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SCHISTOSOMA MANSONI: THE EFFECT OF DEXAMETHASONE ON THE CERCARIA-SCHISTOSOMULUM TRANSFORMATION, *IN VIVO*.

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SUMMARY

Treatment with dexamethasone (DMS) in the early phases of the experimental *Schistosoma mansoni* infection causes an indirect effect on the cercaria-schistosomulum transformation process. This is observed when naive albino mice are treated with that drug (50 mg/Kg, subcutaneously) and infected intraperitonealy 01 hour later with about 500 *S. mansoni* cercariae (LE strain). An inhibition in the host cell adhesion to the larvae, with a simultaneous delay in the cercaria-schistosomulum transformation, is observed. This effect is probably due to a blockade of the neutrophil migration to the peritoneal cavity of mice, by an impairment of the release of chemotactic substances. Such delay probably favors the killing of *S. mansoni* larvae, still in the transformation process, by the vertebrate host defenses, as the complement system.

KEYWORDS: *Schistosoma mansoni*; Peritoneal cavity; Dexamethasone; Neutrophil; Cercaria; Schistosomulun.

INTRODUCTION

The effects of glucocorticoids on the course of many infectious diseases are well known. Glucocorticoids may unbeneficially influence the host-pathogenic organism interaction. However, in the experimental schistosomiasis mansoni, several workers demonstrated that the corticotherapy, when administered in the early phases of *Schistosoma mansoni* infection, causes a reduction in the worm burden [5, 9, 12, 18, 30]. Although this effect seems to be unexpected, it is possible an activity of this drug during the crucial phase of the parasite adaptation to the vertebrate host, the cercaria-schistosomulum transformation (CST) process. This was initially suggested studying the kinetics of the pulmonary phase of *S. mansoni* in mice treated with dexamethasone (DMS). The reduction in the worm burden appeared to occur early, probably at the skin or lung phases of the parasite development [13]. Later, working with mice infected with *in vivo* obtained schistosomules and treated with DMS, no changes were seen [14]. Then, the action of glucocorticoids seemed to be limited to the initial phase of the *S. mansoni* life cycle in mice.

It is very difficult to study the CST in the skin of laboratory animals. On the other hand, the use of the peritoneal cavity of mice for *in vivo* studies of *S. mansoni* development allows easier observations during and after CST, as well as the simple recovery of *in vivo* produced schistosomules [10, 28]. It was shown that these

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larvae, maintained in the peritoneal cavity for 02 weeks, have the same morpho-functional development of the schistosomules found in other sites [25]. This model allows the quick, reproducible and quantitative verification of the kinetics of CST *in vivo* [21], as well as the effect of drugs on this process [19, 20, 22]. During CST in naive mice, a predominant neutrophilic host cell adhesion to the larvae was observed [24]. Moreover, the kinetics of this host cell adhesion parallels CST and does not kill larvae [21], suggesting that these cells may play a role in the *S. mansoni* adaptation to the vertebrate host.

It is known that polymorphonuclear neutrophils (PMN) are important in the initial defense against microorganisms, however little information is available about the interaction between *S. mansoni* and neutrophils that are the first inflammatory cells to infiltrate any site of infection.

This experiment was carried out in the peritoneal cavity of albino mice in order to check the effects to dexamethasone during CST.

MATERIAL AND METHODS

Twenty naive mice (outbred males, weighing about 20 g) were treated with dexamethasone (Decadron® - 50 mg/kg by subcutaneous route) 01 hour before the intraperitoneal inoculation of about 500 *S. mansoni* cercariae (LE strain) shed by laboratory reared-and-infected *Biomphalaria glabrata*. A group of twenty untreated mice was kept as control. Ten mice were sacrificed by cervical dislocation 30 minutes after the cercarial inoculation, and 10 others after 180 minutes. The same was repeated with the control group. The peritoneal cavity was washed with saline and the larvae were concentrated by centrifugation and inspected under a dissecting microscope, as previously described [28].

The larvae were initially classified as cercariae and tail-less organisms (living or dead), with or without cell adhesion (10 or more cells per larva). To separate cercarial bodies, which remain alive in fresh water, from schistosomula, which die in such conditions, 5 ml of distilled water was dropped into the Petri dish containing the larvae and after 10 minutes the live and dead larvae were recounted. Schistosomules showed absence of motility and herniation of the acetabulum, in contrast to the tail-less cercariae still presenting the glycocalyx [21].

The percentage of living cercariae recovered from the treated and control groups were appropriated changed to arc sin $\sqrt{\chi}$ and the Student's t test was then performed.

