Acute Phase Response of Albumin and Haptoglobin in Experimental Infection of the Olive Baboon, *Papio Anubis*, with *Schistosoma Mansoni*

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Summary

Following infection of baboons with *Schistosoma mansoni* cercariae, a rapid four-fold increase in mean serum haptoglobin concentrations was observed. These concentrations dropped to near pre-infection levels following curative treatment of the infected animals with praziquantel. On challenge of the animals with a second cercarial dose, haptoglobin concentrations demonstrated a more gradual increase that did not attain the heights of the initial infection. Albumin concentrations displayed an inversely disproportionate relationship to those of haptoglobin and decreases were much less pronounced. Whereas albumin was not a sensitive indicator of schistosomiasis mansoni, haptoglobin proved to be useful in detecting the acute infection and in determining prognosis of the disease in the olive baboon.

Introduction

Infection of the olive baboon (*Papio anubis*) with a single cercarial dose produces an acute disease characterised by gross liver inflammation and a hepatic granulomatous response, besides severe clinical symptoms (*Damian et al, 1992; Farah et al, 2000*). Determination of liver pathology is therefore useful in assessing prognosis of the disease and monitoring the success of treatment. Unfortunately, the determination of liver pathology often requires invasive methods. There is therefore great need for the development of alternative non-invasive methods for determining the same.

The acute phase response (APR) refers to the inflammatory response that occurs in animals shortly after any tissue injury by infective, immunologic, neoplastic, traumatic, parasitic or other means (*Kushner & Mackiewicz, 1987; Stadnyk & Gauldie, 1991*). The APR is initiated at the site of injury by mononuclear cells, which release a broad spectrum of inflammatory mediators including the pro-inflammatory cytokines TNF-α, IL-1, IL-6 and IFN-γ. These mediators initiate changes in the homeostatic control of the diseased animal. The changes include highly increased protein catabolism and increased synthesis of acute phase proteins (APPs) in the liver (H*einrich et al, 1990; Jensen & Whitehead, 1998; Hirvonen, 2000*). The acute phase response (APR) is prolonged if an acute inflammation develops into a chronic condition (*Baumann & Gauldie, 1994*). Otherwise, as tissue injury resolves, anti-inflammatory mediators such as glucorticoids, IL-4 and IL-10 downregulate the APR (*Gruys et al, 1994; Pannen & Robotham, 1995*).

In a study by Mungatana *et al* (*2006*), a single infection of mice models with *Schistosoma mansoni* cercariae produced marked increases in haptoglobin. However, natural infections in humans probably occur with repeated exposures to cercarie; hence the need to replicate this situation when doing primate studies. Challenge by multiple infec-

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tions may produce an attenuation of the APR. Salonen *et al* (*1996*) found that repeated challenge of the mammary gland with *E.coli* was followed by a suppressed APR. Most of the pathology in schistosomiasis mansoni occurs in the liver. As the acute phase proteins (APPs) are largely produced in the liver, repeated challenge infections would result in a reduced APR from an already damaged liver. Furthermore, Farah *et al* (1997) observed that granulomatous response in the liver was modulated after multiple exposures of the baboon to *S. mansoni* cercariae. This suggests that cytokine production is modulated following multiple (challenge) exposures, resulting in an attenuated APR. Profiling of these APPs may therefore be useful in distinguishing acute from chronic infections, and in assessment of liver pathology. Though much work has been done in investigating the APR in various infections, little appears to have been done to study it in schistosomiasis.

In the present study, serum concentrations of albumin and haptoglobin were measured in baboons following sequential infection, treatment and a second challenge infection, in order to evaluate their use as diagnostic and prognostic indicators for the disease. The disease developed by the Kenya olive baboons (*Papio anubis*) is similar to that seen in man (*Farah et al, 1997*) and thus, the results of this work may be extrapolated for studies in human medicine.

Materials and Methods

Experimental Procedure

The experimental protocol was approved and monitored by the ethical committee of the Institute of Primate Research, Kenya. The experiment lasted 298 days.

