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Antibody Responses to Hydatid Cyst in Experimentally Infected Lambs

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Abstract: By now, very little is known about events occurring during early infection and innate immunity to infection with *E. granulosus* following ingestion of the infective egg stage and establishment of the primary cyst. In this study, ten lambs of 4 - 6 months old of mixed sexes were divided into 2 groups of 5 lambs (one test and one control group). Each lamb in test group received 2000 intraperitoneally protoscolices and each lamb in the control group was injected only PBS. Sera were collected every two weeks until 24 weeks after challenge from each group and serum antibodies were tested by ELISA. Also white blood cells were measured every 2 weeks during this trial. All lambs were killed after 6 months and the internal organs were inspected for hydatid cysts. The results showed that the production of antibody was higher in three lambs of the test group in comparison with control group. In these lambs, hydatid cysts were observed in internal organs. The number and also the average size of hydatid cysts in these lambs were different. In two lambs of the test group, the level of antibody was increased gradually for a few weeks and was then constant. No cyst was observed in these lambs. The numbers of leukocytes including: eosinophils, lymphocytes, neutrophils and monocytes were increased in all of the lambs challenged with protoscolices in comparison with control group. The number of these cells was higher in lambs of test group with no cyst. In conclusion, this study showed that the production of antibody is not able to completely to protect against hydatid cyst and humoral immunity might be correlated with susceptibility to disease in lambs.

Key word: Innate immunity % Humoral immunity % Protoscolices % ELISA % Lamb

INTRODUCTION

Cystic echinococcosis (CE) is a widespread chronic endemic helminthic disease caused by infection with metacestodes (larval stage) of the tapeworm *Echinococcus granulosus*. CE affects humans and a wide range of livestock species [1, 2]. Although the host-parasite interplay, in most cases of echinococcosis appears to be harmonious and clinically asymptomatic for a long period after infection, the host does produce a significant immune response against the early stages of infection, while the parasite adapts highly effective evasive strategies to aid in survival. After the oncosphere locates a target organ, the small hydatid cyst that commences development is immediately confronted by the host immune responses, which are mainly cell-mediated, especially involving infiltration of macrophages and eosinophil cells and low-level polarized Th1 responses. Antibody responses are weak and are, normally, undetectable in the early two to three weeks following infection. In experimentally infected sheep, antibodies to hydatid antigens can be detected as early as 4 to 6 weeks postinfection and persist for at least 4 years [3]. It has been shown that sheep produce specific immunological response during natural *E. granulosus* infections [4]. However, it seems that these responses do not lead to raised serum antibody level in many animal or they are not maintained throughout the course of the infection [5]. The understanding of the mechanisms on the polarization of different subsets of immune cells may be relevant for the rational design of immune intervention and vaccination protocols [6]. The established parasite produces significant quantities of antigens that modulate the immune responses and these include polarized Th2 responses, balanced with Th1 responses. The coexistence of elevated Th1 cytokines, especially interferon (IFN)-(

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and Th2 cytokines including IL-4, IL-5, IL-6 and IL-10, has been recorded in most hydatid patients where cytokine levels have been measured. When a cyst dies naturally, or it is killed by chemotherapy treatment, or is removed by surgery, Th2 responses drop rapidly and Th1 responses become dominant [7]. Collectively researchers finding indicated that in hydatidosis a strong Th2 response correlate with susceptibility to diseases, whereas a Th1 response correlates with protective immunity [8].

The aim of this study was to analyze the humoral response in experimentally infected lambs with protoscolex and also to evaluate the changes of white blood cells during this trial by differential white cell count test (DIFF) in these lambs.

MATERIALS AND METHODS

Preparation of Samples: Hydatid fluid was isolated from livers or lungs of sheep with hydatid cyst in sterile conditions. Hydatid fluid was centrifuged at 5000 g for 30 min at 4°C to remove protoscolices. Isolated protoscolices were washed with hank's solution three times.

Challenge and Sera Collection: Ten lambs with 4-6 months old of mixed sexes were divided into 2 groups of 5 (one immunized and one control group). Each lamb in test group was challenged with 2000 protoscolices, intraperitoneally [9]. Each lamb in control group was injected with PBS. Serum samples were collected every two weeks until 24 weeks after challenge from each group and serum antibodies were tested by ELISA.

