

***In vivo* IL-12 and IL-8 production in hydatid patients and their possible diffusion in hydatid cysts**

Manel Amri, Dalila Mezioug, Saliha Ait-Aissa, Chafia Touil-Boukoffa

Equipe "Cytokines et NOSynthase", Laboratoire de biologie cellulaire et moléculaire, FSB-USTHB, Université Bab-Ezzouar, PB 32, 16111, Algiers, Algeria

Correspondence : Pr C. Touil-Boukoffa, Laboratoire de biologie cellulaire et moléculaire, FSB-USTHB, Université Bab-Ezzouar, PB 32, 16111, Algiers, Algeria
<touilboukoffa@yahoo.fr>

Accepted for publication June 4, 2008

ABSTRACT. Cystic echinococcosis (CE) is caused by infection with the larval stage of the cestode *Echinococcus granulosus*. It is one of the world's major zoonotic infections. Variability and severity of clinical expression of this parasitosis are associated with duration and intensity of infection. They are also related to the variety of human immunological responses to the hydatid antigens. The aim of this work is to study the inflammatory response associated with human hydatidosis by evaluating the possible roles of the proinflammatory cytokines in hydatid patients. We investigated the patterns of IL-12 and IL-8 in serum from Algerian hydatid patients. Serum IL-12 and IL-8 levels are significantly higher in patients with hydatidosis than in control subjects. Furthermore, cytokines secretion correlates with disease status (cystic localizations and clinical stage). These data indicate that infection with *E. granulosus* is associated with high levels of circulating IL-12 and IL-8. Moreover, our data, to our knowledge, constitute the first report of IL-12 and IL-8 diffusion into the hydatid cyst. Our results underline the permeability of the cyst wall to the soluble immune system of the host. The relationship between cyst fertility and cytokine infiltration indicates a strong host-parasite interaction. All these findings have important implications for the diagnosis of hydatidosis in humans.

Keywords: IL-12, IL-8, human hydatidosis

Echinococcus granulosus is one of the world's major zoonotic infections. The larval stage is the causative agent of human hydatidosis. It constitutes a serious public health problem in various parts of the world, particularly in Algeria [1-3]. Human hydatidosis usually manifests as unilocular cyst(s) mainly located in the liver and/or lungs or other viscera of the intermediate host. The slow-growing cysts are able to survive for several years in chronic infections. In many cases, it appears to be refractory to the immunological responses of the host. Generally, the early phases of

infection are asymptomatic and the diagnosis is based on the identification of cyst structures by radiology [4, 5]. Immunodiagnosis is considered to be an additional approach to confirm clinical findings [6, 7]. Treatment modalities for cystic echinococcosis include chemotherapy and surgery. However, surgery still remains the main therapeutic approach throughout the world [5, 8].

It seems that the variability and severity of the clinical expression of this parasitosis are associated with duration and intensity of infection. There are also related to the variety of human immunological responses to the several antigens. The study of cytokines with their multiple regulatory functional outcomes will be useful in designing strategies to develop early immunodiagnosis of hydatid disease. It could also open new perspectives in the understanding of the pathogenesis of this disease. In previous studies, we have shown the presence of IFN (a mixture of IFN- α , β and γ), TNF- α , and IL-6 activities in hydatid patients showing hepatic and pulmonary cystic localization. This production correlates with immunoreactivity versus parasitic antigen (Antigen 5) [9]. These cytokines would play a role in the initiation, intensity and duration of the immune response in human hydatidosis. Nevertheless, the exposure of these mechanisms requires the identification of other cytokines implicated in the immune process.

Abbreviations:

a	after
b	before
bLu	broken cyst in lungs
CE	cystic echinococcosis
cLi	partial calcified cyst in the liver
Ki	kidney
HCF	hydatid cyst fluid
HuIFN- γ	human IFN- γ
Li	liver
L-NMMA	N-monomethyl- L-arginine
Lu	lungs
O	ovary

To evaluate the role of inflammatory cytokines in the immune response against hydatidosis, we investigated *in vivo* IL-12 and IL-8 production in hydatid patients with different cyst localizations (before and after surgery). We have also study the IL-12 and IL-8 levels in hydatid fluid and their correlation with cyst location and fertility.

