PERIPHERAL LYMPHOCYTE SUB – POPULATIONS ANALYSIS IN HYDATIDOSIS PATIENTS WITH DIFFERENT CLINICAL PARAMETERS

Ban N. Al-Qadhi* ,Tariq. I. Al-Jeboori, Nada. M. Al-Bashir*****
*Department of Biology, College of Science, University of Baghdad. Baghdad- Iraq.
** Department of Microbiology, College of Medicine, University of Al-Nahrain. Baghdad- Iraq.
**Medical Research center, University of Al-Nahrain. Baghdad- Iraq.

Abstract

Lymphocyte sub-population analysis had been done by indirect and direct immunostaining (CD-markers) technique for eighty- two hydatidosis patients with different clinical parameters. This technique showed, a significant decrease in percentage of CD3, CD4 cells in hydatidosis patients compared to healthy control group,while the results showed that the level of CD8 cells was higer than the control group. The CD4/ CD8 ratio was lower in hydatidosis patients in comparison to the control group.Furthermore, the percentage of CD14 cells of hydatidosis patients was significantly lower than that of the control group,and there was insignificant increase in CD22 of hydatidosis patients in comparison to that of control group.In general,this study showed that there was no significant difference in all types of CD cells percentage between hydatidosis patients in relation to different clinical parameters,except in relation to the size of the cysts.

تحليل أنواع أجيال الخلايا اللمفاوية المحيطية في مرضى الأكياس العدرية ذوي الدلائل الطبية المختلفة

*** بان نوري القاضي ،** طارق إبراهيم الجبوري و*** ندى محمد طه البشير**
*قسم علوم الحياة ،كلية العلوم ،جامعة بغداد. بغداد– العراق.
**قسم الاحياء المجهرية, كلية الطب ،جامعة النهرين. بغداد– العراق.
***مركز البحوث الطبية ، جامعة النهرين. بغداد– العراق.

الخلاصة

تم التعرف على أنواع أجيال الخلايا اللمفاوية المحيطية لاثنين وثمانين مريض مصاب بالأكياس العدرية ذوي الدلائل الطبية المختلفة بطريقة التصبيغ المناعي المباشرة وغير المباشرة. بينت هذه الدراسة وجود انخفاض معنوي في نسبة خلايا الـCD3 والـCD4 في مرضى الأكياس العدرية مقارنة بمجموعة السيطرة بينما كانت خلايا الـCD8 أعلى من مجموعة السيطرة.نسبة خلايا CD8/CD4 كانت اقل في مرضى الأكياس العدرية مقارنة بمجموعة السيطرة ,علاوة على ذلك كان هناك انخفاض معنوي في نسبة خلايا الـCD14 لمرضى الأكياس العدرية مقارنة بمجموعة السيطرة وكان هناك ارتفاع غير معنوي في نسبة خلايا الـCD22 في مرضى الأكياس العدرية مقارنة بمجموعة السيطرة.بشكل عام أظهرت هذه الدراسة عدم وجود فروق معنوية بين كل أنواع الخلايا اللمفاوية المعلمة(CD-cells) بين المرضى بالدلائل الطبية المختلفة ما عدا حجم الأكياس.

Introduction

Hydatidosis is the parasitization of tissue by the larval stage of different species of the genus *Echinococcus* ,and represents a public health problem with important economic implications (1). Diagnosis of hydatidosis is mainly based on two phenomena: Analysis by morphological methods (radiology, echography , and nuclear magnetic resonance imaging) and analysis by immunological methods (detection of antibodies, antigens, circulating immune complexes, delayed hypersensitivity and lymphoproliferative assays) (2). Peripheral blood lymphocytes (PBL) also can give an idea of immunological status in patients with many diseases. Surface phenotyping criteria are the main index has been used to clarify lines of immune defense mechanisms.

Lymphocytes and other leukocytes express a large number of different molecules on their surface. Such molecules which can be used to distinguish cell populations , are called markers and many of them can be identified by specific monoclonal antibodies (3). Therefore , the aim of this study was to determine the lymphocyte subpopulation involvement in the host response to hydatidosis infection by the use of CD – markers (CD3, CD4, CD8, CD14 , CD22) technique.

