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Parasitological and histopathological effects of immunosuppression in guinea-pigs (*Cavia porcellus*) experimentally infected with *Schistosoma haematobium*

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Abstract

The parasitological and histopathological effects of immunosuppression in guinea-pigs (*Cavia porcellus*) experimentally infected with *Schistosoma haematobium* were studied. A total of 16 guinea-pigs were divided into four groups (four per group): non-immunosuppressed, non-infected group (NN); immunosuppressed, non-infected group (IN); immunosuppressed, infected group (II); non-immunosuppressed, infected group (NI). The IN and II groups were immunosuppressed with 5 mg/kg prednisolone while the II and NI animals were infected with 200–300 *S. haematobium* cercariae. Excretion of eggs in urine/faeces, worm burden and histopathology of some vital organs of the guinea-pigs were studied. Eggs of *S. haematobium* were observed in the urine of the NI and II groups from 9 weeks post-infection and in faeces from 10 and 13 weeks post-infection for the NI and II groups, respectively. However, II animals excreted more viable eggs in urine and faeces than those of the NI group. Worm recovery at 14 weeks post-infection showed that NI and II guinea-pigs had more female worms than male worms and a greater proportion of worm recovery for NI animals was of immature worms. Significant differences ($P < 0.05$) existed between female, male and immature worm burden of the two groups but not in their total worm burden ($P > 0.05$). Histological changes, which were notably reactions to adult *S. haematobium* worms, were observed in the organs of the NI and II groups but these changes were seen more in the organs of the immunosuppressed, infected (II) than in the non-immunosuppressed, infected (NI) guinea-pigs. The results suggest that immunosuppression before infection increased worm survival and had a moderate effect on liver and bladder histology of *S. haematobium* infected guinea-pigs.

Introduction

Schistosomiasis is a clinical term for infection related to trematode parasites that are endemic in at least 76 tropical and sub-tropical countries (Despommier & Chen, 2004). Most pathological alterations caused by the five

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human-infecting species of *Schistosoma* (*Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. mekongi*) are caused by eggs of schistosomes trapped within tissues (Cheng, 1999).

Despite the wide-ranging clinical importance of *S. haematobium* throughout Africa and the Middle East, very few studies have been carried out in the laboratory with this species of schistosome. This is largely due to the absence of a small laboratory animal model in which pathology resembles the human infection with this parasite (Lewis *et al.*, 2008) and because the parasite's life cycle is difficult to maintain under laboratory conditions (Imbert-Establet *et al.*, 1992). With the exception of a few species of primates, the only hosts on which several generations of parasite can be maintained are the golden Syrian hamster (*Mesocricetus auratus*) and jird (*Meriones unguiculutus*) (Imbert-Establet *et al.*, 1992).

Guinea-pigs (*Cavia porcellus*) are poor hosts of *S. haematobium* because the number of parasites is drastically reduced in the early stages of infection in this animal, and no eggs are passed from either urine or faeces, as a result of establishment of only male worms (Kuntz & Malakatis, 1955). Attempts to infect guinea-pigs with the parasite by other researchers also resulted in failure to establish infection (Martins, 1958).

Several researchers have studied the effects of immunosuppression on helminth infections in resistant animals or those with low susceptibility (Weinstein, 1955; Parker, 1961; Miller, 1966; Presson *et al.*, 1988). Immunosuppressant drugs, such as corticosteroids, act on the immune system by impeding functions of leucocytes, which destroy foreign bodies and help to keep the immune system functioning properly. Interference with leucocyte function yields a side-effect of increased susceptibility to infection (Eustice & Eustice, 2006).

The present study was undertaken to determine parasitological and histopathological effects of immunosuppression with prednisolone in guinea-pigs experimentally infected with *S. haematobium.*

Materials and methods

Collection of guinea-pigs, snails and parasites

A total of 16 guinea-pigs weighing 0.32–0.60 kg were obtained from Oba Market, Enugu State, Nigeria. Guinea-pigs were acclimatized for 4 weeks in a well-aerated room and had free access to water and a standard diet *ad libitum*. Intermediate hosts of *S. haematobium*, *Bulinus truncatus,* were collected from Nigercem in Ebonyi State, Nigeria. Snails were transported to the departmental laboratory and subsequently infected with *S. haematobium* miracidia from urine showing haematuria, according to the method of Christensen *et al.* (1984). Harvesting of cercariae was carried out according to the method described by Christensen *et al.* (1984).

Experimental design

The 16 guinea-pigs were divided into four main groups of four animals each: non-immunosuppressed, non-infected group (NN); immunosuppressed, non-infected group (IN); immunosuppressed, infected group (II); non-immunosuppressed, infected group (NI). Immunosuppression was induced using 5 mg/kg prednisolone administered orally daily for five consecutive days before infection. Guinea-pigs were infected by subcutaneous injection of 200–300 *S. haematobium* cercariae.

