Effect Of Toxin Fractions Isolated From Protoscoleces And Hydatid Cyst Fluid Of Sheep Origin On Phagocytosis In Mice

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Abstract

Effect of toxin fractions of protoscoleces (P) and hydatid cyst fluid (HCF), isolated from hydatid cysts of sheep origin on phagocytosis was investigated. Mice of the species *Mus musculus* were injected with these TFs, at different concentrations (2.5&5µg), prior to infection with secondary hydatid disease. Results show that neutrophils express a different affinity towards TFs of different concentrations and different sources (P and HCF). Phagocytosis was higher in +ve control compared to –ve control, and was higher at concentration 2.5µg compared to 5µg in P and was the other way around in HCF at 5µg, indicating that there is a correlation between concentration of TFs, their source and their effect.

Key words: toxin fraction, hydatid, phagocytosis, mice

Introduction

Parasites develop and survive in an environment which is often hostile to them. When facing aggressive conditions, parasites are able to use various and complex strategies to evade host's immune response[1].Both parasites and hosts at the same time attack each other and must defend themselves. Success of the relationship between the two partners is, at least in part, based on an equilibrium between the attack and defense systems. The term immunohomeostasis describes this equilibrium[2].

Although the mechanisms used by *Echinococcus granulosus* to evade the host's immune response are poorly understood, it is well known that infected hosts generally develop a strong immune response to the parasite. However, once the metacestode is established and while it remains undamaged, the host is usually unable to destroy it, suggesting that interference with potentially effective host immune responses could be an important parasite evasion strategy[3].

Survival of established hydatid cysts in the host could be related to cytotoxicity of the cysts, as has been first suggested by Deschiens and poirier[4].Recently, cytotoxicity of hydatid cyst fluid (HCF) on host cells and tissues has been demonstrated *in vitro* and *in vivo* (for references see[5,6].

In a previous work, Salih and Abbu[7]presented a preliminary evidence that toxin fractions (TFs) are strain-specific and can be used to differentiate between strains of *E. granulosus*. One year later, Salih and Abbu[5] studied phagocytosis in mice treated with TFs isolated from HCF obtained from cysts of sheep, cattle and human origin, using two concentrations(10 and 50µg). These two concentrations have been used by Al-Ezzy[8] as immunomodulators in mice against infection with secondary hydatid cysts in which phagocytosis has been studied as one of the criteria taken into consideration. In the present study, the effect of TFs, isolated from protoscoleces and HCF obtained from cysts of sheep origin,at two concentrations(2.5and5µg),on phagocytosis, is investigated, aiming at demonstrating which source of TFs and which concentration is more effective in stimulating phagocytosis in mice infected, experimentally , with secondary hydatid disease.

Materials And Methods

1. Source of hydatid cysts

Hydatid cyst of sheep origin were obtained from infected livers of sheep in Nineveh slaughter house.

2. Isolation of cyst fluid

Protoscoleces were collected from the cysts, aseptically, according to Smyth[9].Viability was estimated according to Smyth and Barrett[10] and only cysts with viability rate more than 90% were used. After centrifugation at 7600g(10000 rpm), using a cryofuge 6-4 (heraeus) for 10 minutes at 4°C, the supernatant (HCF) was separated and kept in sterile containers in refrigerator at–20°C until used.

3. Separation of protoscoleces and cyst fluid toxin fractions

Cysts fluid fractions(CFFs) were separated according to Janssen et al.[11]. Ammonium sulphate was added to the cyst fluid and supernatant of homogenated protoscoleces* each one alone, (49.35 gm/100ml) and the fluid was left in the refrigerator at 4°C for 24 hrs to give enough time for precipitation of protein. The fluid was centrifuged. An equal volume of chloroform was added to the supernatant. Two layers were formed after centrifugation. The chloroform layer was separated and half volume of methanol (chloroform: methanol =2: 1,v/v)was added and centrifuged under the same conditions mentioned above. The supernatant was dried by rotary evaporator. The chloroform-methanol soluble fractions (CMSFs),or TFs, were kept in refrigerator at-20°C until used. At use, they were dissolved in few drops of chloroform and completed by phosphate buffer saline (PBS). Similarly, TFs of P was also extracted.

