RESEARCH ARTICLE

Interleukin-17A correlates with interleukin-6 production in human cystic echinococcosis: a possible involvement of IL-17A in immunoprotection against *Echinococcus granulosus* infection

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ABSTRACT. Hydatidosis is a parasitic disease caused by the development, in humans and other mammals, of the larval form of Taenia, *Echinococcus granulosus*. It is one of the world's major zoonotic infections. This study aimed to examine interleukin-6 (IL-6), interferon-γ (IFN-γ) and interleukin-17A (IL-17A) production in patients with cystic echinococcosis (CE), and the role of IL-17A in the modulation of the immune response against the extracellular parasite, *E. granulosus*. A relationship between IL-6, IL-17A production and C reactive Protein (CRP) levels was also assessed. IL-6, IFN-γ, IL-17A and CRP production were determined in serum from Algerian hydatid patients. Cytokine production was also measured in supernatants from cultures of peripheral blood mononuclear cells (PBMCs) from hydatid patients stimulated by a major parasitic antigen (antigen-5). The increased activity of IL-6, IFN-γ and IL-17A were observed in most serum samples from patients. In contrast, healthy controls showed only minor levels. Similarly, high levels of CRP were detected. Our *in vitro* results indicate a positive correlation between IL-6, IFN-γ and IL-17A production in PBMC culture supernatants. However, IL-6, IFN-γ and IL-17A activity was low in serum and supernatants of PBMC cultures from relapsing patients, and there was no evidence of an immune response against parasitic antigen. Collectively, our results show that IL-17A was produced during human cystic echinococcosis, and was involved in the host defense mechanisms against the extracellular parasite *E. granulosus*. Our data suggest that IL-17A plays an immunoprotective role in this parasitic, helminth infection.

Key words: human cystic *Echinococcosis*, cytokines, interleukin-17A, immunoprotection

Human hydatidosis is severe, chronic, parasitic disease, caused by the larval stage of the cestode *Echinococcus granulosus* [1]. It constitutes a serious public health problem in various parts of the world, particularly in Algeria [2]. This parasitic, helminth infection usually manifests as unilocular cyst(s) mainly located in the liver and/or lungs or other viscera of the intermediate host. It is characterized by a prolonged coexistence of the parasite, *E. granulosus* and the host with no effective rejection reaction. The variability and severity of the clinical expression of this parasitosis are associated with the duration and intensity of infection. They are also related to the variety of human immunological responses to hydatic antigens [3]. The clinical evolution of these cysts is silent for several months, and the symptoms are not specific. Diagnosis is difficult and surgery constitutes the only treatment. Parasitic infections are often associated with cytokine production [4-11].

Interleukin-6 (IL-6) is a pleiotropic cytokine with central roles in immune and inflammatory reactions [12, 13]. IL-6 first binds to the IL-6 receptor (IL-6R), this complex then associates with gp130, inducing dimerization and the initiation of signaling through signal transducer and activator of transcription-3 (STAT3) [14-18].

A lineage of IL-17-producing CD4+ T helper (Th17) cells [19-23], which are distinct from Th1 and Th2 cells, was recently discovered and was shown to be crucial in autoimmune diseases and defense against extracellular bacteria [24, 25]. Th17 cells produce IL-17 (IL-17)-A and F, IL-6, IL-21, IL-22, and TNF-α, which, in turn, act on fibroblasts, macrophages, and endothelial and epithelial cells to elicit inflammatory mediator and chemokine release [26, 27]. Th17 differentiation is thought to be mediated by the combined effects of the transcription factors RORγt and RORα, which are dependent on STAT-3, requires IL-1β,

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IL-6, IL-21, TGF-β [28-31], and the expression of the CCR6 chemokine receptor [32]. The Th17 response has been linked to the pathogenesis of several inflammatory and autoimmune diseases, such as multiple sclerosis, psoriasis, rheumatoid arthritis, colitis, autoimmune encephalitis [33] and schistosomiasis [34].

C-reactive protein (CRP) is an acute phase marker most commonly used to detect inflammation in the body, and to monitor the activity of a range of inflammatory conditions. Infection and inflammation are the most common causes of an elevated CRP level [35]. A positive correlation between CRP and IL-6 has been previously reported in several diseases [36].

We have previously shown the role of cytokines Th1 and Th2 in human hydatidosis [11]. Our analysis of circulating cytokine production in serum from hydatid patients showed the immunoprotective role of Th1 cytokines, especially IFN-γ and IL-12, and pathological role of Th2 cytokines during *E. granulosus* infection.

The present study was focused on determining the role of IL-17A in the immune response against *E. granulosus* infection. We investigated IL-6, IFN-γ and IL-17A production in serum from Algerian hydatic patients with liver and lung hydatid cysts. IL-6, IFN-γ and IL-17A were also determined in PBMC culture supernatants from patients stimulated by a major parasitic antigen (antigen-5). We have also assessed a relationship between IL-6, IL-17A production and CRP levels.

PATIENTS AND METHODS

Subjects

Blood samples were obtained from 65 (27 males and 38 females) Algerian, hydatid patients, tested before and after surgery (one week before and 72 h after surgical removal). Patients were admitted to the Mustapha Bacha Hospital (Department of surgery, Algiers, Algeria). The age range of these patients with cystic echinococcosis was 22-64 years, with a mean of 33 ± 12 years. Patients with concomitant diseases were excluded. None of the patients had ever received a blood transfusion and no pharmacological treatment had been given before had bleeding occurred. The clinical diagnosis was confirmed surgically by the presence of cysts in each case (Department of Surgery and Laboratory of Parasitology). Hydatid cysts were localized in the liver (n = 27) and lung (n = 23). We observed 15 relapse cases (two years after surgery). Healthy controls (n = 25 from the same region in Algeria) were adult volunteer blood donors, none of whom showed positive immunoreactivity when tested with passive hemagglutination, immunoelectrophoresis and ELISA using antigen-5. Healthy control subjects were between 20 and 47 years old, with a mean age of 28 ± 11 years. The serological response to *E. granulosus* was evaluated in each case using passive hemagglutination, immunoelectrophoresis and ELISA for total IgG tested against major parasitic antigen (antigen-5) prepared from fertile human cysts. Each patient had given a written, formal consent for the study required by the ethic committee of the National Agency of Research Development in Health (ANDRS), which supported our project.