Parasites

Biomphalaria pfeifferi snails were obtained from Kangundo Division, Machakos District, Kenya. The snails were screened by exposure to strong light to ensure that they did not have any schistosomes. The snails were housed in a snail room at the Institute of Primate Research, Nairobi. They were placed in aerated plastic trays at temperatures within 25-28 ŶC for 12 hours of light /12 hours of darkness, and fed on lettuce throughout the experimental period. The snails were then infected individually with 3-6 miracidia, artificially hatched from eggs of *Schistosoma mansoni* harvested from infected baboon faeces. The infected snails were maintained in the same conditions for four weeks, after which they were put in the dark until they were required for cercarial shedding. Cercariae for infection were then obtained by exposing the infected snails to artificial light (100 watt lamp) for 1-3 hours.

Hosts

Five Kenyan olive baboons, *Papio anubis,* weighing about 6.5kg and caught in a high-altitude non-schistosomiasis-endemic region, were used for the study. The animals were quarantined for 90 days, prior to initiation of experiments, during which time they were screened for common bacterial, viral and parasitic infections. They were also tuberculin tested. They were tested for prior patent schistosomiasis infection by the Kato technique (*Katz et al, 1972*) and miracidial hatching test (*Yole et al, 1996*).

As a further safeguard against the possibility of prior exposure to schistosomiasis, serum was obtained from each animal and tested for specific *Schistosoma mansoni* soluble worm antigen preparation (SWAP) immunoglobulin G, using enzyme linked immunosorbent assay (ELISA), as described by Nyindo *et al,* (*1999*). Only animals with antibody concentrations below 22µg/ml $\pm$ 3 standard deviations of the value obtained from a laboratory colony-born baboon and negative on the Kato and miracidial hatching test were used in the study. The animals were individually caged and fed on baboon pellets, fruits and vegetables, while water was provided *ad libitum*.Infection schedules comprised a large cercarial dose, of about 1000 cercariae, given once as a single-dose infection. All infections were done percutaneously by the pouch method, following anaesthesia (*Farah et al, 2000*).

The baboons were all sampled on Day 0 (pre-infection) and on Days 27, 54, 81 and 88 post-infection. They were then all treated with a curative dose of

praziquantel on Day 88 post-infection. This treatment was repeated 14 days later and was accompanied by sampling. All the five baboons were subsequently sampled on Days 123, 136, 164, 221 and 228 post-infection. On Day 228, the baboons were infected with a post-treatment challenge of 1,000 cercariae. The infection was done as previously described. The baboons were then sampled on Days 242, 256, 270, 284 and 298 post-initial-infection.
Uninfected baboons were used as the control and sampled at the same time with the infected baboons.

Blood Collection
The baboons were anaesthetised and 10ml of blood was collected from the inguinal vein of each animal, allowed to clot and centrifuged. The serum separated was then stored at -70ϒC ready for analysis (*Farah et al, 2000*).

Haptoglobin Determination
Haptoglobin was measured using the method described by Makimura & Suzuki (*1982*) with modifications by Conner *et al,* (*1988*). The assay uses purified bovine haptoglobin as standard. This test is based on the ability of haptoglobin to bind to haemoglobin and retain peroxidase activity at acidic pH, whereas free haemoglobin loses its peroxidase activity. On addition of the substrate, peroxidase activity resulted in a proportionate colour change, which was read off on an ELISA plate reader at 450nm.

Albumin Determination
Serum albumin was determined spectrophotometrically using bromocresol green solution as described by Varley (*1964*), with modifications by Keay & Doxey (*1984*). The test uses bovine albumin as a standard. It is based on the formation of a coloured complex by albumin in citrate buffer and bromocresol green. The absorbance of this complex is proportional to the albumin concentration in the sample. Absorbance was measured at 578nm for both samples and standard.