Materials and methods

Subjects

Eighty-two patients infected with hydatidosis from different hospitals in Baghdad were included in this study, 35 of them males and 47 females , age range was 5-70 years with 25 patients clinically diagnosed (pre-operation) and 57 patients surgically confirmed (post-operation) , among the patients 31 had cysts less than 5cm, and 37 had cysts more than 5cm.while the others 14 patients with rupture cysts.

The cysts were found in different sites of the body (liver , lung , heart , spleen and kidney).

On the other hand,thirty- three healthy volunteers were included as the control group. These had negative specific serology which confirmed by Indirect Heamagglutination test using commercial Kit (bio Merieux , France).

Blood samples collection

Four ml of venous blood were collected from each individual (patients and control) in sterile heparinized tubes and mixed well to avoid clotting,and used for the lymphocyte separation assay.

Analysis of lymphocyte sub-populations by immunostaining technique

The Isopaque - Ficol technique , originally described by (4) was used for isolation of lymphocytes as follows:

Four ml of heparinized blood was diluted with an equal volume of phosphate buffer saline (PBS) then layered over 4 ml of lymphocyte separation medium in a sterile glass tube. After centrifugation at 120XG for 30 min. in a centrifuge (18°C),the buffy coat layer of lymphocytes was collected using Pasture pipette.The aspirated lymphocytes were washed three times with PBS.Viable cell count was performed using trypan blue exclusion technique.Dead cells took up the dye within a few seconds and appear blue when viewed under the microscope using a Newbauer counting chamber.

Cell number was adjusted to contain $1x10^6$ cell / ml.10 µl of lymphocytes suspension was placed per well of an immunofluorescent slide, allowed to air-dry at room temperature,then 10µl acetone as a fixative was added,allowed to air –dry at room temperature,then wrapped in foil and stored at -20°C till used.

The immuofluorescent slides pre-coated with lymphocytes were removed from the freezer and allowed to reach room temperature. The coated lymphocytes were probed with 10µl of mouse monoclonal antibodies raised against specific human CD markers (CD3 , CD4,CD8, CD22) diluted in 1:10 PBS separately.

Slides were then incubated at 37°C for 2 hours,non reacted monoclonal (Serotic co.) antibodies were removed by three cycles of washing with PBS, air dried and mounted with glycerin. Slides were examined under 40x magnification of a fluorescent microscope (Olympus) .Their dark green staining identified positively labelled cells.200 cells were counted to determine percentage of reactivity of the tested monoclonal antibodies.

While for analysis of percentage of CD14 the direct immunofluorescent technique was used as follows :

1. Slides coated with lymphocytes were taken out of the freezer and washing steps beings by dipping slides into PBS filled jar with stirring for 10 min. and for just one time.
2. Ten µl of 1:5 diluted of immunfluorescence - labeled monoclonal antibodies which were of

CD14.This type was added applied to duplicate wells,and then incubate slides within a humid chamber at 37°Cwith mild shaking for two hours,and repeat step 1.
3. One to 2 drops of mounting fluid was added onto each well to prevent dehydration of staining fluids and to avoid fading out of fluorochrome - labeled antibodies.
4. Cover slips were placed on the mounting fluid-spotted slides.At this stage slides become ready for examination by fluorescent microscope at 40x to 100x objective lenses immediately or up to one day later as maximal duration. Their dark green staining identified positively labeled cells.100 cells were counted to determine percentage of reactivity of the tested monoclonal antibodies.

Statistical analysis

Statistical manipulations conducted in the analysis of the data including general linear model was used when comparing different clinical conditions followed by least significant difference test (LSD) and Duncan letters for multiple comparisons between the means.These manipulations were carried out according to Statistical Analysis System(5).

Results

Phenotyping of (PBL) had been done by indirect and direct immune-staining methods by using anti - CD3 (total T-cells detection) , anti – C D4 (T-helper/inducer cells detection), anti-CD8 (T-cytotoxic / suppressor cells detection), anti - CD14 (macrophage cells detection) , and finally , anti-CD22 (B-cells detection). The CD4 / CD8 ratio was of special importance because it represents an index that refers to the immunological balance between T-helper cells and T-cytotoxic cells in the immune system, in that the higher CD4 / CD8 ratio the nearer balance point would be to T-helper cells, which means lower cytotoxic activity and higher other forms of cell mediated immunity (CMI) and humoral immunity.