Differential White Cell Count Test : Blood samples were obtained by syringe and collected in tubes containing EDTA. White blood cells were measured every 2 weeks during this trail using differential white cell count test.

Necropsy: Approximately 6 months after the experimental infection, all lambs were euthanized and the internal organs were inspected for hydatid cysts. The carcasses were dressed and examined superficially for the presence of hydatid cysts. The heart and kidneys were sliced and the omentum and spleen examined. The liver and lungs were examined extensively; 2 and 4-5-cm sections of the liver and lungs, respectively, were prepared and palpated.

ELISA : ELISA was performed in a 96-well microtitration plates (Polysorb, PISHTAZ TEB). The plate was coated overnight with secreted antigens of *E. granulosus* (diluted in 10 mM carbonate buffer [pH 9.6] in order to give protein concentrations of 2.5µg/ml for detection of *E. granulosus*-specific antibody. All the solutions were used at 300 µl per well. One hundred µl of sera samples at a 1:10 dilution in PBS containing 1% v/v of Bovine serum albumin (PH=7.4) was loaded into duplicate wells and incubated for 1 hour at room temperature (RT). Duplicate positive and negative control sera were used. The wells were washed by ELISA washer five times with PBS-Tween 20 (0.05%). Then, 100 µl of horse radish peroxidaze (HRP)-labeled polyclonal antibodies against sheep IgG at a 1:2,000 dilution in PBS+1% BSA was loaded into all the wells and incubated for 1 hour at RT. The plate was washed as described above to remove the excess conjugate. For color development, 100 µl of 3,3',5,5'-Tetramethylbenzidine (TMB) was added to each well as a substrate and the reaction was terminated after 15 min by addition of 100 µl of 1M of HCL solution to each well. The absorbance at 490 nm was monitored in ELISA reader.

RESULTS

Humoral Immune Response: Results showed that the level of antibody in test group was higher than in control group, although the level of antibody was variable in lambs of test group. The results showed that the production of antibody was higher in three lambs of test group which were challenged with protoscolices in comparison with control group (Figure 1). In these lambs, hydatid cysts were observed in internal organs. The number and also the average size of hydatid cysts in lambs were different. Mean size of hydatid cysts in challenged lambs were 3.7 cm. In two lambs of test group, the level of antibody was increased gradually for a few weeks and was then constant (Figure 1). No cyst was observed in these lambs.

Differential WBC Count : In this study, white blood cells were measured until 24 weeks post-challenge with an interval of 2 weeks. The number of eosinophils (Figure 2), lymphocytes (Figure 3), neutrophils (Figure 4) and monocytes (Figure 5) were variable in all of lambs challenged with protoscolices in comparison with control group, although, the number of cells was higher in two lambs of test group with no cyst (Figure 2, 3, 4, and 5).

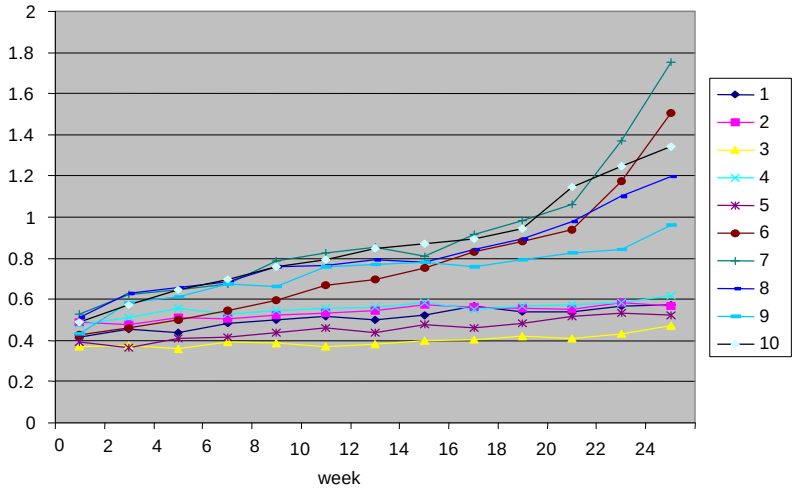

Fig. 1: The level of antibody in test and control groups until week 24. Number 1-5 indicate control and number 6-10 indicate test group, respectively