Serum collection

Blood samples collected from healthy donors and patients were centrifuged at 3,000 rpm for 10 min to obtain the serum. Hemolysed serum was excluded from this study. All serum samples were immediately stored at -80°C until analysis.

Antigen-5 preparation

Hydatic cyst fluid collection

Crude, hydatid cystic fluid (HCF) samples were collected by aseptic puncture of fertile hydatid cysts removed by surgery from hydatic patients. The hydatid cysts were localized in the liver. The HCF was filtered to eliminate the remaining hydatid membranes. It was centrifuged at5000 rpm/min (15 min, +4°C), and the sediment checked for the presence of protoscolices. Finally, the cyst supernatant was stored at -20°C until antigen-5 preparation.

Antigen-5 preparation

Antigen-5 was prepared by chromatography on sephadex G-200. Eluted peaks were detected by spectroscopy recording at 280 nm and were analyzed by immunoelectrophoresis and ELISA using a specific rabbit hyperimmune serum against anti-antigen-5 for the detection. Antigen-5 was localized in the second eluted peak corresponding to a molecular weight of about 65 kDa. The eluted fractions were pooled, lyophilized and used for induction of IL-6 and IL-17A production.

Mononuclear cell preparation and cell cultures

Mononuclear cells were prepared from peripheral blood of patients with liver hydatidosis (n = 27) and healthy donors (n = 25).The mononuclear cells were separated by Ficoll-Hypaque density (1.077 mg/mL, Sigma, Saint Louis, USA) gradient centrifugation at 2600 rpm over 15 min. The mononuclear cell fraction at the interface was collected and washed twice with RPMI 1640 medium (1600 rpm, 10 min). Cell viability was assessed by trypan blue dye exclusion and adjusted to 5.10^6 cells/mL in RPMI 1640 medium supplemented with 5% fetal calf serum, inactivated at 56°C for 30 min, 15 mM Tris pH 7.5, 2 mM glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin and 10 μg/mL gentamicin. Cells were then cultured in 48-well plates (final volume of 0.5 mL) in the presence or absence of antigen-5 (10 μg/mL) and incubated for 18-20 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. Cultures were centrifuged at 12 000 rpm for 15 min and supernatants were collected and frozen at -80°C until cytokine determination.

Cytokine quantification (ELISA)

Levels of IL-17A, IFN-γ and IL-6 in serum and supernatant of PBMC cultures were quantified by enzyme-linked immunosorbent assays (ELISA) (LABSYSTEM) as recommended in the manufacturer's instructions (Immunotech, Coulter Company-France for IFN-γ and IL-6 and Invitrogen for IL-17A). Optical density was measured at 405 nm. These assays detected only human cytokines.

CRP

CRP levels were measured in serum from patients with liver hydatidosis before and after surgery (n = 27) *via* a system of automated analyzers based on nephelometry (Behring Nephelometer Analyzer, Behring, Germany). They were expressed as mg/L.

Statistical analysis

All data were expressed as means and standard deviations (mean ± SD). Student's test was used for comparisons between different means. Differences were considered statistically significant for p value ≤ 0.05.

RESULTS

1-IL-6, IFN-γ and IL-17A levels in serum from hydatid patients with liver and lung hydatidosis, before and after surgical removal of cysts

Analysis of circulating cytokine levels revealed that those of IL-6, IFN-γ and IL-17A were significantly increased in all patients in comparison with healthy controls.

Serum from patients with liver cyst had significantly higher IL-6 (145.24 ± 11.5 pg/mL) and IFN-γ (18.99 ± -2.31 IU/mL) levels before surgery than healthy donors serum (6.32 ± 3.11 pg/mL for IL-6 and 2.05 ± 0.83 IU/mL for IFN-γ) ($^{***}P<0.0001$) (*figures 1A-B*). Similarly, IL-17A (93.44 ± 7.83 pg/mL) levels measured in the same serum from hydatic patients are higher compared to levels from control subjects (8.01 ± 3.01 pg/mL) ($^{***}P<0.0001$) (*figure 1C*). We noted with interest that IL-6, IFN-γ and IL-17A production correlated with immunoreactivity against parasitic antigen, suggesting that immunostimulation by the parasitic antigen is involved in the immune response leading to IL-IL-6, IFN-γ and IL-17A production.

In order to determine a possible relationship between cyst location and cytokine production *in vivo,* serum IL-6, IFN-γ and IL-17A levels were assessed. We observed that patients with liver cysts showed the most elevated serum IL-6, IFN-γ and IL-17A levels (145.24 ± 11.5 pg/mL; 18.99 ± -2.31 IU/mL and 93.44 ± 7.83 pg/mL respectively, before surgery *versus* 183.46 ± 13.2; 8.93 ± 1.44 IU/mL and 125.36 ± 7.9 pg/mL respectively, after surgery) as compared with patients with lung cysts (105.76 ± 8.9 pg/mL; 13.32 ± 1.66 IU/mL and 75.63 ± 6.73 pg/mL respectively, before surgery *versus* 133.54 ± 10.42 pg/mL; 6.14 ± 1.34 IU/mL and 97.56 ± 8.13 pg/mL respectively, after surgery) (*figures 1A-C*). These results suggest that cystic location is strongly related to cytokine induction.