PATIENTS AND METHODS

Patients

Fifty one blood samples were obtained from Algerian patients with *Echinococcus granulosus* infections (30 ± 13 years old, 68.63% men), tested before and after surgery (one week before and 24 h-72 h after surgical removal). Clinical diagnosis was surgically confirmed by the presence of cysts in each case (Department of Surgery and laboratory of parasitology, Mustapha Bacha Hospital, Algiers, Algeria). Subjects having secondary infection and other acute or chronic diseases were not included in this study. Ten healthy controls (mean age 25 ± 2 years, 70% women) (from the same region in Algeria) were included in this study. They did not present inflammatory disease or any sign of infection at the time of blood sample collection. In addition, none of the subjects had ever received blood transfusions or any medication. All participants gave their informed consent for the present study, which was carried out according to the guidelines of local Ethics Working Group.

Serum collection

Blood samples collected from healthy donors and patients were centrifuged at 2000rpm for 10 minutes to obtain the serum. Haemolysed serum was excluded from this study. All serum samples were stored at -70°C until cytokine determination. The serum samples were classified into several groups. This classification was based on clinical stage (b: before surgery and a: after surgery- $n = 51$), cyst location (Li + Lu: liver + lungs- $n = 10$; Li: liver- $n = 09$; Lu: lungs- $n = 11$; Ki: kidney- $n = 4$); and cyst states (cLi: partially calcified cyst in the liver- $n = 06$; bLu: broken cysts in the lungs- $n = 11$).

Hydatid cyst fluid collection

Thirty seven hydatid cyst fluid (HCF) samples were obtained by aseptic puncture of hydatid cysts removed by surgery from hydatid patients. The hydatid cysts were mainly localized in the liver (Li, $n = 15$), lungs (Lu, $n = 19$) and less frequently in the ovary (O, $n = 3$). The HCF was filtered in order to eliminate the remaining hydatid membranes. It was centrifuged at 3000rpm (15min, $+4^{\circ}\text{C}$) and the sediment checked for the presence of protoscolices. Finally, the supernatant of each cyst was stored at -70°C until cytokine determination. The hydatid fluids are classified into several groups. This classification is based on fertility (“+++”: high fertility- $n = 13$; “++”: medium fertility- $n = 15$; and “-”: no fertility- $n = 9$), and cyst location (Li: liver- $n = 15$; Lu: lungs- $n = 19$; and O: ovary- $n = 3$).

Cytokine detection

Levels of IL-8 and IL-12 in serum and cyst fluid samples were evaluated by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (Immunotech). The plates were read on an ELISA reader (LABSYSTEM). These assays detected only human cytokines.

STATISTICAL ANALYSIS

All results were expressed as mean \pm SD (standard deviation). Data analysis was performed with the SYSTAT 11. Student's *t* test was used for comparisons between different groups (patients/controls, stimulated/unstimulated cells). Differences were considered to be statistically significant for *p* value < 0.05 .

