and killing them. While cell adherence to schistosomules was observed *in vivo*, the number of schistosomules attacked was small. According to Bloch (1984), the reason for the discrepancy may be that *in vivo* the parasites are metabolically more active and alter their surface membranes more rapidly, so that cell adherence is not so effective as in *in vitro* environment. Lozzi et al. (1996) showed that *S. mansoni* primary and re-infection induced an influx of eosinophils to regional lymph nodes. However, only in the reinfected animals, were the eosinophils able to adhere to the larval surface, damaged larval being found inside eosinophilic infiltrates. These authors questioned the significance of this adhesion, since it could either signify a role in larval death or a phenomenon secondary to larval degenerative changes.

Why helminths have been responsible for evolutionary pressure to eosinophil response in most of their hosts? Is it any particular type of adaptative immune response (Mitchell 1991) that favors the host and the parasite? (Fig. 5).

The eosinophil is an ubiquitous cell, able to hide its purposes for more than 100 years (thousands or millions of years?). What is its role in the normal physiology? To answer this question it will be fundamental to do phylogenetic and *in vivo* studies, together with molecular biology approaches. In this context, McNagny et al. (1996) detected that avian retrovirus-transformed eosinophils and their precursors express a 100 KD cell surface glycoprotein, which presents homology to human melanotransferrin. By analogy to saxiphilin, melanotransferrin may have evolved to bind and inactivate toxic substances present in intestine or generated during kidney filtration or eosinophil maturation. Further experiments are required to elucidate the molecule's true function (McNagny et al. 1996).

Finally, studies performed in shistosomiasis (and other parasitic diseases) have brought numerous information on the various facets of eosinophil functions and led to a better knowledge of eosinophil physiopathology, applicable to other diseases involving eosinophils and specially allergic diseases (Capron & Capron 1992). In fact, infections by parasites can be useful models to study eosinophil physiology and physiopathology, because the parasites may function as natural eosinophil stimulants or depressors. According to Samter (1980), eosinophils continue to be "nominated but not elected cells". Actually the function of eosinophils is complex, varied and still unknown, but can be artificially schematized as in Fig. 6.

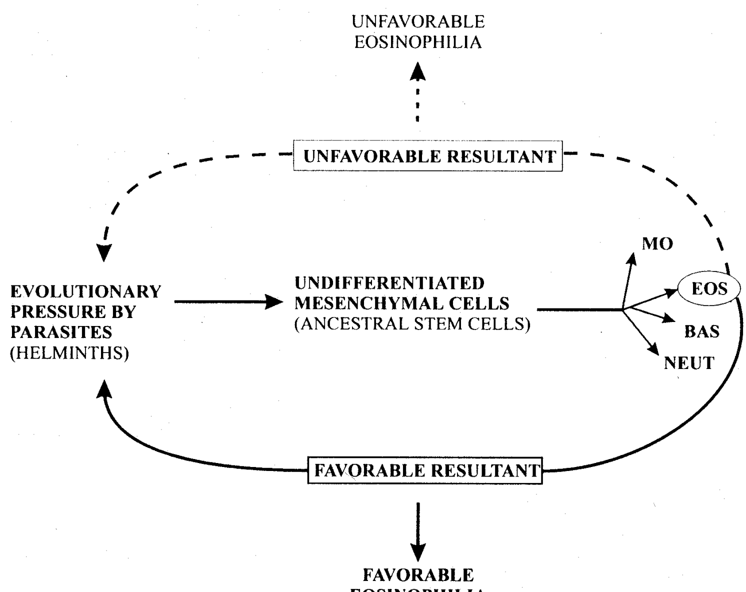

Fig. 5: it appears that evolutionary pressure by helminths on ancestral cells, stimulated the eosinophil differentiation, provoking a particular type of adaptative cellular (immune) response. A variety of examples demonstrate that eosinophils can favorably or unfavorably contribute to the host-parasite interactions.

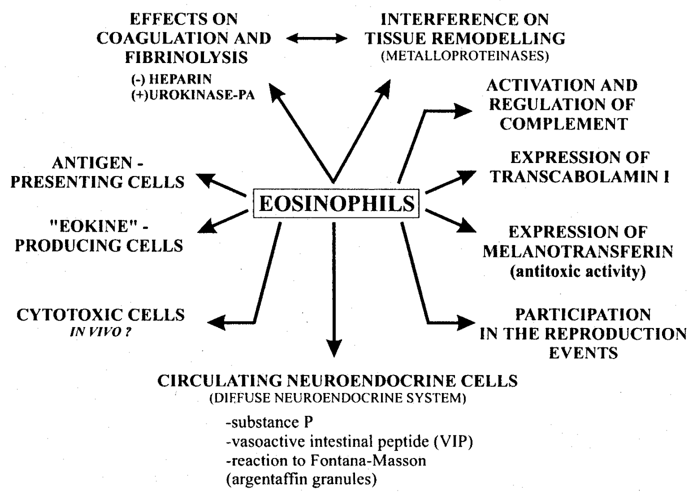

Fig. 6: many functional hypothetical capabilities of eosinophils, which are probably integrated during eosinophil participation *in vivo* processes, such as inflammation and wound healing (Gansler 1956, Pepper & Lindsay 1960, Tchernitchin et al. 1967, Gleich et al. 1974, Okun et al. 1974, Dahl & Venge 1979, Venge et al. 1979, Hibbs et al. 1982, Adrouny et al. 1984, Davis et al. 1984, Zittoum et al. 1984, Ackerman et al. 1987, Weiler & Gleich 1988, Weinstock et al. 1988, Luque & Montes 1989, Weinstock & Blum 1990a, b, Weller et al. 1991, Kroegel et al. 1994).

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