Statistical Analysis
Statistical analysis of the data was performed using SPSS and Excel software programmes. Excel was used to graphically depict trends in the analytes measured in infected animals and in uninfected controls. The data was analyzed for statistical significance at $p<0.05$ by one-way ANOVA with Duncan's Multiple Range Test (DMRT).

Results
Changes in the mean serum haptoglobin concentrations (±SEM) in *S. mansoni* infected baboons and the uninfected controls are depicted in Figure 1. The mean haptoglobin concentrations started to increase by Day 27 post-infection and showed a four-fold increase from pre-infection concentrations of 1.68±0.23g/l to 8.2±0.81g/l by Day 88 post-infection when treatment was instituted. Following treatment, the levels slightly increased to 8.4g/l±0.86g/l on Day 102. After this, they gradually decreased to near pre-infection levels of 2.3±0.71g/l on Day 228, when the animals were challenged with a second dose of *S. mansoni* cercariae. This resulted in haptoglobin levels showing a slow but gradual increase to reach a concentration of 6.1±0.95g/l on Day 298, when the experiment was terminated. The haptoglobin concentrations following initial infection, and thereafter following the challenge infection, were statistically different from pre-infection concentrations, except those of Day 228 post-infection. Mean haptoglobin concentrations of the infected baboons were also significantly different ($p <0.05$) from those of uninfected controls at all sampling points.
Changes in the mean serum albumin concentrations (±SEM) in *S. mansoni* infected baboons and uninfected controls are depicted in Figure 2. The mean albumin concentrations in infected baboons showed a gradual decrease from a pre-infection value of 47 ± 2.8g/l to 34 ± 3.9g/l at Day 88, when the baboons were treated curatively with praziquantel. Following treatment, the albumin levels showed minimal changes from pre-treatment concentrations up to the time of a second challenge

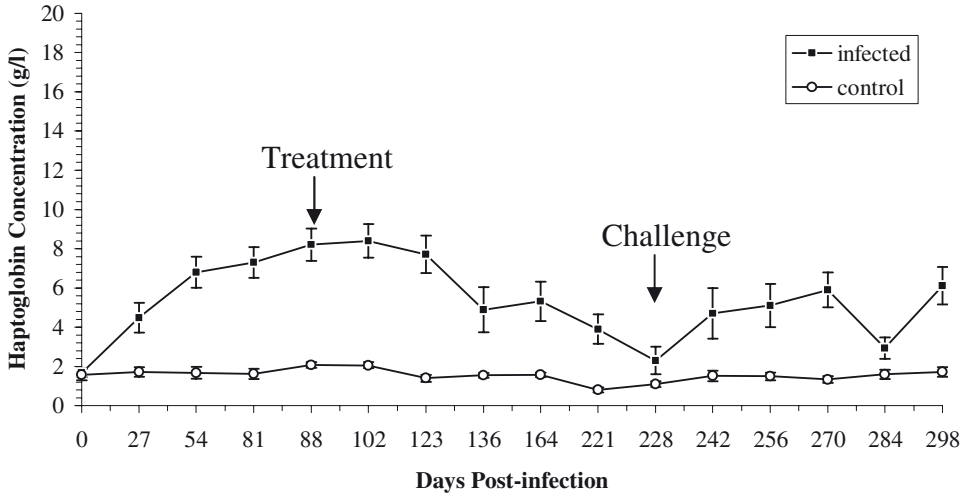

Figure 1. Mean Serum haptoglobin concentrations in control and S. mansoni infected baboons.

with *S. mansoni* cercariae. Following the challenge infection, the mean albumin concentrations of the challenged baboons then gradually started decreasing by 242 days post-infection to reach mean serum concentrations of 22.8 ± 1.5g/dl on Day 298 post-infection when the experiment, was terminated. The mean serum albumin concentration in the infected baboons was significantly dif-