RESULTS

IL-8 and IL-12 levels in patients compared to healthy donors

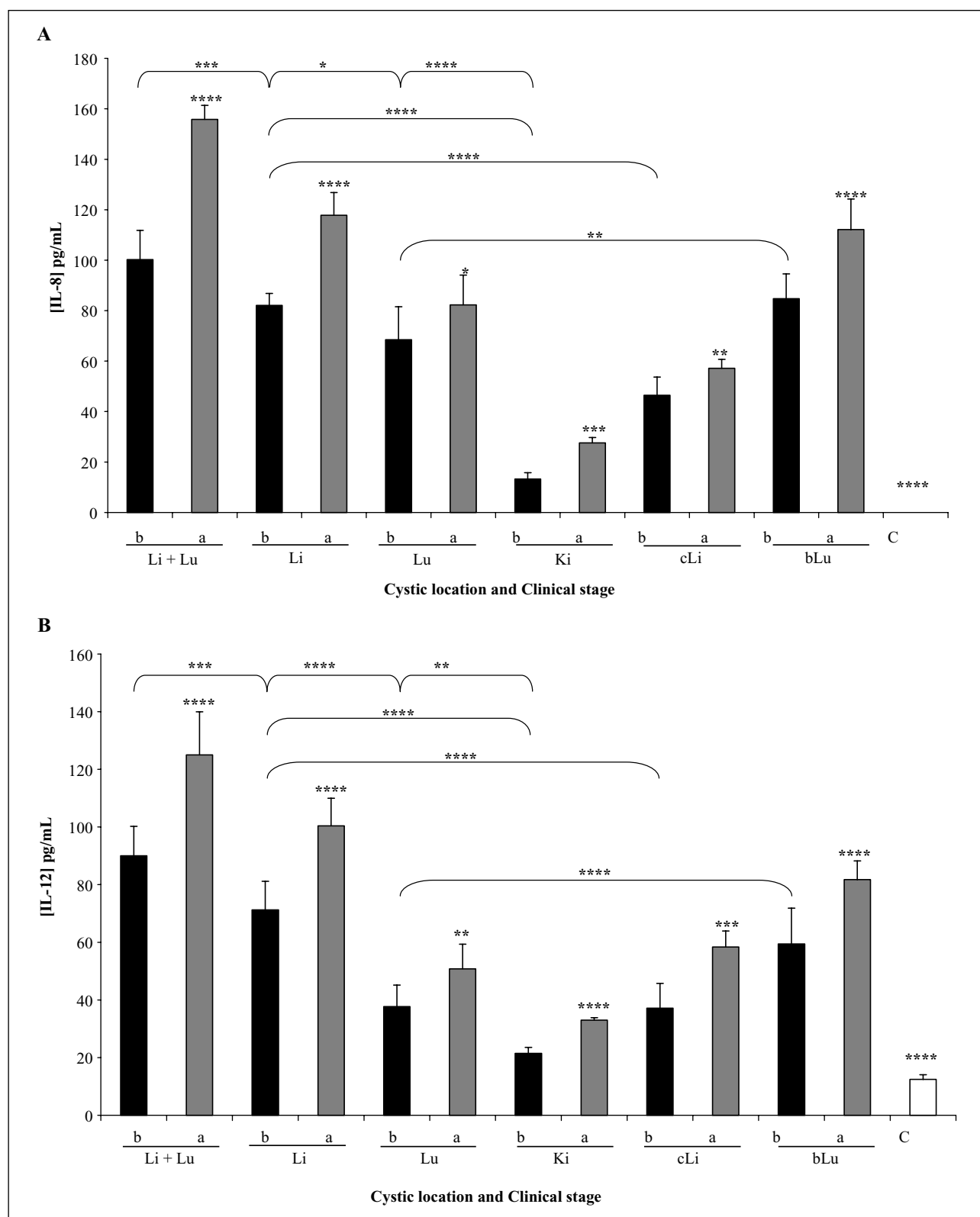
Analysis of circulating cytokine production reveals that levels of IL-8 and IL-12 are elevated in all hydatid groups. Further, we observe a distinct pattern in patients and normal donors. As shown in *figure 1A*, patient serum samples (before surgery) have significantly higher IL-8 levels at all locations (*e.g.* 100.2 ± 11.5 pg/mL of IL-8 in liver plus lung) than serum samples from healthy donors (0 pg/mL) ($p < 0.0001$). Similarly, IL-12 levels in the hydatid patients (before surgery) are higher compared with levels in uninfected control subjects (*e.g.* 90 ± 10.23 pg/mL of IL-12 in liver plus lung *versus* 12.47 ± 1.58 pg/mL; $p < 0.0001$) (*figure 1B*). The same significant difference between patients and controls is also shown for all cyst locations.

Correlation between IL-8/ IL-12 levels and disease stage

In order to determine a possible relationship between disease progression and cytokine production *in vivo*, serum IL-12 and IL-8 levels were assessed before and after surgery. The clinical stage seems to influence IL-8 and IL-12 production. Analysis of cytokine production before and after surgery shows that the IL-8 and IL-12 production observed following surgery is higher in all cases (*e.g.* in multiple cysts localization: 100.2 ± 11.5 pg/mL *versus* 155.8 ± 5.75 pg/mL of IL-8- $p < 0.0001$; and 90 ± 10.23 pg/mL *versus* 125 ± 14.94 pg/mL of IL-12- $p < 0.0001$) (*figure 1*). The same significant difference between clinical stages of disease (before and after surgery) is also shown for all cyst locations.

Correlation between IL-8/IL-12 levels and cyst location

Our results indicated in *figure 1* suggest that cystic location is related to cytokine production. Interestingly, we observed that patients with multiple hydatidosis (liver and lung cysts) showed the most elevated serum IL-8 and IL-12 levels (100.2 ± 11.5 pg/mL and 90 ± 10.23 pg/mL respectively, before surgery). The hepatic cystic location is in