Total T-lymphocytes (CD3)

Hydatid disease patients have shown CD3 cells Percentage (65.72 ± 1.57%) which was significantly lower (P<0.05) than the control group (76.36 ± 2.10%) (Table 1).But , there was no significant difference (P>0.05) in CD3 cells percentage between hydatid disease patients in relation to different clinical parameters(sex, age, operation and site of infection) (Table 2), except in relation to the size of cyst (Table 2). There was a significant increase (P<0.001) in CD3 cells percentage (72.63 ± 1.41%) in patients with small hydatid cysts in comparison to patients with large cysts (58.98 ± 2.38 %) (Table 3).

T-helper / inducer lymphocytes (CD4)

The percentage of CD4 cells was (45.49 ± 1.19%) in hydatidosis patients and it was significantly lower (P < 0.05) than that of the control group (51.73 ± 1.40 %) (Table 1) . However, there was no significant difference (P > 0.05) in CD4 cells percentage between hydatidosis patients in relation to different clinical parameters (Table 2). On the other hand, and in relation to cysts size , small cysts showed significant increase (P<0.001) in CD4 cells percentage (51.16 ±1.98%) more than the large cysts (40.04 ±1.82%) (Table 3).

T- cytotoxic / suppressor lymphocytes (CD8)

The percentage of CD8 cells (36.14 ± 0.94%) of hydatidosis patients was significantly higher (P<0.05) than that of the control group (32.54 ± 1.22%) (Table 1). But, there was no significant difference (P>0.05) in CD8 cells percentage between hydatidosis patients in relation to all clinical parameters including the cyst size (Tables 2) and Table (3).

CD4/CD8 ratio

CD4 / CD8 ratio was lower in hydatidosis patients (1.25) than that of the control group(1.58) (Table 1).

Macrophage lymphocytes (CD14)

The percentage of CD14 cells of hydatidosis patients (7.89 ± 0.17%) was significantly lower (P<0.002) than that of control group (9.20 ± 0.42%) (Table 1). While , there was no significant difference (P>0.05) in CD14 cells percentage of hydatidosis patients in relation to different clinical parameters (Table 2), except in relation to the size of the cyst.The small cysts showed significant increase (P<0.005) in CD14 percentage (8.42 ± 0.09%) in comparison to the ones with large cysts (7.34± 0.37%) (Table 3).

B-Lymphocytes (CD22)

There was a relative increase in CD22 cells percentage of hydatidosis patients (11.45 ± 0.58 %) in comparison to CD22 cells percentage of the control group (10.37 ± 0.47%) but this increase was not significant (P > 0.05) (Table 1). However , all different clinical parameters showed no significant differences (P > 0.05) between hydatidosis patients including the size of the cyst (Tables 2) and Table (3).

Table 1:Mean percentage ±SE of CD3,CD4,CD8,CD14 andCD22 lymphocytes populations in the sera of hydatidosis patients and control group

CD% / Group	CD3 M±SE	CD4 M±SE	CD8 M±SE	CD14 M±SE	CD22 M±SE	CD4/ CD8 ratio
control	76.36 ±2.10 * a	51.73 ±1.4 a	32.54 ±1.22 * b	9.20 ±0.4 a	10.37 ±0.47 a	1.58
patients	65.72 ±1.57 b	45.49 ±1.19 b	36.14 ±0.94 a	7.89 ±0.17 b	11.45 ±0.58 a	1.25

*a,b Duncan letters

Table 2:Mean percentage ±SE of CD3,CD4,CD8,CD14andCD22 lymphocyte populations for hydatidosis patients in relation to sex,age,operation and site of infection.