In this study, we explored the effect of the surgical removal of cysts on IL-6, IFN-γ and IL-17A levels. Analysis of cytokine production, before and after surgery, in serum from hydatid patients with liver hydatidosis showed that the IL-6 and IL-17A levels observed following surgery were higher, 72 h after surgical removal of cysts (145.24 ± 11.5 pg/mL *versus* 183.46 ± 13.2 pg/mL for IL-6 and 93.44 ± 7.83 pg/mL *versus* 125.36 ± 7.9 pg/mL for IL-17A), than those of IFN-γ, which declined rapidly (18.99 ± -2.31 IU/mL *versus* 8.93 ± 1.44 IU/mL). Thirty days after surgery, IL-6, IFN-γ and IL-17A levels were very low and almost comparable to the detected levels in healthy donors (15.01 ± 5.6 pg/mL for IL-6; 4.47 ± 1.22 IU/mL for IFN-γ; 10.69 ± 3.73 pg/mL for IL-17) (*figures 2A-C*). The reduction in IL-6, IFN-γ and IL-17A levels correlated with the loss of immunoreactivity against antigen-5, suggesting that this antigen was involved in triggering the host response.

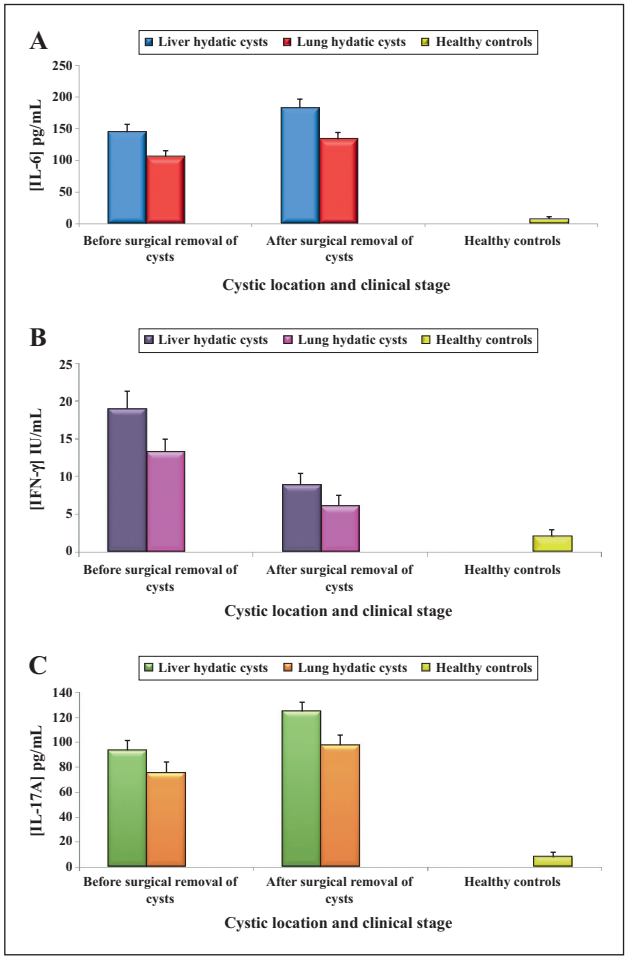

Figure 1

IL-6 (A), IFN-γ (B) and IL-17A (C) levels in serum from Algerian hydatid patients with liver (n = 27) and lung (n = 23) hydatidosis, one week before surgery and 72 h after surgical removal of cysts, and from healthy controls (n = 25). Cytokine levels are quantified as described in Methods and materials, and are presented as the mean ± SD pg/mL corresponding to the results obtained from triplicate assays. Differences are statistically significant for p value ≤ 0.05 between two groups.

In contrast, patients who relapsed two years after surgery exhibited no significant IL-6 (4.63 ± 1.6 pg/mL), IFN-γ (1.03 ± 0.52 IU/mL) or IL-17A (3.13 ± 2.23 pg/mL) levels compared to levels detected in patients and healthy donors. These patients displayed no immunoreactivity against antigen-5 (*figures 2A-C*).

Our results indicate that, in addition to the clinical stage and cystic location, there is a relationship between disease progression and cytokine production.

CRP levels were also examined in serum from patients with liver hydatid disease before and after surgery. High CRP levels were detected in serum from patients before surgery in comparison with healthy donors (*table 1*). Seventy two hours after surgical removal of the cysts, the mean levels of CRP showed significant increases. These elevated serum CRP levels returned to normal thirty (30) days after surgery (*table 1*). CRP levels correlated significantly with IL-6

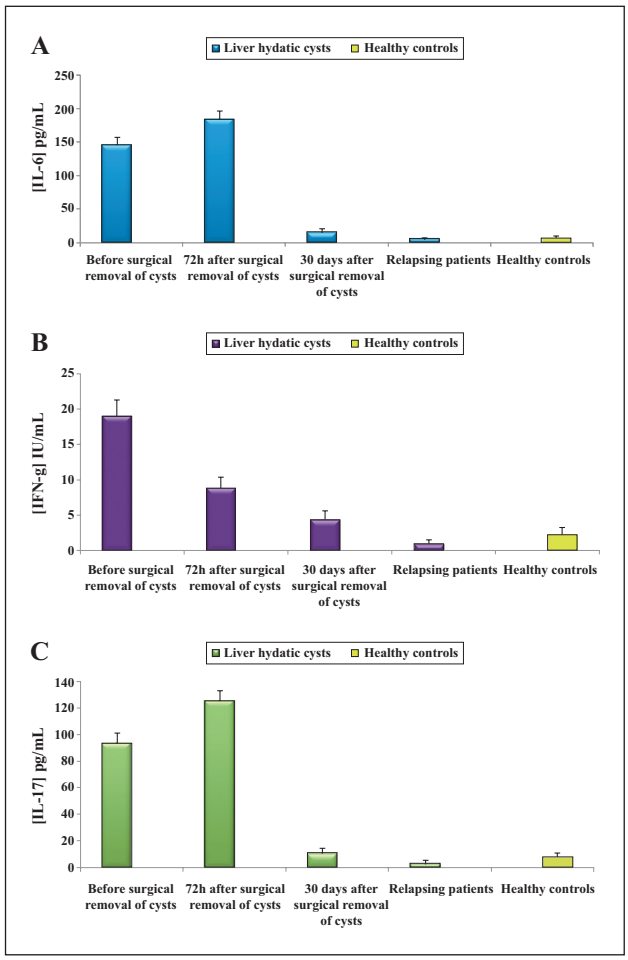

Figure 2

Effect of surgical removal of the cysts on IL-6, IFN-γ and IL-17A levels.