**Figure 1**

Serum levels of IL-8 (A) and IL-12 (B) in Algerian hydatid patients (b: before surgery; and a: after surgery- $n = 51$) and healthy controls (C- $n = 10$). Li+Lu: liver + lungs- $n = 10$; Li: liver- $n = 09$; Lu: lungs- $n = 11$; Ki: kidney- $n = 4$; cLi: partially calcified cyst in the liver- $n = 06$; bLu: broken cysts in lungs- $n = 11$. Cytokine levels were quantified as described in *Materials and Methods* and are presented as the mean \pm SEM pg/mL. Significant differences between two groups (e.g. patients/controls) are indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and **** $p < 0.0001$). Differences were statistically significant for all groups.

second position (with 82.11 ± 4.7 pg/mL of IL-8 and 71.28 ± 9.9 pg/mL of IL-12, before surgery) followed by lung (with 68.5 ± 13.04 pg/mL of IL-8 and 37.73 ± 7.43 pg/mL of IL-12, before surgery).

We observed with interest that in patients with a rare cystic location (in kidney), serum IL-8 and IL-12 levels are very low, but remain higher than those measured in healthy controls (with 13.25 ± 2.5 pg/mL of IL-8 and 21.5 ± 2.04 pg/mL of IL-12, before surgery; $p < 0.0001$).

Furthermore, we have noted that the partial calcification of cysts reduces cytokine production measured in patients bearing intact hepatic cyst (46.42 ± 4.27 pg/mL versus 82.11 ± 4.7 pg/mL of IL-8 and 37.17 ± 8.59 pg/mL versus 71.28 ± 9.9 pg/mL of IL-12; $p < 0.0001$).

In contrast, those patients with a complication such as the breakdown of cysts show significantly higher levels of IL-8 (84.72 ± 9.86 pg/mL) and IL-12 (59.43 ± 12.41 pg/mL) in comparison with those bearing an intact pulmonary cyst (with 68.5 ± 13.04 pg/mL of IL-8; $p = 0.003$ and 37.73 ± 7.43 pg/mL of IL-12; $p < 0.0001$) (figure 1).

IL-8 and IL-12 levels in cystic fluid

Thirty-seven hydatid fluid samples were aseptically taken from fertile, hydatid cysts and acephalocysts of patients (see Patients and methods). Our observations show a correlation between cytokine levels and cyst fertility. The ELISA sandwich method revealed significant IL-8 and IL-12 levels in fluids from all fertile cysts located in the liver (with 1547.71 ± 98.68 pg/mL of IL-8 and 136 ± 9.86 pg/mL of IL-12) or lungs (with 793.17 ± 39.73 pg/mL of IL-8 and 103.17 ± 12.58 pg/mL of IL-12). In infertile hepatic cysts; levels of cytokines significantly decrease to 134 ± 10.15 pg/mL of IL-8 ($p < 0.0001$) and 55 ± 5 pg/mL of IL-12 ($p < 0.0001$). The same observations were also noted in pulmonary cysts (with 793.17 ± 39.73 pg/mL of IL-8 and 10 ± 1.41 pg/mL of IL-12) (figure 2).

Moreover, we have noted with interest that cyst location is related to the cytokine levels in cysts. In fertile cysts, an hepatic location shows the highest levels of cytokines (with 1547.71 ± 98.68 pg/mL of IL-8 and 136 ± 9.86 pg/mL of IL-12) as compared to a pulmonary location (with 793.17 ± 39.73 pg/mL of IL-8; $p < 0.0001$ and 103.17 ± 12.58 pg/mL of IL-12; $p < 0.001$) (figure 2). The same observations were also noted in infertile cysts (figure 2).

DISCUSSION

Production of IL-8 and IL-12 in hydatid patients

To our knowledge, very little information is available concerning the role of IL-8, and IL-12 in human hydatidosis. Further, no previous studies have directly examined IL-12 and IL-8 production in Algerian hydatid patients, or their relationship to cyst location and disease stage. The present study is designed to assess the serum levels of IL-12 and IL-8 in Algerian patients with hydatidosis, and in healthy donors from the same community, as a control subjects. Our results provide evidence that levels of IL-12 and IL-8 are higher in hydatid patients.

This increase is in agreement with previous data on the role of cytokines in host anti-hydatid defence, and underscores

the ability of the larval stage of *Echinococcus granulosus* to trigger cytokine production [9-12].