RESULTS

The data are summarized in Figure 01. About 30% of the inoculated cercariae were recovered from the peritoneal cavity of all mice. It was observed that DMS

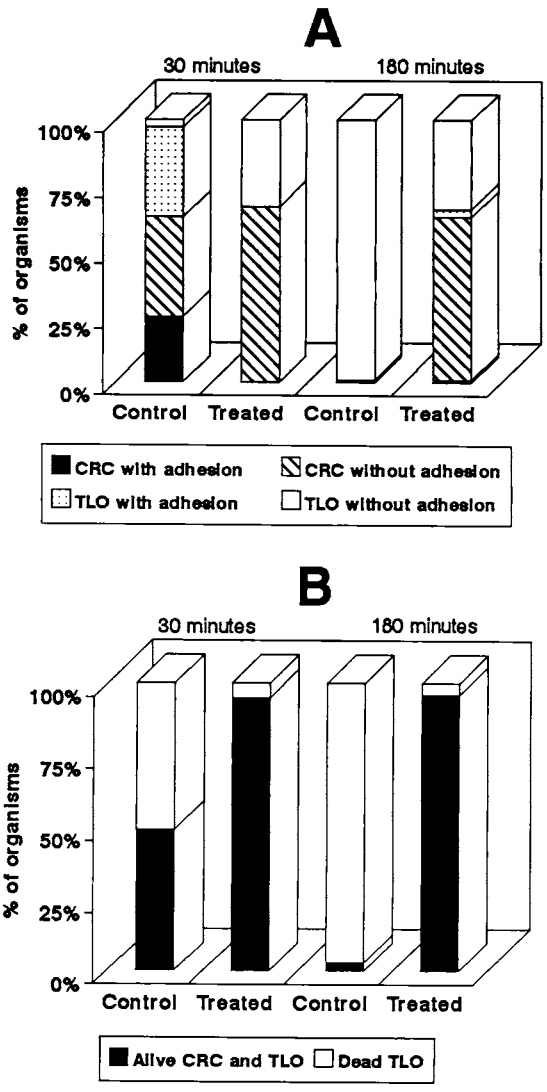

Fig. 1 - Percent distribution of *S. mansoni* larvae recovered from dexamethasone treated animals: A) As recovered in saline; B) The same recovered larvae in A, 10 min. after exposure to water to separate tailless cercariae from true schistosomules. CRC = Cercaria; TLO = Tail-less organisms.

induces a significant ($p<0.01$) blockade of cell adhesion to the larvae recovered 30 minutes after and also until 180 minutes of intraperitoneal inoculation (Fig. 01a). In addition to the inhibition of host-parasite interaction, a significant ($p<0.01$) delay of CST was verified in 30 and 180 minutes after the inocula (Fig. 01b), demonstrating clear parallelism between cell adhesion and larvae transformation in the peritoneal cavity of mice.

DISCUSSION

The *in vivo* peritoneal cavity model allows detailed observations on CST [19, 20, 21, 22, 23]. In this cavity, cercariae of *S. mansoni* trigger an intense process of cell adhesion to their surface that nevertheless does not kill the larvae but is parallel to the transformation process [21]. Three hours after intraperitoneal inoculation, all larvae are found to be schistosomules with no cells attached to their surface. Among these cells there are polymorphonuclear neutrophils (PMN) that seem to phagocitate the glycocalyx of the cercariae [24] and thus facilitate their transformation into schistosomules [23], allowing then to escape from the host defense mechanisms. The glycocalyx must be lost, since this structure activates the complement system by the alternative pathway [11].

Similar observations were made with an *in vivo* microscopy in the subcutaneous tissue in Algire chambers, inserted into the dorsal skin of C3H/HeJ mice and cheek pouches of hamsters [3]. The mobilization of the granulocytes to cercariae begins in this approach after 45 minutes of inoculation into the chamber, peaking during the third or fourth hour. In that study, the tail-less organisms with cell adhesion were considered as schistosomules [3]. However, a progressive decrease of the granulocyte adhesion to these "schistosomules" was observed by the end of the second day.

All the observed schistosomules in the tissue were found to be alive and free of host cells. Tail-less larvae might be considered cercariae having granulocytes attached to their surface or schistosomules, according to several criteria, and one of the possible mechanisms may be the presence of unremoved part of the glycocalyx. After the remotion of this cercarial envelope the larvae are free from the cells and can be considered as true schistosomules, as demonstrated elsewhere [23]. Thus, this kinetics of host cell adhesion to the larvae in the subcutaneous tissue of rodents is very similar to that observed in the peritoneal cavity of mice, although more slow because of the inherent conditions of the skin.