IL-6 (A), IFN-γ (B) and IL-17A (C) levels in serum from Algerian hydatid patients with liver hydatidosis (n = 27), 72 h, 30 days and two years after surgical removal of cysts (patients who relapsed two years after surgery, n = 15) and healthy controls (n = 25). Serum was analyzed in triplicate for human IL-6, IFN-γ and IL-17 by ELISA, and cytokines levels are presented as the mean ± SD pg/mL. Differences are statistically significant for p value ≤0.05 between two groups.

(r = 0.91; p<0.001), IL-17A (r = 0.85; p<0.001) and a positive immune response against antigen-5 (*table 1*). Our results suggest that increased serum CRP levels may be due to an increased production of IL-6 and probably IL-17A.

Antigen-5-mediated IL-6, IFN-γ and IL-17A production by PBMC from hydatidic patients

IL-6, IFN-γ and IL-17A production was measured in PBMC culture supernatants from hydatic patients with liver hydatidosis (n = 27), before and after surgery, and healthy donors (n = 25). Before antigen stimulation, significantly higher IL-6 (27.42 ± 4.2 pg/mL and 44.28 ± 5.03 pg/mL, before and after surgery respectively), IFN-γ levels (16.53 ± 2.03 IU/mL and 11.76 ± -1.40 IU/mL respectively, before and after surgery) and IL-17A (19.12 ± 2.68 pg/mL and 36.51 ± 3.72 pg/mL, before and after surgery respectively) were observed in patient PBMC supernatants in comparison with healthy donor PBMC supernatants (5.06 ± 1.8 pg/mL; for IL-6; 1.34 ± 0,81 IU/mL for IFN-γ and 3.22 ± 0.55 pg/mL for IL-17A. There were significantly higher IL-6 and IL-17A levels in post-surgical patient supernatants compared to pre-surgical supernatants (*figures 3A,C*).

After antigen-5-stimulation of PBMC cultures from hydatid patients and healthy donors, we observed a subsequent increase in IL-6 and IFN-γ levels in patient supernatants (46.25 ± 3.93 pg/mL and 71.37 ± 4.35 pg/mL for pre- and post-surgical supernatants respectively for IL-6; 24.03 ± 2.42 IU/mL and 19.77 ± 1.76 IU/mL for pre- and post-surgical supernatants respectively for IFN-γ), and only 9.14 ± 2.12 pg/mL and 2.23 ± 1.01 IU/mL***P<0.0001 for IL-6 and IFN-γ control PBMC supernatants respectively. Interestingly, IL-17A production measured in the same supernatants, showed a marked difference in stimulated PBMCs from hydatid patients (32.28 ± 3.21 pg/mL and 59.63 ± 3.93 for pre- and post-surgical supernatants respectively), in comparison to those observed for PBMCs from healthy donors (6.56 ± 1.24 pg/mL;***P<0.0001). We observed with interest, a concomitant increase in IL-6, IFN-γ and IL-17A production in the PBMC supernatants stimulated by antigen-5 (*figures 3A,C*).

In contrast, relapsing patients did not respond to antigen stimulation and their PBMCs did not secrete significant amounts of IL-6, IFN-γ or IL-17A when stimulated with parasitic antigen (antigen-5). In fact, patients who relapsed two years after surgery exhibited no significant IL-6, IFN-γ and IL-17A levels in supernatants before or after antigen-5 stimulation of PBMC cultures compared to those detected in patients and healthy donors (3,42 ± 1.02 pg/mL and 3.78 ± 1.07 pg/mL for IL-6; 1,29 ± 0.33 IU/mL and 1.22 ± 0.45 IU/mL for IFN-γ; 2,42 ± 0.73 pg/mL and 2.62 ± 0.81 pg/mL for IL-17A, respectively, before and

Table 1

Comparison of serum cytokine and CRP levels (mean ± SD) in subjects studied.

Serum	Cystic localization	Clinical stage	Serological reaction against antigen-5	[IL-6]pg/mL	[IL-17A] pg/ mL	CRP (mg/L)
n = 27	Liver	b	+	145.24 ± 11.5	93.44 ± 7.83	19.63 ± 7.3
n = 27	Liver	a	+	183.46 ± 13.2	125.36 ± 7.9	42.33 ± 11.7
n = 27	Liver	c	-	15.01 ± 5.6	10.69 ± 3.73	6.88 ± 2.4
Healthy						
donors n = 25		-	6.32 ± 3.11	8.01 ± 3.01	4.26 ± 0.45	

CRP levels correlated significantly with IL-6 (r = 0.91), IL-17A (r = 0.85), and a positive immune response against antigen-5

b: before surgery; a: after surgery; c: thirty (30) days after surgery; CRP: C reactive protein; (+): positive

Serological reaction against antigen-5; (-): positive serological reaction against antigen-5