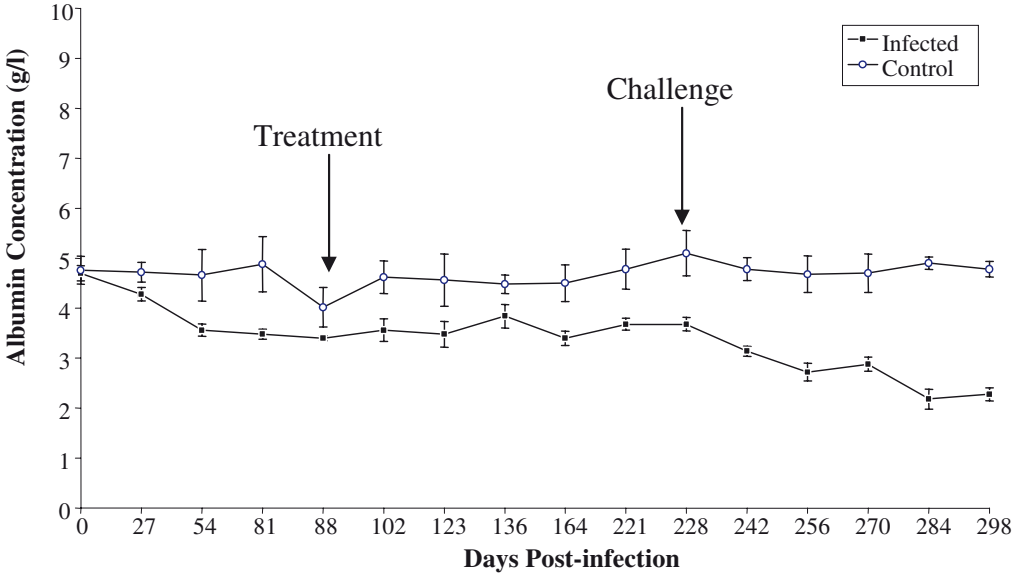

Figure 2. Mean Serum albumin concentrations in control and S. mansoni infected baboons.

ferent from control levels throughout the experiment ($p<0.05$). Post-infection concentrations only became statistically different (at $p<0.05$) from pre-infection concentrations on Day 54 post-infection. All post-challenge concentrations were, however, significantly different from pre-infection concentrations. The decrease in mean albumin levels was more marked following the second challenge infection as compared to the decrease following the initial infection. A possible explanation for this may be that following the challenge infection, the liver was further damaged, further impairing its ability to synthesise albumin. Indeed, even subsequent to curative treatment, albumin levels were not observed to rise back to normal pre-infection concentrations, hinting at some residual pathology of the liver.

At termination of the experiment, all infected animals appeared to have loss of body condition, ascitis, hepatomegally, splenomegally and slightly enlarged mesenteric lymph nodes. The livers of the animals demonstrated moderate density of granulomas and tissue fibrosis.

Discussion

Over the last few decades, acute phase proteins (APPs) have become the biomarkers of choice in human (*Kushner & Mackiewicz, 1987; Malle & De Beer, 1996*) and veterinary (*Alseemgeest et al, 1994*) medicine for monitoring inflammation and infection. Although acute phase assays are not specific for one disease, this seeming disadvantage is shared with other long established diagnostic tests such as temperature and heart rate measurements that have nevertheless proved useful over centuries of use (*Murata et al, 2004*). Furthermore, the application of APP measurements in diagnostics and determination of disease prognosis would be of great value because the APPs are not species-specific, they are easy to measure and are not adversely affected by physiological variations. They are also good indicators of early disease and residual pathology (*Solter et al, 1991; Gruys et al, 1993; Murata et al, 2004*).

The baboons are the most frequently used non-human primates in schistosomiasis research since they acquire natural infections (*Fenwick, 1969*), develop disease and acquire protective immunity similar to that in man (*Nyindo & Farah, 1999*). Farah *et al,* (*1997*) demonstrated that the pathogenetic process of *S. mansoni* could be faithfully produced in baboon models and that granuloma formation in the liver peaks around weeks 6-7 post-infection.