4. Experimental design

30 Parasite-free, laboratory-bred, 5-6 weeks old male, BALB/c mice were used in the present study. They were injected, intraperitonealy (i.p.), with approximately2000protoscoleces and hydatid fluid toxin fractions isolated from hydatid cysts of sheep origin as follows:

Experiment 1

5 mice were not treated with TFs and not infected with protoscoleces (-ve control group).

Experiment 2

5 mice were injected with approximately 2000 protoscoleces only, but not with TFs (+ve control group).

Experiments 3-6

In each experiment,5mice were injected with at the concentrations2.5and5µg of protoscoleces origin (experiment 3 and 4, respectively) and the same concentrations of HCF(experiments5and 6, respectively).

5. Phagocytosis

Phagocytosis was studied using nitro blue tetrazolium (NTB) reduction by neutrophils, 10µl of the dye was mixed with the same

*protoscoleces were suspended with phosphate buffer saline(1:4v/v),then freezed and thawed two times prior homogenization with ultra sonicator at 24 waves for 3-6 minutes, finally the liquid was centrifuged at 14000rpm for15minutes to separate the supernatant (crude extract of P).

volume of blood obtained from investigated hosts, and incubated at 37°C for30 minutes. Then, blood films were prepared, stained with leishman stain and examined under the microscope. Neutrophils which showed positive results (reduced the dye) were counted according to park et al. [12] as follows:

$$\text{Phagocytic index} = \frac{\text{No. of neutrophils reduce pigments}}{\text{Total no. of neutophils}}$$

6. Statistical analysis

Complete Randomized Design (CRD) and Duncan's Multiple Range Test were used to establish the difference between the means at the level $p < 0.05$.

Results

Phagocytic activity of microphages (neutrophils), represented by their ability to reduce the NTB pigments to formazan particles, in male mice treated with toxin fractions isolated from protoscoleces(P) and hydatid cyst fluid(HCF)of sheep origin, compared to–ve and +ve control groups, are shown in table(1). It is obvious from this table that infecting mice with whole protoscoleces (establishing secondary hydatid cysts) seems to have stimulated phagocytosis (+ve C group) when compared with the–ve C group.On the other hand, infecting mice with secondary hydatid cysts after treating them with toxin fractions from both sources (Pand HCF) caused a significant decrease in phagocytosis, However, this decrease seems to be dependent on the concentration of the TFs and on their source (PorHCF). highest decrease was obtained with HCF TFs at 2.5 µg of the same source.

Table (1) phagocytosis in mice treated with toxin fractions isolated from protoscoleces (P) and hydatid cyst fluid (HCF) of sheep origin.

Groups	Phagocytic index at times (days) post infection, (Mean ± SE)			
	Concentration µg	3	15	30
P	2.5	23.0 ± 4.92 d	15.25 ±3.20 efg	6.0 ± 0.91 hij
	5.0	21.0 ± 2.04 de	11.75 ± 2.10 ghi	6.0 ± 1.73 hij
HCF	2.5	17.75 ± 2.87 d-g	11.50 ± 1.05 ghi	4.75 ± 1.55 ij
	5.0	19.5 ± 3.01 def	13.0 ± 2.38 fgh	8.0 ± 1.78 hij
-ve C		2.75 ± 0.75 j	3.25 ± 0.48 j	4.25 ± 0.48 j
	+ve C	74.25 ± 2.02 a	65.0 ± 1.78 b	55.75 ± 1.65 c

Means with different letters vertically and horizontally represent significant difference at $p \leq 0.05$

Discussion

A good deal of advanced work on host-parasite relationship has been accomplished during the last decade among which immunological studies on cestodes, in general, and metacestodes of *E. granulosus*, in particular, have been commenced [for references see 3, 13]. However, there is still a lot to be learned on immunological reactions which take place during development of *Echinococcus* strains in their hosts [14].