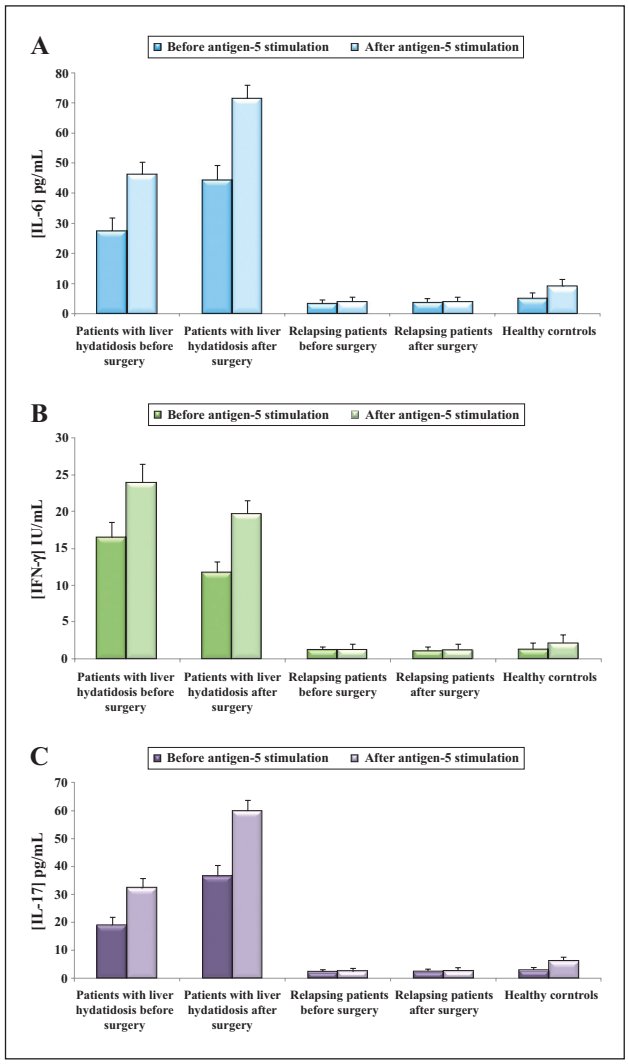

Figure 3

Correlation between IL-6, IFN-γ and IL-17A levels in supernatants of peripheral blood mononuclear cell (PBMC) cultures from hydatid patients stimulated with antigen-5. IL-6 (A), IFN-γ (B) and IL-17A (C) levels produced in the PBMC supernatants from hydatid patients with liver hydatidosis (n = 27), and from relapsing patients (patients who relapsed two years after surgery, n = 15) were tested before and after surgery (one week before surgical removal of cysts and 72h after), and healthy donors (n = 25) in response to antigen-5 (10μg/mL). IL-6, IFN-γ and IL-17A levels are presented as the mean ± SD pg/mL corresponding to the results obtained from triplicate assays. Differences are statistically significant for p value ≤ 0.05 between two groups.

after surgical removal of the cysts), and after antigen-5 stimulation of PBMC cultures (4.04 ± 1.17 pg/mL and 4.08 ± 1.2 pg/mL for IL-6; 1.42 ± 0.55 IU/mL and 1.31 ± 0.66 IU/mL for IFN-γ;2.80 ± 0.85 pg/mL and 2.96 ± 0.92 pg/mL for IL-17A, respectively, before and after surgical removal of the cysts) (*figures 3A,C*).

DISCUSSION

The results reported here show the involvement of IL-6, IFN-γ and IL-17A in the host anti-hydatic response. The increased IL-6, IFN-γ and IL-17A levels observed in serum from hydatid patients before surgery, suggested the implication of these cytokines in host defence mechanisms against the parasitic infection.

Our results are in line with those reported by many authors showing the role of IL-6 and IFN-γ in the host, anti-hydatic response [6, 7, 9, 11, 37-39]. Several intra-cellular parasitic diseases, such as infection by *Leishmania* [4, 8, 40, 41] and *Plasmodium* [42, 43], showed similar high circulating IL-6 and IFN-γ levels in patient serum. IL-6 is also induced by viral infections, auto-immune diseases, chronic inflammation, lymphoid malignancy or lipopolysaccharides [44].

IL-17 is part of a relatively new cytokine family that exhibits potent inflammatory activities, both alone and in concert with other cytokines. Increased expression of IL-17 has been reported in many clinical conditions, particularly rheumatoid arthritis and lung infections [45].

Emerging data suggest the crucial role of Th17 cells in mounting an immune response for both intracellular and extracellular pathogens. Both IL-17A and IL-17F are induced in several models of infections, suggesting the involvement of this newly described helper subset [26-46].

IL-17 was shown to be important in the protection against *Leishmania braziliensis*, *Leishmania donovani* [47], and *Toxoplasma gondii* infections [48]. Many pathogens, including Mycobacteria and *Pneumocystis carinii,* induce IL-23 secretion from infected macrophages and dendritic cells that helps in the generation of Th17 cells. In addition to fungal pathogens [49], IL-17 is also essential for the protection against gram-negative bacteria, including *Klebsiella pneumoniae* [50] and *Pseudomonas aeruginosa* [51]. A role for IL-17 in Lyme disease has also been reported: both human and mouse T cells produced increased amounts of IL-17 in response to the *Borrellia burgdorferi* infection.

Although it is well established that Th1 cells play a protective role in immunity to many pathogens, there is very limited information on the role of IL-17 in protective immunity towards infection. It has been suggested that a major function of IL-17 is to stimulate the production of CSF (GM-CSF and G-CSF), which increases the production of neutrophils, monocytes, and CXCL chemokines such as CXCL8 (IL-8), CXCL1, and CXCL6 (GCP2), which serve as potent chemoattractants for neutrophils [52], or CXCL10 (IP10), which acts as a chemoattractant for Th1 cells [53]. It is well established that neutrophils are important, both as effectors and as modulators of the immune response via secreted molecules such as IL-12. IL-17 also increases the production of IL-6, which has proinflammatory and regulatory effects on the immune response.

It has been demonstrated that Th17 may bridge the gap between innate and adaptive immunity and could attract other subsets of Th cells and cells of the innate compartment, including neutrophils and macrophages, to sites of infection, to enable efficient clearing of the pathogens. Th17 cells are required for a recall response of mice vaccinated with *M. tuberculosis* antigen, as they promote the recruitment of Th1 in the lungs by stimulating the release of mediators such as CXCL10 [54]. Th17 cells could play the same role in *L. donovani* infections. Naturally-resistant subjects with enhanced IL-17 responses would thus react more rapidly to *L. donovani*, attracting strong effectors of innate immunity and recruiting Th1 cells to tissues. These cells would, in turn, enhance the microbicidal activity of phagocytes. Thus, Th17 and Th1 cells may play complementary roles in protection against *L. donovani,* with both being required for complete protection. In this association

between Th1 and Th17 responses, Th1 cells may play the important role of downregulating the Th17 response after the infection is controlled, thereby preventing tissue damage resulting from out-of-control expansion of the Th17 cell population [55].