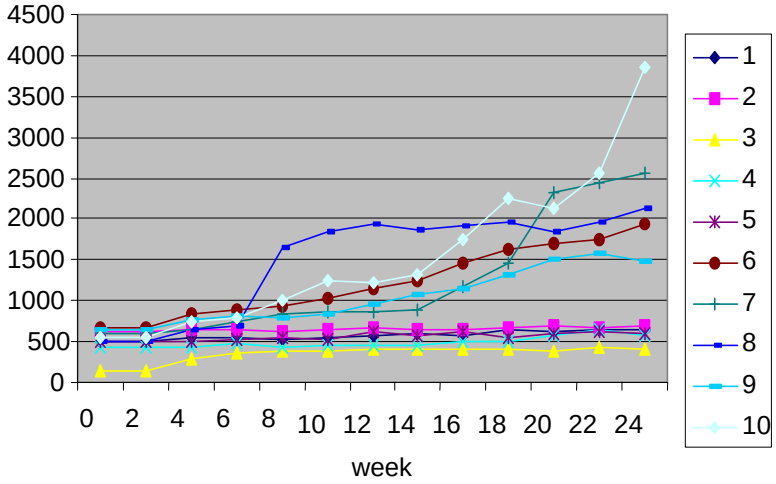

Fig. 2: The numbers of eosinophils, in test and control groups until week 24. Number 1-5 indicate control and number 6-10 indicate test group, respectively

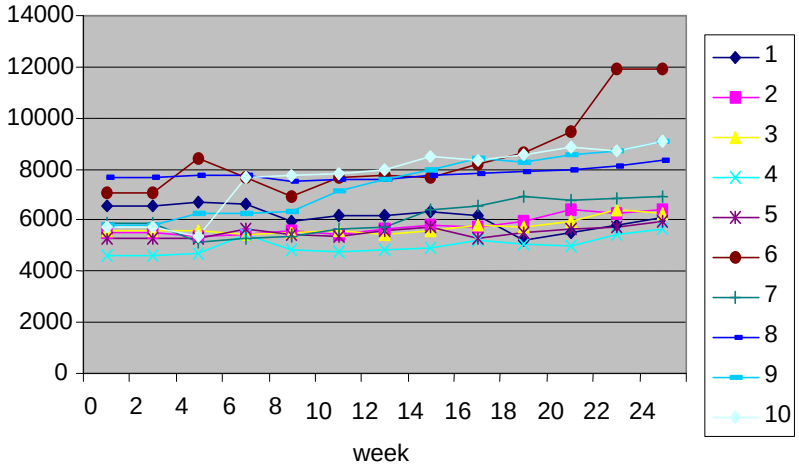

Fig. 3: The numbers of lymphocyte, in test and control groups until week 24. Number 1-5 indicate control and number 6-10 indicate test group, respectively

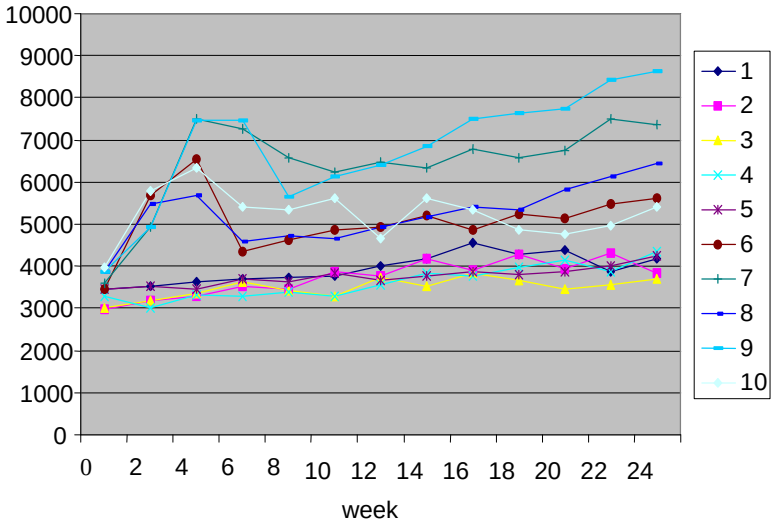

Fig. 4: The number of neutrophils, in test and control groups until week 24. Number 1-5 indicate control and number 6-10 indicate test group, respectively