CD-marker typing	Sex		Age/years			Operation		Site of infection			
	♂	♀	<10	11-40	>40	Pre	Post	A	B	C	D
CD3 ±SE	66.95 ±2.65 a*	64.48 ±2.45 a	67.32 ±2.56 a	65.17 ±2.45 a	65.3 ±3.7 a	65.53 ±2.92 a	67.38 ±1.73 a	67.19 ±3.28 a	66.45 ±3.7 a	63.94 ±2.5 a	62.3 ±4.5 a
CD4 ±SE	44.90 ±2.3 a	46.08 ±2.27 a	44.34 ±3.08 a	45.40 ±2.24 a	47.00 ±1.99 a	44.82 ±2.52 a	47.83 ±1.89 a	47.12 ±2.65 a	48.24 ±3.25 a	46.53 ±2.02 a	39.10 ±7.4 a
CD8 ±SE	35.35 ±0.93 a	36.94 1.66 ±a	37.63 ±3.52 a	34.93 ±0.99 a	33.52 ±1.14 a	35.75 ±1.08 a	36.36 ±1.46 a	35.70 ±1.54 a	35.7 ±1.67 a	36.95 ±1.95 a	35.55 ±2.45 a
CD14 ±SE	7.92 ±0.24 a	7.86 ±0.26 a	8.36 ±0.23 a	7.93 ±0.21 a	7.30 ±0.51 a	7.48 ±0.27 a	8.25 ±1.19 a	7.71 ±0.49 a	8.01 ±0.26 a	8.02 ±0.24 a	7.4 ±0.7 a
CD22 ±SE	11.83 ±0.94 a	11.08 ±0.7 a	10.5 ±1.2 a	11.18 ±0.68 a	13.5 ±1.71 a	10.98 ±0.48 a	11.87 ±1.01 a	13.06 ±1.24 a	10.95 ±1.34 a	8.02 ±0.24 a	11.7 ±0.2 a

A=multi-organs. B=Liver. C=Lung. D=Kidney *a Duncan letters

Table 3:Mean percentage ± SE of CD3,CD4,CD8,CD14 andCD22 lymphocytes populations in the sera of hydatidosis patients in relation to cysts size.

Cyst size	CD3 M±SE	CD4 M±SE	CD8 M±SE	CD14 M±SE	CD22 M±SE	CD4/CD8 ratio
<5cm	72.63 ±1.41 a	51.16 ±1.98 a	34.12 ±1.01 a	8.42 ±0.09 a	12.29 ±1.31 a	1.49
>5cm	58.98 ±2.38 b	40.04 ±1.82 b	36.14 ±1.78 a	7.34 ±0.37 b	10.63 ±0.62 a	1.10
P-value	<0.0001	<0.001	>0.05	<0.005	>0.05	-

*a,b Duncan letters

Discussion

Peripheral blood lymphocyte (PBL) Phenotyping is a mirror image of immunity and can give an idea of the immunological status in patients with hydatid disease .So we intended to disclose the aspects of this important immunological feature for hydatidosis patients, in order to examine what differences are significantly present in comparison with control group.

The immune suppression of hydatid disease, which is reflected by low levels of CD3 total lymphocytes , was shown in this study. This result supported a previous report which has revealed that hydatidosis patients have low levels of circulating CD3 cells (6).

This immune suppression is represented by reduction of T- lym- phocytes proliferation (7). Lymphoproliferation has been demonstrated in the early stages of experimental hydatid infection , whereas T cell depletion occurs in later stages (8). This reduction may result from reduced cell surface expression of the CD3 antigen which results in inhibition of signal transformation and inhibit lymphocyte activation (9). While , (10) suggested that the suppression may be mediated by macrophages, either by defective antigen processing and presentation or by elaboration of suppressive mediat - ors like prostglandins.

In general, protective immunity against infection with an extra cellular pathogens is considered to be essentially cell mediated , dependent on the interaction of macrophages with T - lymphocytes (11). But the depression of immune response to protozoan and helminth parasite is associated with disturbances of the normal fine structure of lymphoid organs and disruption of cell traffic (12).