The clinical stage and antigenic burden appeared to influence IL-6, IFN-γ and IL-17A production. As reported above, IL-6, IFN-γ and IL-17A levels were high in presurgical patients. Seventy two hours after surgery, circulating IL-6 and IL-17A levels increased. Nevertheless, thirty days after surgical removal, circulating IL-6, IFN-γ and IL-17A levels were very low. The reduction in IL-6, IFN-γ and IL-17A levels correlated with the loss of immunoreactivity against antigen-5, suggesting that this antigen was involved in triggering the host response. These results indicate that the reduction in IL-6, IFN-γ and IL-17A production, and the loss of immunoreactivity, were due to the fact that surgical removal of the cysts drastically reduced the availability of soluble parasitic antigen, especially the major antigen 5.

The higher serum IL-6 and IL-17A levels observed 72 h after surgery, in the absence of postoperative complication such as infection, was probably due to surgical stress and subsequent activation of immune responses. Cruickshank *et al.* and Yamauchi *et al.* reported that surgical intervention induces a cytokine response depending on the type of operation and severity of surgical trauma [56, 57]. Several authors have showed the effect of surgery on components of the human immune system. It has been demonstrated that surgery induced a series of inflammatory responses such elevation of body temperature and leukocytosis, and increased acute phase reactants [58, 59]. Current evidence suggests that these responses are mediated by proinflammatory cytokines such as IL-6 and probably IL-17, which are produced locally and enter the blood stream very soon after surgery.

Serum levels of CRP were also determined before and after surgical intervention. Substantial amounts of CRP were detected in serum from hydatid patients before surgery. It was significantly increased 72 h after surgical removal of the cysts. The most probable reason for this was surgical stress. A positive correlation between CRP, IL-6 and IL-17A was found, confirming the relationship between inflammation due to cystic echinococcosis and activation of monocytes. Our results suggest that echinococcosis causes activation of monocytes *via* increased production of IL-6 and IL-17A and in turn increasing CRP production. Such activation was enhanced during surgery as surgical stress can trigger the production of inflammatory cytokines.

A positive correlation between CRP and IL-6 has been previously reported in several diseases [60, 61].

Our results support the role of IL-6 [62], and probably IL-17A, in the induction of CRP synthesis. Many authors have demonstrated that IL-6 and IL-17 have a very important role in the mediation of immunoinflammatory events [63, 64].

Andru *et al.* and Mackiewicz *et al.* reported that IL-6 acts on liver cells as a hepatocyte-stimulating factor, and induce various acute-phase proteins such as CRP, β_2 –fibrinogen, haptoglobin and hemopexin, in synergy with IL-1 and TNF-α [65, 66]. IL-17 has pro-inflammatory properties and induces fibroblasts, endothelial cells, macrophages, and epithelial cells to produce several inflammatory mediators, leading to inflammation.

In addition to the clinical stage, our results indicate that cyst location also was related to the intensity of cytokine induction. Patients bearing hepatic cysts have higher serum cytokine levels in comparison with those with cysts in the lungs. The data were in agreement with a previously published report indicating that cyst location determines the host immune response. Cesbron *et al.* reported that hepatic hydatid cysts were expected to provide the highest amounts of antigen, probably because of the good vascularity of this major filtering organ [67]. Moreover, Vidor *et al.* and Craig and Nelson showed that hepatic cysts are more permeable than pulmonary cysts [68, 69].

IL-6, IFN-γ and IL-17A production was also measured in peripheral blood mononuclear cell (PBMC) culture supernatants from hydatic patients. PBMC from patients with liver hydatidosis, re-stimulated by the parasitic antigen, antigen-5, responded actively to the antigen stimulation by an increased release of IL-6, IFN-γ and IL-17A, as compared to healthy controls and relapsing patients.

These results confirm the involvement of parasitic antigen in the immune response leading to IL-6, IFN-γ and IL-17A induction.

Concomitant increases in IL-6, IFN-γ and IL-17A levels, produced in the PBMC supernatants from patients, were observed with interest. The role of IL-6 in Th17 differentiation has been clearly demonstrated [71, 72]. Our results indicate that stimulation with antigen-5 induced Th1 and Th17 cells. Furthermore, the cytokines secreted by these T cell subtypes may contribute to protection against *E. granulosus*.

In our study, significantly lower IL-6, IFN-γ and IL-17A levels were observed both in serum, and in supernatants of PBMC cultures from relapsing patients who did not display any immune response towards parasitic antigen (antigen-5). These data support the notion of a strong relationship between disease progression and cytokine production.

The concomitant decrease in IL17A and IFN-γ in the relapse stage is probably related to the development of evasion strategies by the macroparasites leading to host defense inhibition. This evasion mechanism seems to be related to the reduction in the ability of Th17 and Th1 cell subsets to respond to the parasitic antigen in the relapse stage of hydatidosis. The decrease in IL-17A and IFN-γ induction indicate a reduction in sensitivity, and probably a lack of memory aptitude for the parasitic antigen in relapsing patients.

This mechanism remains to be clarified in adapted experimental models of echinococcosis. In our previous study, we showed that Th1 cytokines are related to a good prognosis in hydatidosis. In contrast, Th2 cytokines correlated with susceptibility to the disease and were associated with clinical complications (relapsing) and secondary localizations [11]. Our current study seems to suggest a beneficial, preventive effect of IL17 A and IFN-γ against *E. granulosus* in humans.

Our results are in agreement with recent data reported by Jiao-Yu *et al.*, showing no differences in IFN-γ and IL-17A serum levels between the patients with chronic cystic echinococcosis and healthy controls. This observation supports the hypothesis that during chronic cystic echinococcosis infection, altered TLR2 and TLR4 expres-

sion might be involved in cytokine modulation, which allows the parasite to escape host immunosurveillance and promotes chronic *E. granulosus* infection [70].Touil-Boukoffa *et al.*, showed that leukocytes from relapsing patients did not secrete significant amounts of IFN-γ when stimulated with antigen-5, which is linked to a decreased CD4+ T-cell count [38].