Haptoglobin has been described as a sensitive, specific and efficient disease marker in humans and several animal species including cattle, sheep and dogs (*Solter et al, 1991; Alsemgeest et al, 1994: Skinner & Roberts, 1994*). It is also less likely to give false positive and negative results in comparison to other indicators such as haematology (*Skinner & Roberts, 1994; Solter et al, 1991*). These findings have been echoed in the present study, which has found that there are indeed significant increases that occur in serum concentrations of haptoglobin, during the acute phase of *S. mansoni* infection.

Haptoglobin showed increases of up to 400% of pre-infection concentrations on Day 102 after the initial infection. Following curative treatment, haptoglobin levels were seen to drastically decline to near-control concentrations. However, normal pre-infection concentrations were not realized. This may have been possibly due to residual tissue pathology, especially that of the liver. Indeed, in *S. mansoni* infected mice, it was found that residual liver fibrosis hindered the attainment of pre-infection concentrations of haptoglobin even after curative treatment (*Mungatana et al, 2006*).

Following challenge with a second cercarial dose, a gradual increase in mean haptoglobin concentrations was observed at all sampling points except for Day 284. The anomaly observed in the mean haptoglobin concentration on Day 284 could have been due to factors that attenuate the acute phase response such as nutritional status and stress factors (*Kushner & Mackiewics, 1987; Alsemgeest et al, 1994*). Post-challenge increases were, however, not

as pronounced as those following the first infection, probably because the liver, which is the main source of haptoglobin, had not completely recovered from fibrosis, thus reducing its potential to produce the acute phase proteins (APPs). Moreover, repeated challenge has been shown to result in modulation of the granuloma in *S. mansoni* infected baboons, possibly due to changes in cytokine patterns as shown by Farah *et al* (*1997*). This demonstrates that there are differences between acute phase and chronic phase profiles of the haptoglobin, as has been observed in other chronic infections *(Salonen et al, 1996*).

Albumin was seen to gradually decrease following the initial infection, though to levels only slightly lower than those of control serum and more markedly following the challenge by a second infection. Post-treatment recovery of the albumin levels was also very gradual and virtually undetectable. These observations could be explained by the fact that the liver has large reserves of albumin synthetic capacity. In addition, albumin generally has a long plasma half-life (20 days in humans).

Acute phase proteins (APPs) might have pathophysiological functions during disease conditions such as schistosomiasis. Haptoglobin can influence the pathogenesis of disease in several ways. In the circulation, it binds iron, an effect that in the long run could improve iron conservation (*Ngure et al, 1997; Wigful, 2000*), which would mitigate the impact of anaemia sometimes seen in schistosomiasis. Also, the peroxidase activity of the haemoglobin-haptoglobin complexes might locally inactivate inflammatory cell products, which cause tissue damage in chronic disease (*Koj, 1974*). Haptoglobin, at acute phase response (APR) concentrations, has been shown to be able to significantly depress T-cell stimulation (*Oh et al, 1990*). The effects of low serum albumin are related to maintenance of fluid in the circulating compartment (albumin provides 80% of plasma oncotic pressure). With reduced levels of serum albumin, fluid may escape into the tissues to cause oedema or into body cavities to cause ascitis. This, in addition to periportal fibrosis, could have be a contributing factor in the ascitis seen in the pathology of the disease.

This study demonstrated that an acute phase response, accompanied by a decrease in serum albumin and an increase in serum haptoglobin, occurs in *S.mansoni* infection of baboons. Although changes in albumin levels were not easily noticeable, it should be noted that the increases in haptoglobin levels could be demonstrated in circulation fairly early in the infection, long before the development of definitive signs and symptoms. Moreover, the acute phase protein responded sensitively to success of treatment and also indicated the presence of residual pathology. Haptoglobin therefore proved to be a good indicator of acute infection, success of treatment, residual tissue pathology and chronic disease.

The results of this study have shown that the determination of the severity of tissue pathology and response to treatment in *S. mansoni* infection may indeed be enhanced by the measurement of serum haptoglobin. However, it will be important to identify the mechanisms responsible for the development of these acute phase reactions.

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