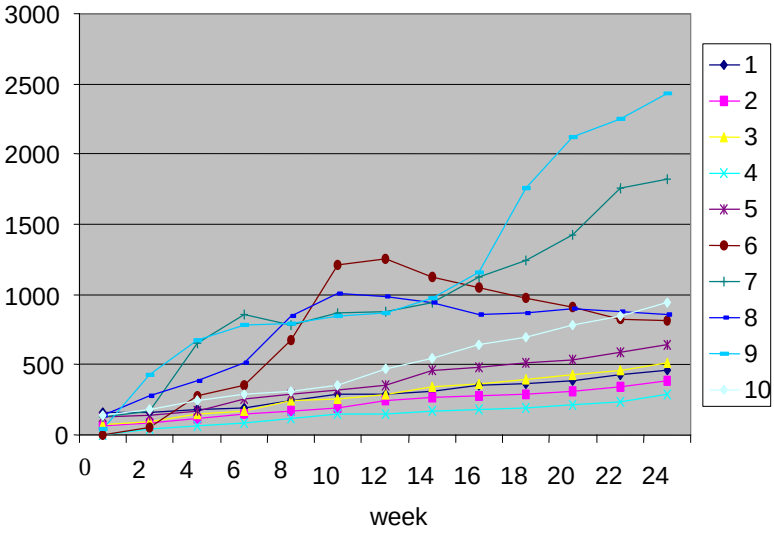

Fig. 5: The number of monocytes, in test and control groups until week 24. Number 1-5 indicate control and number 6-10 indicate test group, respectively

DISCUSSION

By now, very little is known about events occurring during early infection and innate immunity to infection with *E. granulosus* following ingestion of the infective egg stage and establishment of the primary cyst [10].

In our study**,** there was an increase in eosinophils, lymphocytes, neutrophils and monocytes in lambs challenged with protoscolices mainly in two of them. Because two lambs of the test group did not show any hydatid cyst in internal organs in the end of experiment, our results indicated that the innate immunity might play an important role in early infection with protoscolices. Early infections may be associated with a significant cellular inflammatory response [11] that may cause pathologic changes [12], since there is an increase in leukocytosis, mainly of eosinophils, lymphocytes and macrophages [13]. At the early stages of the disease, there is a marked activation of cell-mediated immunity to the parasite [14]. In experimental infection, less than %10 of protoscolices survive to form cysts [15]. The majority of parasite killing occurs weeks postinfection. Activated macrophages are involved in the killing of *Echinococcus* protoscolices [16]. In the early stages of echinococcal

development, cellular responses may play a crucial role in protection against infection. Early experiments in vitro showed that neutrophils, in association with antibody, can bring about the killing of *E. granulosus* oncospheres [17], suggesting a possible role for antibody-dependent cell mediated cytotoxicity (ADCC) reactions, although antibody levels against this stage are low and/or cell-mediated immunity may induce killing. It is generally accepted that *Echinoccocus* is unaffected by the immune response during the developing stage. However, natural infections in sheep indicate that some cysts can be killed during the latter stages of development, with the relatively frequent occurrence of dead, calcified metacestodes or necrotic cysts [18]. These are due to the degeneration of the primary cyst, leaving the cavity full of host leukocytes and protoscolex-derived daughter cysts [19]. There is no direct evidence that the death of such cysts is due to an immunological phenomenon, but it is a likely possibility. If a progression in cyst degeneration does take place, then the immune response may play a role in the death of the parasite. This may signify increased immunological stimulation with cyst progression. Unfortunately, there are no detailed studies of immunological events associated with the degeneration of different types of cyst and therefore, unknown mechanisms may be involved [3].

In our study, the level of antibody in three lambs which received protoscolices was higher than in the control group after 6 months. Although, the level of antibody was increased in these lambs, some hydatid cysts were observed in internal organs. In two lambs of the test group, the level of antibody was increased gradually for e few weeks and then was constant to the end of this experiment. No cyst was observed in these lambs. This result indicated that in hydatidosis the production of antibody is not able to completely protect against hydatid cyst. Increasing the level of antibody during hydatidosis could indicate the sensitivity of lambs to hydatid cyst. Our results were correlated to the results of [20-22] Vuitton 2003; Ortona *et al*. 2003 and Fraize *et al.,* 2005. Results of these studies indicated that in Echinococcus spp infection in intermediate host, Th2 response is associated with susceptibility to the disease, whereas a Th1 response is associated with protective.

In conclusion, our data indicated that the innate or cellular immunity might be more effective than humoral immunity against the hydatid cyst. This is an important area that needs to be further explored as it may provide an understanding of the mechanisms of protection against the hydatid cyst.

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