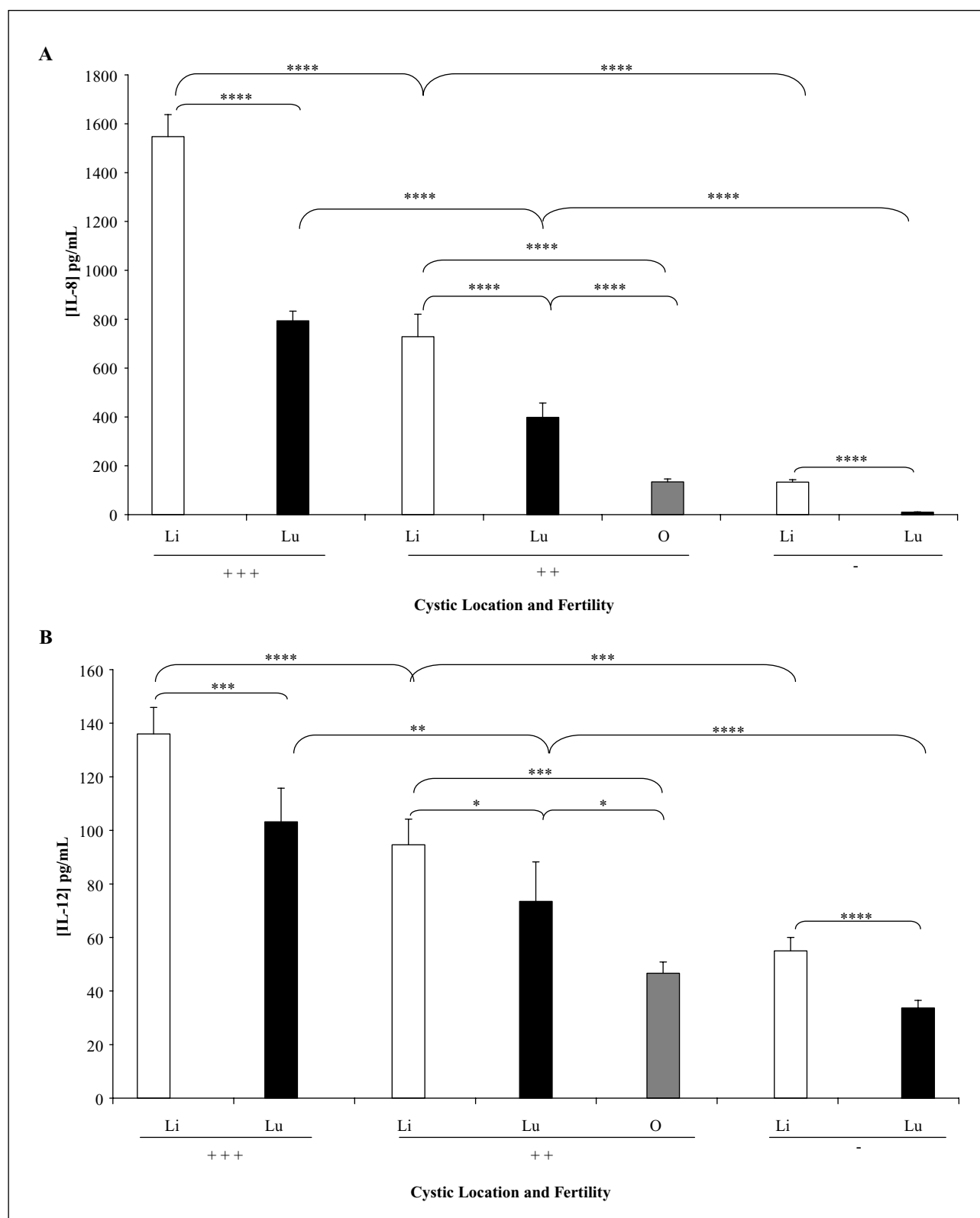
The role of cytokines in infection by various parasites is usually been investigated by measurement of cytokine levels in patients' serum. This approach has a several advantages; one of them is the feasibility of assaying many samples quickly and easily. Refik *et al.* observed that IL-8 is increased in 11/28 (39.3%) of patients with cystic echinococcosis following surgery [13]. However, in this study, the patient population, and the disease stage when blood samples were obtained differed considerably from those in our study, which may explain the differences in the patterns of cytokine response. Rigano *et al.* detected IL-12 p40 mRNA almost exclusively in PBMC isolated from successfully treated patients at the end of chemotherapy [14]. We have previously measured antigen-driven cytokine production in patients with hydatidosis (before and after surgery). Our results showed high *in vitro* production of antigen-driven IL-12 and IL-8 by PBMC isolated from these patients [15]. The same observation was also reported by Dreweck *et al.* in a related infection by *Echinococcus multicularis*. These authors showed that substantial amounts of IL-8, and IL-12 were detected in alveolar echinococcosis patients [16]. Several intra-cellular parasitic diseases, such as infections by *Plasmodium*, showed similar serum IL-8 and IL-12 level increase. Expression of IL-8 mRNA has been demonstrated in placental malaria infection [17]. In addition, significantly elevated serum levels of IL-12, and IL-8 were found in severe cases of malaria versus healthy controls [18]. Courtioux *et al.* also reported that high levels of IL-8 were associated with parasite detection in human infection with *Trypanosoma brucei gambiense* [19].