In this study the percentage of CD4 T-lymphocyte subset of hydatidosis patients showed a significant decrease compared to the control group,these cells (CD4 T-cells) depend for their activation on antigen presented by MHC class II, and the polarization of CD4 to Th1 or Th2 is important in pathological control of the disease. Both subsets of CD4 T-cells (Th1 and Th2) are involved in hydatidosis (13), and CD4 T-cells number can be either host protective or disease promoting , depending on its subsets and their cytokines. However, it is reported that chronic helminthic infections cause chronic immune activation and strong Th2 type cytokine profile (14). This polarization towards the Th2 subtype and its cytokine profile was reported in cases of hydatid disease (15).So the decrease of CD4 T- cells in this study may be due to cell apoptosis resulting in selective deletion of Th1 cytokines as (16) showed in their study on the Leishmania donovani.

In immunohistochemical study done by (17) showed that most cases having progressive hydatid cysts have relatively small number of CD4 T-cells in the pericystic adventitia. Furthermore, (18) reported in their study on impeded Th1 CD4 memory T- cell generation in chronic liver infection with E. multilocularis (AE), that frequencies of circulating CD4 T - cell secreting IFN-γ , IL-2 , TNF-α were low. However , the number of specific CD4 memory T-cells was not increased in cured AE patients

after complete surgical removal of the metacestode. Finally, many factors may influence the differentiation of CD4 T-cells, those include the balance of cytokines, antigen dose, antigen presenting cells and host background (19).

The functional contribution of CD4 and CD8 T-cells are not defined.Here we studied in vitro the phenotype of circulating T-cell subsets in hydatidosis patients by CD-markers analysis .There was a relatively significant increase of CD8 cells compared to healthy control subjects.This result is in agreement with other findings for immune response in chronic persisting helminth infection (20), they showed elevated numbers of activated CD8 T-cells in the peripheral blood of patients with chronic alveolar echinococcosis. This elevation in CD8 may be due to cross priming of CD8 T-cells in infectious disease (21) . Antigen presenting cells (APC) can acquire exogenous antigens by phagocytosis and present them to CD8 cells in the contex of MHC-1 molecules (22) .

Some of these reports have proposed the existence of a subset of APC located throughout the lymphoid tissues with this specialized function. Dendritic cells were shown to be able to stimulate MHC-1 restricted T-cell response by exogenous routes (23) . A non specific immunosuppression was also demonstrated by (24) during prolonged infection with E.granulosus and despite the overall reduction in Th-1 cells , there was a considerable suppressor activity. Furthermore, other studies indicated that E.granulosus may possess an ability to alter the composition of lymphocyte population in favor of non- specific T- cell suppressor activity (25) .

Other reports proposed that successful establishment of the parasite and regulation / depression of the anti - parasite immune response may be causally related and that non – specific , parasite mitogen induced polyclonal expansion of the T - cell population, with consequent activation of T-suppressor cell clones,which may be the mechanism of immune regulation (26).

Because IL-12 and TNF are mainly produced by macrophages we can correlate the percentage of macrophages in this study with their cytokine production levels in previous studies.According to this point, we can consider that the decrease of CD14 cells in our results corresponds with a significant decrease in TNF and IL-12 (27,15).

(28) investigated the role of macrophages in resolving hydatid disease in experimental infection and they found, that activated macrophages are involved in the killing of Echinococcus protoscolices and the majority of killing occurs within the first two weeks post infection. However, studies in vitro indicated that macrophage-dependent killing of protoscolices can be increased by INF-γ (29),and decreased by some cytokines such as IL-10 (28).Helminthes can also inhibit dendritic cell migration(30) , and prevent their activation by antigens that normally would promot IL-12 production (31).

In the current study,CD22(B-lymphocytes) showed relatively an increase but not significant in B-lymphocytes in comparison to the control group.The increased number of B-cells may result from polyclonal activation of these cells (32).

Previous work with murine experiment by (33) showed the relatively modest increase in B-cell numbers and tend to be obscured by the T-cell response,and early expantion of B-cell population in advance of specific germinal center formation suggesting that initial B-cell proliferation obscured by the T-cell response,and early expantion of B-cell population in advance of specific germinal center formation suggesting that initial B-cell proliferation is a non specific mitogen - induced effect, thus suggesting that B- cell mitogenicity may be independent of T-cell help.

Finally , CD4 / CD8 ratio is considered an index of immune suppression, in the current study the results showed that there was a relative decrease in this ratio in hydatidosis patients in comparison to the control group.