PMN are relatively nonspecific easily activated phagocytic cells, as it is required for their function as the first line of defense [26]. They promptly respond to a wide range of substances including leukotriene B_4, Platelet activating Factor (PAF), C5a complement fragment and lypopolysacharides of Gram-negative bacteria (LPS).

Glucocorticoids inhibit the release of many biological active substances, including PMN chemo-attractants [15]. They have no effect on *in vitro* PMN migration [4], but reduce the chemotactic activity of inflammatory exudates [17]. Current thinking suggests that part of the anti-inflammatory activity of the glucocorticoids can be explained through the blockade of the formation of eicosanoids [2] that is mediated by the induced synthesis of lipocortin, a phospholipase A_2 (PLA_2) inhibitor. So, the release of arachidonic acid-derived chemotactic factors (such as leukotriene B_4) could be inhibited.

On the other hand, DMS could control inflammation by the differential modulation of C3, factors B and H secretions [6] and therefore bring a control over the local production of complement derived inflammatory active products such as anaphylotoxins C3a and C5a, and possibly the local deposition of iC3b.

It is plausible that an inflammatory stimulus can directly activate plasma systems or injure local cells with liberation of chemotactic factors, but the activity of factors as leukotriene B4, C5a, LPS and others on cell migration is not selective [8]. However, in the last decade many evidence of association of resident macrophages with the selective migration of PMN to an inflamed site (similar to "alarm cells") were found. This has been shown either with macrophages from the subcutaneous tissues or from the peritoneal cavity of rats [29]. Macrophages release chemotactic factors for PMN that stimulate their migration both *in vitro* and *in vivo* (as in the peritoneal cavity of rats). The release of these chemotactic factors by peritoneal macrophages is also blocked by DMS both *in vitro* and *in vivo* experiments [7].

It is generally agreed that PMN migration to an inflammatory exudate has at least two defined stages: adhesion to endothelial cells and migration to the extra vascular space, oriented by chemotactic substance(s). Glucocorticoids have been suggested to interfere in both stages [7]. This event, in part, is probably mediated by blockade of macrophage release of chemotactic factors, which under normal conditions attract the PMN to the inflammatory site and may cause adhesion to endothelial cells [7].

Thus, the inhibition caused by DMS on the PMN adhesion to the *S. mansoni* larvae during the CST process verified in the present study may be a result of an inespecific blockade of PMN migration to the peritoneal cavity of mice, by an impairment of chemotactic factors released by peritoneal macrophages.

This observation may be relevant to understanding the interaction of the parasite with the host immune effector mechanism, since the survival of the parasite depends upon their ability to evade the effector mechanism of the host's immune system.

Although not essential, the PMN can accelerate the CST[24]. Then, using DMS, the glycocalyx coating larvae may remain longer than the usual period of time and the invading organisms will be killed by host defenses, as the complement system. This system is activated by this cercarial surface structure and kills the larvae [11] because the high doses of gluococorticoids seem to block the removal of glycocalyx by neutrophils, despite this drug induces depression of several complement components [1], but it is not yet known if the magnitude of this depression has major physiological significance [27]. The reduction in the worm burden in mice treated with DMS is then an early phenomenon as initially postulated by COKER [5], that indirectly affects the CST and the adaptation of *S. mansoni* to the vertebrate host. However, a direct deleterious effect of glucocorticoids upon the early stages of parasite development [12] can not be excluded in this model as an additional effect that would favour a reduction in the worm burden.

RESUMO

Schistosoma mansoni: o efeito da dexametasona na transformação da cercária em esquistossômulo, *in vivo*.

O tratamento com dexametasona (DMS) nas fases iniciais da infecção experimental com *S. mansoni* leva a um efeito indireto sobre o processo de transformação da cercária em esquistossômulo, quando camundongos isentos de infecção são tratados com esta droga (50 mg/kg, subcutâneamente) e, 01 hora depois, são infectados intraperitonealmente com cerca de 500 cercárias de *S. mansoni* (cepa LE). Foi observada uma significativa redução na adesão de células do hospedeiro às larvas, com um atraso simultâneo no processo de transformação das cercárias em esquistossômulos. Este efeito é, provavelmente, devido a um bloqueio inespecifício da migração neutrofílica para a cavidade peritoneal, através de um bloqueio da liberação de substâncias quimiotáticas. Tal atraso pode permitir a morte das larvas de *S. mansoni* (ainda em processo de transformação) pelas defesas do hospedeiro vertebrado, como o sistema do complemento.

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