IL-8 is a member of the C-X-C chemokine family. IL-8 is a proinflammatory, chemoattractant cytokine produced mainly by activated monocytes/macrophages [20-23].

IL-12 was originally termed natural killer cell stimulatory factor or cytotoxic lymphocyte maturation factor. This is produced mainly by activated macrophages/monocytes. It has a very important role in the initiation and regulation of the innate cellular immune response [24-26].

Taken together, these observations and our results suggest that IL-12 initiates a protective T helper type 1 immune response in hydatid disease. Emery *et al.* showed that IL-12 is of crucial importance in inhibiting larval growth after establishment of the *Echinococcus multicularis* metacestode in the liver. These authors suggest that this cytokine could be of potential value in the treatment of human alveolar echinococcosis [27]. Other studies have also shown that IL-12 can be used as an adjuvant in conjunction with soluble antigens to enhance Th1 immune responses and to induce protection against re-infection by shistosomes [28].

IL-12 exerts its influence through upregulating IFN- γ [25, 26, 29]. IFN- γ has been shown to have *in vitro* anti-hydatid activity. Our *in vitro* experiments using L-NMMA (inhibitor for NOS) and anti-HuIFN- γ have shown that IFN- γ -induced nitric oxide production appears to be a critical mechanism for hydatid infection control [12]. IFN- γ also enhances the production of IL-12, creating a positive reinforcement loop which enhances Th1 type immunity [29]. Among the other possible mechanisms, IFN- γ may also

**Figure 2**

Hydatid cyst fluid levels of IL-8 (A) and IL-12 (B) in cyst samples surgically removed from Algerian hydatid patients ($n = 37$). The hydatid fluids are classified into several groups. This classification is based on fertility (“+++”: high fertility- $n = 13$; “++”: medium fertility- $n = 15$; and “-”: no fertility- $n = 9$) and cysts location (Li: liver- $n = 15$; Lu: lungs- $n = 19$; and O: ovary- $n = 3$). Cytokine levels were quantified as described in *Materials and Methods* and are presented as the mean \pm SEM pg/mL. Significant differences between two groups (*e.g.* liver cyst/lung cyst) are indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and **** $p < 0.0001$). Differences were statistically significant for all groups.

acts by induction of chemoattractants for immune cells. IL-8 release may provide a potential mechanism for attraction of effector cells into the invaded tissue by *Echinococcus granulosus*. A further study is needed to investigate the effect of IFN- γ on IL-8 and IL-12 production elicited by hydatid antigens.

Influence of clinical stage

The higher serum IL-8 and IL-12 levels observed after surgery support the notion of a relationship between clinical stage and cytokine induction. Our results imply that the increase in cytokine levels correlates with the inflammatory state following surgical removal of cysts. These observations are in line with those reported by many other authors showing that major surgery induces a series of inflammatory responses such as elevation of body temperature and erythrocyte sedimentation rate, leukocytosis and increased acute phase reactants [30, 31]. Current evidence suggests that these responses are mediated by pro-inflammatory cytokines such as IL-8 and IL-12, which are produced locally and enter the blood stream very soon after surgery [32].

Influence of cyst location

In addition to the clinical stage, our results indicate that cyst location is related to the intensity of cytokine induction. Patients carrying multiple cysts (in liver and lungs) have higher serum cytokine levels. This increase might be associated with the presence of more than one cyst. Cytokine production is also higher in patients with liver cysts in comparison with those bearing cysts in the lungs. This difference may be attributed to several factors. Hepatic hydatid cysts are expected to provide the highest amounts of antigen, probably because of the good vascularity of this major filtering organ [33]. Moreover, Vidor *et al.* showed that hepatic cysts are more permeable than pulmonary cysts [34]. The data are in agreement with a previously published report indicating that cyst location determines the host immune response. For instance, a close relationship has been reported between cyst number, characteristics and, above all, location within the liver itself, and increases in blood level of IL-1, IL-2, IL-4 [35] and NO [10, 11].