References

1. Matossian , R. M. **1987.** The immunological diagnosis of human hydatid disease, *Trans. R. Soc. Trop. Hyg.* **71** : 101-104.
2. Gadea , I. , Ayala , G. , Diago, M., Cu at, A. and Garcade Lomas, J. **1999.** Immunological diagnosis of human cystic echinococcosis : Utility of discriminate analysis applied to the Enzyme - linked immunoelectrotrans for blot., *Clin. Diag. Lab. Immunol.*, **6** : 504 - 508.
3. Van Dongen, J. J. and Adriaansen, H. J. **1996**. Immunobiology of leukemia. In : Henderson, E. S. , Lister, J. A. and Greaves,

M. F. (eds). *Leukemia* , 6th ed. pp83-103. Philadelphia : WB Saunders company.

4. Boyum , A. **1968**. Separation of lymphocytes from blood and bone marrow Scand. *J. Clin. Lab. Invest*. , **21** : 77-89.
5. SAS, Institute , **2001**. SAS user's guide : Statistics version, SAS Institute Inc., Cary, **NC., USA.**
6. Hernandez-Pomi, A. , Borras-Salvador, R. and Gisbert, A**. 1997**. Analysis of cytokine and specific antibody profiles in hydatid patients with primary infection and relaps of disease.*Parasite Immunol*., **19** : 553-561.
7. Juma, A. **1999**. Cytogenetic , Biochemical and Bacteriological studies on patients infected with Shistosoma haematobium and those with bladder carcinoma. Ph.D. Thesis College of Medicine, Al-Nahrain University
8. Riley, E. M. , Dixon, J. B. , Kelly, D. F. and Cox, D. A.**1985**. The immune response to Echinococcus granulosus :Sequential histological observation of lymphoreticular and connective tissue during early murine infection. *J. Comp. Pathol*., **95** : 93-104.
9. Rezai, H. , Farrell. J. and Soulaby, E. **1980**. Immunological response of L.donovani infection in mice and significance of T-cell in resistance to experimental leishmaniasis. *Exp. Immunol*. , **40** : 508 - 514.
10. Saha, B., Das, G., Vohra, H., Ganguly, N. and Mishra, G. **1995**. Macrophage T-cell interaction in experimental visceral leishmaniasis : Failure to express contimulato molecules on Leishmania - infected macrophages and its implication in the suppression of cell mediated immunity. *Eur. J. Immunol*.,**25**:2492-2498.
11. Kaufmannn, S. H. and Doherty, P. C. **1997.** Immunity to infection, *Curr. Opin. Immunol*., **9** : 453-455.
12. Cook, R. , Trapp, A. and Williams, J. **1981.** Pathology of *Taenia taeniaformis* infection in the rat hepatic, lymph node and thymic changes. *J.of Comp. Pathol*., **91**:219-226.
13. Bauder, B., Auer, H., Schilcher, F., Gabler, C., Romig, T., Bilger, B. and Aspock, H. **1999**. Experimental investigations on the B and T cell immune response in primary alveolar echinococcosis.*Parasite Immunol*., **21** : 409 - 421.
14. Bentwich,z.,Kalinkovich,A.,Wqeisman,Z.,B orkov,G.,Beyers,N.andD-Beyer,A. **1999.** Can eradication of helminthic infection change the face of AIDS and TB.*Immunol.Today*,**20**:477-534.
15. Rigano, R., Profumo, E., Bruschi, F., Carulli, G., Azzara, A., Ioppolo, S., Buttari, B., Ortono, E., Margutti, P., Teggi, A., and Siracusano, *A.* **2001.** Modulation of human immune response by E.granulosus antigen B and its possible role in evading host defenses. *Infec.Immunity* , **9** : 288-296.
16. Das,G.,Vohra, H., Rao, K., Saha, B. and Mishra , G.**1999.** *Leishmania donovani* infection of susceptible host results in CD4 T-cell apoptosis and decreased Th-1 cytokine production,. *Scand.J.Immunol*.,**49** : 307-310.
17. Sakamoto, T. and Cebrera, P. A. **2003.** Immuohistochemical observation on cellular response in unilocular hydatid lesions and lymph nodes of cattle . *Acta Trop*.,**85** : 271-279.
18. Manfras, B., Reuter, S., Wendland, Th., Boehn, B. and Kern, P. **2004.** Impeded Th1 CD4 memory T cell generation in chronic persisting liver infection with Echinococcus multilocularis .*Journal Int. Immunol*., **16** : 43-50.
19. Baqir, H. **1999.** Humoral and cellular immune response in visceral leishmaniasis : Possible application in diagnosis , epidemiology and vaccine design. Ph. D. Thesis , College of Medicine, Al-Nahrain University.
20. Manfras, B., Reuter, S., Wendland, Th. and Kern, P. **2002.** Increased activation and oligoclonality of peripheral CD8 T cells in the chronic human helminth infection with alveolar echinococcosis. *Infec. Immunity*, **70** : 1168 - 1174.
21. Den-Haen, J. and Bevan, M. **2001**. Antigen presentation to CD8 T-cells: Cross - priming in infectious disease. *Curr. Opin. Immunol*., **13** : 437-441.
22. Rock, K. , Gamble, S. and Rothstein, l. **1990.** Presentation of exogenous antigen with class I major histocompatibility complex molecules. *Sci*. , **249** : 918 - 921.
23. Larsson, M., Fonteneau, F. and Bhardwaj, N. **2001**. Dendritic cells resurrect antigens from dead cells. *Trends Immunol*. , **22** : 141 - 148.
24. Allan, D., Jenkins, P., Connor, R. and Dixon, J. **1981**.A study of immuno regulation of BALB/C mice by *E.granulosus* equinus during prolonged infection. *Parasite . Immunol*., **3** : 137-1
25. Dixon, J., Jenkins, P., Allan, D. and Connor, R. **1978**. Blastic stimulation of unprimed