Parasitological assay

Urine and faeces of guinea-pigs in infected groups were screened for eggs from 9 weeks post-infection. Urine of guinea-pigs was screened according to the method of Christensen *et al.* (1984) while their faeces were screened for eggs according to the method of Imbert-Establet *et al.* (1992). At 14 weeks post-infection, guinea-pigs were killed and worms were recovered by the perfusion of hepatic and mesenteric vessels of animals, as described by Christensen *et al.* (1984).

Histopathology

Specimens of liver and bladder were removed from killed guinea-pigs, fixed in 10% buffered formalin, embedded in paraffin wax and 5 μm thick sections were cut and processed following routine procedures (Drury & Wallington, 1967). Sections were examined under a microscope for changes in histology.

Data analysis

Data analysis was carried out using GENSTAT (VSN International, Hemel Hempstead, Herts, UK). Analysis of variance (ANOVA) was used to test significance level while the LSD (least-significant difference) test was used to compare the mean number of worms recovered.

Results

Screening of eggs in urine and faeces of guinea-pigs

At 9 weeks post-infection, the urine of all the infected guinea-pigs, i.e. the immunosuppressed, infected (II) and non-immunosuppressed, infected (NI) groups, had eggs that hatched to miracidia in the presence of light. However, eggs seen in the NI group were sparse and took a longer time to hatch and, with time, hatching of eggs stopped. Very few non-viable eggs were later seen in the faeces of this non-immunosuppressed group. At 10 weeks post-infection, viable eggs that immediately hatched on exposure to light were only observed in the urine of immunosuppressed, infected (II) guinea-pigs. Excretion of eggs continued in this group of guinea-pigs until 12 weeks post-infection. Thereafter, it was observed that excretion of eggs ceased from the urine of these immunosuppressed guinea-pigs but was observed in their faeces.

Histopathology

Liver sections collected from non-immunosuppressed, non-infected guinea-pigs (NN) showed indistinctly separated hepatic lobules, with sinusoids and cords of parenchyma polyhedral cells (hepatocytes) radially organized around a central vein. Located at the margin of three or more lobules was the portal area which

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contained the portal vein, hepatic artery, bile ductule and lymphocytes, all within a connective tissue network.

Sections collected from immunosuppressed, non-infected guinea-pigs (IN) showed mild to moderate cytoplasmic vacuolation of hepatocytes in the centrolobular area. Sections from immunosuppressed, infected guinea-pigs (II) showed moderate to severe vacuolation of centrolobular hepatocytes, and outright necrosis of the most severely affected ones, which were predominantly at mid-zonal areas of lobules. The lobular architecture was generally distorted and sinusoids distended. Liver sections also showed a remarkable number of hepatocytes in mitosis and, at the portal end of lobules, there was mild to moderate periductular mononuclear leucocytic infiltration. In addition, there were randomly distributed focal areas of mild fibrosis.

Sections collected from non-immunosuppressed, infected guinea-pigs (NI) showed panlobular mild to moderate granular cytoplasmic degeneration and necrosis of hepatocytes with prominent pyknosis of isolated hepatocytes in the centrolobular area. Mononuclear leucocytic infiltration of the portal area was generally mild.

Urinary bladder sections from non-immunosuppressed, non-infected guinea-pigs (NN) showed transitional epithelial lining cells above the lamina propia and submucosa, internal and external longitudinal muscular layers being covered by a serosa. Sections from immunosuppressed, non-infected guinea-pigs (IN) showed histology similar to that of NN animals. For II guinea-pigs, there was moderate to severe cytoplasmic vacuolation of mucosa epithelial cells. In addition, there were focal areas of mononuclear leucocyte aggregation in urinary bladder mucosa. Cytoplasmic vacuolation of mucosa epithelial cells was mild in NI guinea-pigs.

Worm burden

Worm recoveries from the NI and II groups are shown in table 1. Results showed a higher total worm burden in II than NI animals but there was no significant difference ($P > 0.05$). More immature worms were recovered from the NI group of guinea-pigs and statistical analysis showed significant differences between female, male and immature worm burden of the two groups (NI and II) ($P < 0.05$).

Discussion

Guinea-pigs have been shown to be resistant or to have little susceptibility to infection with *S. haematobium* (Martins, 1958). The presence of a very low number of *S. haematobium* eggs (which took a longer time to hatch to miracidia) in the urine of non-immunosuppressed, infected guinea-pigs (NI) 9 weeks post-infection and in the faeces 10 weeks post-infection was contrary to the results of Kuntz & Malakatis (1955). Working on experimental animal models, Kuntz & Malakatis (1955) reported that egg production and subsequent passage in faeces and urine were variable and seemed to depend mostly on individual compatibility or host–parasite relationship. Only moderate quantities of eggs were irregularly passed by mice and hamsters. Cotton rats passed only a limited number of viable eggs, whereas albino rats and guinea-pigs passed none.

Immunosuppression resulted in the excretion of more *S. haematobium* eggs in the urine of the II group for a longer period (9–12 weeks) and eggs hatched immediately on exposure to light. Eggs found in the faeces of the immunosuppressed, infected group were also more in number and hatched to miracidia on exposure to light, contrary to what was observed in the non-immunosuppressed, infected group. Agnew *et al.* (1988) and Imbert-Establet *et al.* (1992), using mice, never found *S. haematobium* eggs in urine but only in faeces from the middle of week 12 and at week 20, respectively.