Detection of cytokines in cystic fluid

To our knowledge, the present study is the first to show the presence of cytokines within hydatid cyst fluid. Ait-Aissa *et al.* had previously observed the presence of nitrite oxide in the cyst fluid [11]. Our results show that, as a consequence of antigenic burden and IFN- γ induction, IL-8, and IL-12 produced may diffuse and move into the cyst. However, our findings do not exclude the possibility of cytokine up-regulation in localized areas of the parasite sequestration (production by neighbouring cells). Further investigations are required to clarify this hypothesis. Furthermore, our results show that cytokine levels are associated with cystic fertility and the viability and vitality of the protoscoleces. In the same way, cytokine levels seem to be variable according to the location of the cyst. All these data suggest that the production and the diffusion of IL-8 and IL-12 inside hydatid cysts may be governed by

anatomical and physiological parameters, including the location of the cyst and cystic fertility.

CONCLUSION

Collectively, this present study provides evidence that IL-8 and IL-12 are actively produced during the course of human *Echinococcus granulosus* infection. Our results also show that cytokine secretion correlates with cystic localizations and clinical stage. Further, circulating cytokines or locally-produced cytokines could diffuse into the cyst. Our results suggest that these two pro-inflammatory cytokines are implicated, at least in part, in the host defence mechanism in hydatid disease. These findings underline the strong relationship between the immune system of host and parasite. Thus, the measurement of serum cytokines may allow for better monitoring and prediction of disease evolution in human hydatid patients. IL-12 is a useful clinical marker for disease activity and the protective Th1 response in hydatidosis. Combined with its role in down-regulating Th2 responses associated with pathology, IL-12 represents a powerful tool with which to manipulate the immune system. It is our hope, in future studies, to evaluate the clinical usefulness of IL-12 in the treatment of human echinococcosis.

Acknowledgments. We wish to thank the technical and surgical staff of the Mustapha-Bacha Hospital of Algiers for providing blood and cyst samples. Special thanks to Professor Hamrioui. We thank all voluntary participants in this study. We are grateful to Dr. Jeanne Wietzerbin for helpful discussions. This work was supported by a grant from the ANDRS (National Agency for Development of Scientific Research).

REFERENCES

1. Shambesh MK. Human cystic echinococcosis in North Africa (excluding Morocco). In: Anderson FL, Ouheli H, Kachani M, eds. *Compendium on cystic echinococcosis in Africa and Middle Eastern countries with special reference to Morocco*. Pravo' Brigham Young University, 1997: 223.
2. Bardonnet K, Benchikh-Elfegoun MC, Bart JM, *et al.* Cystic echinococcosis in Algeria: cattle act as reservoirs of a sheep strain and may contribute to human contamination. *Vet Parasitol* 2003; 116: 35.
3. Seimenis A. Overview of the epidemiological situation on echinococcosis in the Mediterranean region. *Acta Trop* 2003; 85: 191.
4. Suwan Z. Sonographic findings in hydatid disease of the liver: Comparison with other imaging methods. *Ann Trop Med Parasitol* 1996; 89: 261.
5. Schantz PM. Progress in diagnosis, treatment and elimination of echinococcosis and cysticercosis. *Parasitol Intern* 2006; 55: 7.
6. Carmena D, Benito A, Eraso E. Antigens for the immunodiagnosis of *Echinococcus granulosus* infection: An update. *Acta Trop* 2006; 98: 74.
7. Zhang W, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; 47: 24.
8. Craig PS, McManus DP, Lightowers MW, *et al.* Prevention and control of cystic echinococcosis. *Lancet Infect Dis* 2007; 7: 385.