mouse lymphocytes by living protoscolices of *E.granulosus* :A possible connection with transplant immunity. J. *Parasitol*.,**64**:949-950.

26. Nicol, C. and Sewell, M. **1984.** Immunosuppression by larval cestodes of *Babesia microti* infections. *Ann. Trop. Med. Parasitol.*, **78** : 228 - 233.
27. Torcal,J., Navarro - Zorraquino , R ., Lozano, L., Larrad, L., Salinas, J. ,Ferrer, J. ,Roman, J. and Pastor, C. **1996.** Immune response and in vivo production of cytokines in patients with live hydatidosis. *Clin. Exp. Immunol*., **106** : 317 - 322.
28. Jenkins, P., Dixon, J., Rakha, N. and Carter, S. **1990**. Regulation of macrophage - mediated larvicidal activity in *Echinococcus granulosus and Mesocestoides corti* (Cestoda) infection in mice. *Parasitol*., **100**:309-315.
29. Kanazawa, T., Asahi, H., Hata, H., Mochida, K. , Kagei, N. and Stadecker, M. **1993**. Arginine - dependent generation of reactive nitrogen intermediates is instrumental in the in vitro killing of protoscoleas of *E.multilocularis* by activated macrophages.*Parasit. Immunol*., **15**:619-623.
30. Angeli, V., Faveeuw, C., Roye, O. , Fontaine, J. , Teissier, E. and Troltein, F. **2001**. Role of the parasite - derived prostaglandin D2 in the inhibition of epidermal langerhans cell migration during schistosomiasis infection. *J. Exp. Med*., **193** : 1135 - 1147.
31. Semnani, T., Sabzevari, H. Lyer, R. and Nutman, T. **2001**. Filarial antigens impair the function of human dendritic cells during differentiation. *Infec. Immunity*. **69** : 5813 - 5822.
32. Stites, D. P. **1976**. Laboratory methods of detecting cellular immune function. In : *Basic and clinical immunology.* Fundenberg, H. H., Stits, D. P.and Caldwell .J. L. (eds) . pp. 318 - 322. Lange Medical Publication, California.
33. Riley, E. and Dixon, J. **1987.** Experimental *E.granulosus* infection in mice : Immunocytochemical analysis of lymphocyte populations in local lymphoid infection during early infection. *Parasitol*., **94** : 523 - 532.