Liver sections of immunosuppressed, non-infected guinea-pigs (IN) when compared with those of non-immunosuppressed, non-infected (NN) guinea-pigs revealed that immunosuppression caused mild histological changes to the liver of the IN group of guinea-pigs. Urinary bladder sections of IN guinea-pigs, however, had similar histology to those of non-immunosuppressed, non-infected guinea-pigs (NN). This could suggest that the dose of immunosuppressant used had a mild effect on the liver but not on the urinary bladder.

Moderate to severe histological changes were observed in the liver and urinary bladder of immunosuppressed, infected guinea-pigs (II). Compared with immunosuppressed, non-infected guinea-pigs (IN), the damage observed in liver sections of immunosuppressed, infected guinea-pigs (II) was severe, leading to distortion of the lobular architecture. In addition, cytoplasmic vacuolation of mucosa epithelial lining cells observed in urinary bladder sections of II guinea-pigs was absent in immunosuppressed, non-infected guinea-pigs (IN). However, the lesions observed in liver and urinary bladder sections were not characteristic of schistosome infection (i.e. eggs in tissue, granuloma and eosinophilia).

Tissue damage similar to that observed in the liver and urinary bladder of immunosuppressed, infected

Table 1. Worm recovery from guinea-pigs (*Cavia porcellus*) infected with *Schistosoma haematobium*.

	Worm burden				
Groups	Female	Male	Mature	Immature	Total
NI	20.33 ± 2.73	4.67 ± 0.88	26.00 ± 4.36	26.00 ± 2.31	52.00 ± 0.00
II	49.33 ± 6.01	22.30 ± 3.84	71.67 ± 16.86	4.33 ± 1.67	76.00 ± 10.82
LSD	18.32	10.95	17.04	7.91	30.03

NI, non-immunosuppressed, infected group; II, immunosuppressed, infected group; LSD, least significant difference.
F-probability: $P < 0.05$.

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guinea-pigs (II) was also seen in liver and bladder sections of non-immunosuppressed, infected guinea-pigs (NI), but there was a higher level of damage in II than in NI animals. Differences in the level of tissue damage in the NI and II groups could be attributed to a combined effect of immunosuppressant and parasite in the II animals.

Cytoplasmic vacuolation of hepatocytes and mucosa epithelial cells of the urinary bladder was moderate to severe in the immunosuppressed, infected guinea-pigs (II) and mild to moderate in the non-immunosuppressed, infected animals (NI). Mild to moderate mononuclear leucocytic infiltrations in the portal area of the liver of the two groups suggest an allergic reaction to worms inhabiting the portal-mesenteric veins (Meleney *et al.*, 1953), while focal mononuclear leucocytic aggregation observed in mucosa of the urinary bladder of immunosuppressed, infected guinea-pigs could be a reaction to *S. haematobium* eggs which was not sustained. Meleney *et al.* (1953) stated that much tissue reaction to eggs is usually observed in muscularis and serosa of the bladder, but in the present study no observable tissue reaction was found in muscularis and serosa. Regeneration in the form of mitotic cells observed in liver parenchyma of immunosuppressed, infected guinea-pigs (II) could suggest that their liver cells went through tissue injury, which must have been caused by the presence of parasites.

Worm recovery at the end of the study (14 weeks post-infection) included both immature, and mature male and female worms. The worm burden of non-immunosuppressed, infected guinea-pigs was largely of immature worms, while a higher number of mature worms was recovered from immunosuppressed, infected guinea-pigs (II). This agrees with the work of Weinstein (1955), who reported that a greater number of *Nippostrongylus muris* larvae matured to adults in cortisone-immunosuppressed rats than in their non-immunosuppressed counterparts. However, the mean total worm burden of immunosuppressed, infected guinea-pigs (II) was not significantly different ($P > 0.05$) from that of non-immunosuppressed, infected guinea-pigs (NI). Female, male and mature worm burdens of II animals were significantly higher ($P < 0.05$) than those of the NI group. Worm recovery from the present study showed that at week 14 post-infection, when guinea-pigs were killed and perfused, female worms predominated. This is contrary to the result obtained by Kuntz & Malakatis (1955) who recovered only male worms in guinea-pigs experimentally infected with *S. haematobium*. Working with mice infected with the Niger strain of *S. haematobium*, Imbert-Establet *et al.* (1992) reported a preponderance of males over female worms. The presence of female worms could also explain why *S. haematobium* eggs were found in the urine and faeces of the NI and II groups of guinea-pigs.

In conclusion, immunosuppression of guinea-pigs with prednisolone before infection enhanced the survival of both male and female *S. haematobium* worms and production of viable eggs. However, this did not lead to the development of the typical pathology of *S. haematobium* disease in this laboratory host, even though reactions were observed in the liver and bladder, believed to be directed at adult worms and eggs of the parasite.

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