Collectively, the present study provides evidence that IL-17A is produced in Algerian patients during *E. granulosus* infection Its production correlates with IL-6 and IFN-γ production, and varies according to cystic localization, clinical stage, immunoreactivity against parasitic antigen, and disease progression. Our results suggest that IL-17A, in addition to IFN-γ, plays a role in protection against *E. granulosus*. These findings should stimulate the development of new strategies for preventing this severe and devastating parasitic disease responsible for the deaths of large numbers of people in countries in which it is endemic.

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REFERENCES

1. Mc Manus DP, Smyth JD. Hydatidosis: Changing Concepts in Epidemiology and Speciation. *Parasitol Today* 1986; 2: 163.
2. Seimenis A. Overview of the epidemiological situation on Echinococcosis in the Mediterranean region. *Acta Trop* 2003; 85: 191.
3. Kharebov A, Kahmias J, Elon J. Cellular and humoral immune responses of hydatidosis patients to *Echinococcus granulosus* purified antigens. *Am J Trop Med Hyg* 1997; 57: 619.
4. Cox FEG, Liew Y. Cell subsets and cytokines in parasitic infections. *Parasitol Today* 1992; 8: 371.
5. Barcinski MA, Costa-Moreira ME. Cellular response of protozoan parasites to host-derived cytokines. *Parasitol Today* 1994; 10: 352.
6. 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.
7. Touil-Boukoffa C, Sanceau J, Tayebi B, Weitzerbin J. Relationship Among Circulating Interferon, Tumor Necrosis Factor-α and Interleukin-6 and Serologic Reaction Against Parasitic Antigen in Human hydatidosis. *J Interferon Cytokine Res* 1997; 17: 211.
8. Cetre C, Pierrot C, Cocude C, *et al*. Profiles of Th1 and Th2 cytokines after primary and secondary infection by Schistosoma mansoni in the semipermissive rat host. *Infect Immunol* 1999; 67: 2713.
9. Rigano R, Profumo E, Ioppolo S, Notagiacomo S, Teggi A, Siracusano A. Serum cytokine detection in the clinical follow up of patients with cystic echinococcosis. *Clin Exp Immunol* 1999; 115: 503.
10. Torre D, Speranza F, Giola M, Matteelli A, Tambin R, Biondi G. Role of Th1 and Th2 cytokines in Immune Response to Uncomplicated *Plasmodium Falciparum Malaria. Clin Diagn Lab Immunol* 2002; 9: 348.
11. Mezioug D, Touil-Boukoffa C. Cytokine profile in human hydatidosis: Possible role in the immunosurveillance of patients infected with *Echinococcus granulosus.* Parasite 2009; 16: 57.
12. Kishimoto T. The biology of interleukine-6. *Blood* 1989; 74: 1.
13. Hirano T, Akira S, Taga T, Kishimoto T. Biological and Clinical aspects of interleukin 6. *Immunol Today* 1990; 11: 443.
14. Hibi M, Nakajima K, Hirano T. IL-6 cytokine family and signal transduction a model of the cytokine system. *J Mol Med* 1996; 74: 1.
15. Kishimoto T. IL-6 family of cytokines and gp-130 biological and clinical implications. *Eur Cyt Netw* 1996; 7: 429.
16. Liu XH, Kirschenbaum A, Lu M, *et al*. Prostaglandin E (2) stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6 /GP130 /STAT-3 signaling pathway. *Biochem Biophys Res Commun* 2002; 290: 249.
17. Humphrey RK, Beattie GM, Lopez AD, *et al*. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells* 2004; 22: 522.
18. Mitsuyama K, Matsumoto S, Rose-John S, *et al*. STAT3 activation via interleukin 6 transsignalling contributes to ileitis in SAMP1/Yit mice. *Gut* 2006; 55: 1263.
19. Yao Z, Fanslow WC, Seldin MF, *et al*. *Herpesvirus Saimiri* encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 1995; 3: 811.
20. Harrington LE, Hatton RD, Mangan PR, *et al*. Interleukin-17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; 6: 1069.
21. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. *Curr Opin Immunol* 2007; 19: 652.
22. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; 25: 821.
23. Jong E, Suddason T, Lord GM. Development of mouse and human T helper 17 cells. *Clin Exp Immunol* 2010; 159: 48.
24. Park H, Li Z, Yang XO, *et al*. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; 6: 1133.
25. Romagnani S. Human Th17 cells. *Arthritis Res Ther* 2008; 10: 206.
26. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity* 2004; 21: 467.
27. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annu Rev Immunol* 2009; 27: 485.
28. Ivanov II, McKenzie BS, Zhou L, *et al*. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17(+) T helper cells. *Cell* 2006; 126: 1121.
29. Mangan PR, Harrington LE, O'Quinn DB, *et al*. Transforming growth factor beta induces development of the Th17 lineage. *Nature* 2006; 441: 231.
30. Annunziato F, Cosmi L, Santarlasci V, *et al*. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; 204: 1849.
31. Zhi C, Arian L, O'Shea JJ. Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. *Semin Immunol* 2007; 19: 400.
32. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol* 2008; 180: 214.
33. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev* 2008; 223: 87.

34. Rutitzky LI, Stadecker MJ. CD4 T cells producing pro-inflammatory interleukin-17 mediate high pathology in schistosomiasis. *Mem Inst Oswaldo Cruz* 101 Suppl 2006; 1: 327.

35. Black S, Kushner I, Samols D. C-reactive protein. *J Biol Chem* 2004; 279: 48487.

36. Kawasaki Y, Hosoya M, Katayose M, Suzuki H. Correlation between serum interleukin-6 and C-reactive protein concentrations in patients with adenoviral respiratory infection. *Pediatr Infect Dis J* 2002; 21: 370.

37. Rigano R, Profumo E, Teggi A, Siracusano A. Production of IL-5 and IL-6 by peripheral blood mononuclear cells (PBMC) from patients with *Echinococcus granulosus* infection. *Clin Exp Immunol* 1996; 105: 456.