9. Touil-Boukoffa C, Sancéau J, Tayebi B, et al. Relationship Among Circulating Interferon, Tumor Necrosis Factor- α , and Interleukin-6 and Serological Reaction Against Parasitic Antigen in Human Hydatidosis. *J Interferon Cytokin Res* 1997; 17: 211.
10. Touil-Boukoffa C, Bauvois B, Sancéau J, et al. Production of Nitrite Oxide (NO) in Human Hydatidosis. Relationship between Nitrite and IFN γ levels. *Bioch* 1998; 80: 739.
11. Ait-Aissa S, Amri M, Bouteldja R, et al. Alterations in interferon-gamma and nitric oxide levels in human echinococcosis. *Cell Mol Biol* 2006; 52: 65.
12. Amri M, Ait-Aissa S, Belguendouz H, et al. In vitro anti-hydatid action of IFN- γ in dependent on the nitric oxide pathway. *J Interferon Cytokin Res* 2007; 27: 781.
13. Refik M, Mehmet M, Durmaz R. Postoperative changes in serum cytokines profile and nitric oxide levels in patients with cystic echinococcosis. *Parasite* 2005; 12: 265.
14. Rigano R, Profumo E, Buttari B, et al. A. Cytokine gene expression in peripheral blood mononuclear cells (PBMC) from patients with pharmacologically treated cystic echinococcosis. *Clin Exp Immunol* 1999; 118: 95.
15. Amri M, Mezioug D, Ait-Aissa S, et al. Purification de deux antigènes de l'échinococcose humaine par gel filtration et chromatographie d'affinité: impact sur la production de l'IL-8, l'IL-12 et le NO. *JSAC* 2005; 15: 187.
16. Dreweck CM, Soboslay PT, Schulz-Key H, et al. Cytokine and chemokine secretion by human peripheral blood cells in response to viable *Echinococcus multilocularis* metacystode vesicles. *Parasite Immunol* 1999; 21: 433.
17. Abrams ET, Brown H, Chensue SW, et al. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *J Immunol* 2003; 170: 2759.
18. Lyke KE, Burges R, Cissoko Y, et al. Serum Levels of the Proinflammatory Cytokines Interleukin-1 Beta (IL-1 β), IL-6, IL-8, IL-10, Tumor Necrosis Factor Alpha, and IL-12(p70) in Malian Children with Severe *Plasmodium falciparum* Malaria and Matched Uncomplicated Malaria or Healthy Controls. *Infect Immun* 2004; 72: 5630.
19. Courtioux B, Boda C, Vatunga G, et al. A link between chemokine levels and disease severity in human African trypanosomiasis. *Intern J Parasitol* 2006; 36: 1057.
20. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989; 84: 1045.
21. Taub DD, Oppenheim JJ. Chemokines, inflammation, and the immune response. *Ther Immunol* 1994; 1: 229.
22. Petering H, Götze O, Kimmig D, et al. The biologic role of interleukin-8: functional analysis and expression of CXCR1 and CXCR2 on human eosinophils. *Blood* 1999; 93: 694.
23. Zeilhofer HU, Schorr W. Role of interleukin-8 in neutrophil signaling. *Curr Opin Hematol* 2000; 7: 178.
24. Scott PS. IL-12: Initiation cytokine for cell-mediated immunity. *Science* 1993; 260: 496.
25. Trinecheri G. Interleukin-12: a cytokine produced by antigen presenting cells with immunoregulatory functions in the generation of T helper cells type 1 and cytotoxic lymphocytes. *Blood* 1994; 84: 4008.
26. Del Vecchio M, Bajetta E, Canova S, et al. Interleukin-12: biological properties and clinical application. *Clin Cancer Res* 2007; 13: 4677.
27. Emery I, Leclerc C, Sengphommachanh K, et al. In vivo treatment with recombinant IL-12 protects C57BL/6J mice against secondary alveolar echinococcosis. *Parasite Immunol* 1998; 20: 81.
28. Mountford AP, Shires VL, Anderson S. Interleukin-12 and protective immunity to schistosomes. *Braz J Med Biol Res* 1998; 31: 163.
29. Schroder K, Hertzog PJ, Ravasi T, et al. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* 2004; 75: 163.
30. Bernard SL, Lorerg J. Age and immune response to a surgical stress. *Arch Surg* 1983; 118: 405.
31. Lennard TWJ, Shenton BK, Borzitta A, et al. The influence of surgical operations on components of the human immune system. *Br J Surg* 1985; 721: 771.
32. Sakamoto K, Arakawa H, Mita S, et al. Elevation of circulating interleukin 6 after surgery: factors influencing the serum level. *Cytok* 1994; 6: 181.
33. Cesbron JY, Capron M, Capron A. Le diagnostic immunologique de l'hydatidose humaine. *Gastroenterol Clin Biol* 1986; 10: 415.
34. Vidor E, Piens MA, Abbas M, et al. Hydatid cyst fluid biochemistry (*Echinococcus granulosus*). Influence of localization on cyst permeability. *Ann Parasitol Hum Comp* 1986; 61: 333.
35. Torcal M, Navarro-Zorraquino R, Lozano L, et al. Immune response and in vivo production of cytokines in patients with liver hydatidosis. *Clin Exp Immunol* 1996; 106: 317.