Phagocytic cells are of two types, polymorphoneuclear neutrophils (microphages), in blood, and mononuclear cells (macrophages), distributed in the tissues of the host´s body [15]. In general, they represent the first line of defense in the body of the host.

The present study was conducted to demonstrate the changes in the phagocytic activity of neutrophils (microphages) in the blood of mice treated with TFs, isolated from P and HCF of sheep origin prior to infection with secondary hydatid disease. Riott *et al*. [15] pointed out that neutrophils comprise over 95% of the circulating granulocytes. In the blood, one of the mechanisms by which these cells destroy the foreign bodies is trapping and engulfing them by chemotaxis, adherence, ingestion and finally digesting them by lysosomes present in their cytoplasm [16].

In hydatid disease, toxicity comes from factors present in the HCF, secreted by the germinal layer(protoscoleces are buds of germinal layer), these factors have been found to be lipid-like in nature as it was possible to isolate them by chloroform extraction only [17]. Salih and Abbu [5] presented an evidence that TFs of HCF possess an activity against microphages in mice, supporting the findings of Janssen *et al*. [18, 19] that TFs of HCF can also be found active outside the living parasite as a whole, indicating a clear susceptibility of microphages to Tfs. However, for all foreign bodies to be engulfed by phagocytic cells, it is a prerequisite that they should be hydrophobic, and since TFs of hydatid cyst have been shown to possess a lipid-like properties i.e.

hydrophobic in nature [17], they can be readily attracted to the phagocytic cells.

In the present study, Phagocytosis was lower when neutrophils were treated with TFs of HCF origin than those of P origin, indicating that these cells express a different affinity (different susceptibility) towards TFs of different origin (different source) and express higher affinity towards lower concentration of TFs of HCF and vice versa for TFs of P origin. The only explanation available is that neutrophils could have been damaged due to treatment with TFs which could be, in turn, due to changes in the metabolic activity of these cells and loss of rigidity in their membranes, as has been speculated by Janssen *et al*. [18].

The main conclusion which could be drawn from the results of the present study is that, there is an obvious correlation between the concentration of TFs, the source from where they are obtained from and their effect. However, further work is needed to clarify the mechanism by which these fractions destroy phagocytic cells and the possibility of using any of these fractions, at a certain concentration, in inducing an immunomodulation of the host immune response against infection with hydatid disease which may open up new clues in the fight against this disease.

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تأثير الأجزاء السامة المعزولة من الرؤيسات الاوليه والسائل العدري من أصل أغنام على معامل البلعمه في الفئران

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الملخص

درس تأثير الأجزاء السامة للرؤيسات الاولية والسائل العدري المعزولة من الأكياس العدريه من أصل أغنام، على عملية البلعمه. حقنت فئران بيض من نوع *Mus musculus* بالأجزاء السامة ،بتراكيز مختلفة، قبل إحداث الإصابة بداء الأكياس العدريه الثانوي. تبين النتائج أن البلاعم الصغيرة (العدلات) تعبر بألفه مختلفة تجاه الأجزاء السامة بتراكيزها المختلفة ومصادرها المختلفة (رؤيسات أوليه وسائل الكيس العدري). كانت عمليه البلعمه أعلى في ضابط السيطرة الموجبة مقارنة بنظيره ضابط السيطرة السالبة وكانت أعلى عند التركيز ٢,٥ مايكروغرام مقارنة بالتركيز ٥ مايكروكرام في الرؤيسات الأولية وكانت على العكس من ذلك في سائل الكيس العدري عند التركيز ٥ مايكروغرام ، مشيرة بان هناك علاقة بين الأجزاء السامة ومصدرها وتأثيرها.