38. Touil-Boukoffa C, Bauvois B, Sancéau J, Hamrioui B, Wietzerbin J. Production of nitric oxid (NO) in human hydatidosis: Relationship between nitrite production and interferon-γ levels. *Biochimie* 1998; 80: 739.

39. Refik M, Mehmet N, Durmaz R. Postoperative Changes In Serum Cytokines Profile And Nitric Oxide Levels In Patients With Cystic Echinococcosis. *Parasite* 2005; 12: 265.

40. Caceres-Dittmar G, Tapia FJ, Sanchez MA, *et al*. Determination of the cytokines profile in American cutaneous leishmaniasis using the polymerase chain reaction. *Clin Exp Immunol* 1993; 91: 500.

41. Melby PC, Andrade-Narvaez FJ, Darnell BJ, Valencia-Pacheco G, Tryan W, Palomocetina A. In creased expression of proinflammatory cytokines in chronic lesions of human cutaneous leishmaniasis. *Infect Immunol* 1994; 62: 837.

42. Kern P, Hemmer CJ, Van Damme J, Gruss HJ, Dietrich M. Elevated tumor necrosis factor alpha and interleukin-6 as markers for complicated *Plasmodium falciparum* malaria. *Am J Med* 1989; 87: 139.

43. Prada J, Malinowski J, Muller S, Bienzle U, Kremsner PG. Hemozoin differentially modulates the production of interleukin-6 and tumor necrosis factor alpha in murine malaria. *Eur Cytokine Netw* 1995; 6: 109.

44. Van Snick J. interleukin-6: an Overview. *Annu Rev Immunol* 1990; 8: 253.

45. Wu Q, Martin RJ, Rino JG, Breed R, Torres RM, Chu HW. IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory *Mycoplasma pneumoniae* infection. *Microbes Infect* 2007; 9: 78.

46. Ishigame H, Kakuta S, Nagai T, *et al*. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. *Immunity* 2009; 30: 108.

47. Pitta MG, Romano A, Cabantous S, *et al*. IL-17 and IL-22 are associated with protection against human kala azar caused by *Leishmania donovani. J Clin Invest* 2009; 119: 2379.

48. Kelly MN, Kolls JK, Happel K, *et al*. Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against *Toxoplasma gondii* infection. *Infect Immun* 2005; 73: 617.

49. Zelante T, De Luca A, Bonifazi P, *et al*. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol* 2007; 37: 2695.

50. Ye P, Garvey PB, Zhang P, *et al*. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. *Am J Respir Cell Mol Biol* 2001; 25: 335.

51. Priebe GP, Walsh RL, Cederroth TA, *et al*. IL-17 is a critical component of vaccine-induced protection against lung infection by lipopolysaccharide heterologous strains of *Pseudomonas aeruginosa. J Immunol* 2008; 181: 4965.

52. Kelly MNJK, Kolls K, Happel JD, *et al*. Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against *Toxoplasma gondii* infection. *Infect Immun* 2005; 73: 617.

53. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008; 28: 454.

54. Khader SA, Bell GK, Pearl JE, *et al*. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nature Immunol* 2007; 8: 369.

55. Pitta MGR, Romano A, Cabantous S, *et al*. IL-17 and IL-22 are associated with protection against human kala azar caused by *Leishmania donovani. J Clin Invest* 2009; 119(8): 2379-87.

56. Cruickshank AM, Fraser WD, Burns HJ, Van Damme J, Shenkin A. Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. *Clin Sci* 1990; 79: 161.

57. Yamauchi H, Kobayashi E, Yoshida T, *et al*. Changes in immune-endocrine response after surgery. *Cytokine* 1998; 10: 549.

58. Lennard TWJ, Shenton BK, Borztta A *et al*. The influence of surgical operations on components of the human immune system. *Br J Surg* 1985; 721.

59. Sakamoto K, Arakawa H, Mita S, *et al*. Elevation of circulating interleukin 6 after surgery: factors influencing the serum level. *Cytokine* 1994; 6: 181.

60. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; 265: 621.

61. Kawasaki Y, Hosoya M, Katayose M, Suzuki H. Correlation between serum interleukin-6 and C-reactive protein concentrations in patients with adenoviral respiratory infection. *Pediatr Infect Dis J* 2002; 21: 370.

62. Castell JV, Gomez-Lechon MJ, David M, *et al*. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Letters* 1989; 242: 237.

63. Seghal PB, Grienger G, Tosata G. Regulation of the acute phase and immune responses: Interleukin-6. *Ann NY Acad Sci* 1989; 1: 557.

64. Hirano T. Interleukin 6 and its relation to inflammation and disease. *Clin Immunol Immunopathol* 1992; 62: 60.

65. Andru T, Bauer J, Gerok W. Effects of cytokines on the liver. *Hepatology* 1991; 13: 364.

66. Mackiewicz A, Scooltink H, Heinrich P, Rose-John's M. Complex of soluble human IL-6up regulates expression of acut-phase proteins. *J Immunol* 1992; 149: 2021.

67. Cesbron JY, Capron M, Capron A. Le diagnostic immunologique de l'hydatidose humaine. *Gastroentérol Clin Biol* 1986; 10: 415.

68. Vidor E, Piens MA, Abbas M, Pettavy AF. Biochimie du liquide hydatique, influence de la localisation sur la perméabilité des kystes. *Ann Parasitol Hum Comp* 1986; 61: 330.

69. Craig PS, Nelson GS. The detection of circulating antigen in human hydatid disease. *Ann Trop Med Parsitol* 1984; 78: 219.

70. Jiao-Yu S, Wei-Zheng J, Hai-tao L, Tuerhongjiang T, Ren-Yong L, Hao W. TLR2, TLR4 expression in peripheral blood mononuclear cells of patients with chronic cystic echinococcosis and its relationship with IL-10. *Parasite Immunology* 2011; 33:692-696.

71. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. *Cur Opinion Immunol* 2007; 19: 281.

72. Gaffen SL. An overview of IL-17 function and signaling. *Cytokine* 2008; 43: 402.