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IL-10, IFN-γ AND TNF-α IN ACUTE AND CHRONIC HUMAN FASCIOLIASIS

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ABSTRACT

The three cytokines, IL-10 (Th2 product), IFN-γ (Th1 product) and TNF-α work in concert. The present work was undertaken to study the level of these cytokines during the acute and chronic stages of human fascioliasis in an attempt to assess the involvement of Th1 and Th2 cells in regulation of the immune response in this disease. Sera of twenty six *Fasciola* patients were obtained and divided into two groups: twelve with acute and fourteen with chronic fascioliasis, sera of a control group were studied simultaneously. During the acute stage, a significant increase was observed in IL-10, IFN-γ as well as TNF-α and specific antibody level. In the chronic phase, a significant increase of IL-10 level was observed. IFN-γ showed a lower level as compared to the acute stage but TNF-α was still high. Accordingly, in fascioliasis in the early phase of infection B cells, macrophages, Th1 and Th2 cells were all activated. They cooperate in overcoming the parasite and work to the benefit of the host. With time and after maturation of the evading worms, Th2 action predominates. IL-10 (Th2 cytokine) which is antagonistic to IFN-γ (Th1 product) and consequently to TNF-α limits the immunopathology that may be caused by the latter.

INTRODUCTION

Parasitic infections typically stimulate both antibody and cell mediated immune responses. Review of literature showed that the studies on the

immune response in human fascioliasis are limited. So, the role of either humoral or cellular mechanisms remains uncertain and poorly understood. Generally, multiple subpopulations of T-lymphocytes play important roles in the induction and regulation of the immune responses. In fact, expression of protective immunity and of pathologic changes are regulated by a complex network of coordinated responses: a T-cross regulatory circuit (Pancre et al., 1994). The cell clones have been divided based on function and lymphokine secretion into Th1 and Th2. Cher and Mosmann (1987) reported that some antigens appear to preferentially induce Th1, some others induce Th2 and some induce both. B cells present antigen to Th2 cells. This triggers the release of different cytokines among which IL-10, which in turn induces B cell activation and differentiation into plasma cells producing antibodies (Rousett et al., 1992). IL-10 may be anti-inflammatory and immunosuppressive (Roitt et al., 1998). A macrophage internalizes material and interacts with Th1 cells which produces cytokines including IFN-γ. IFN-γ in turn activates the macrophage and can act to limit parasite survival (Roitt et al., 1988). TNF-α is the prime effector cytokine of the macrophages. It is produced by other cells: T cells and natural killer cells. It is a principal mediator of inflammatory and immunopathologic responses (Peakman and Vergani 1997). The three cytokines IL10, IFN-γ and TNF-α work in concert. During an immune response release of IFN-γ activates macrophages, augments TNF-α release and regulates antibody responses. IL-10 suppresses the production of TNF-α by macrophages, this indirectly inhibits production of IFN-γ by T-cells (Peakman and Vergani 1997).

The aim of the present work is to study the level of IL-10, IFN-γ and TNF-α in acute and chronic human fascioliasis and accordingly to assess the involvement of Th1 and Th2 cells in regulation of the immune response in this disease.

MATERIAL AND METHODS

Patients and sera: Twenty six *Fasciola* patients were enrolled in the present study. Their age ranged from 16 to 50 years, ten were males and sixteen were females. They were divided into two groups: (1) Twelve patients with acute fascioliasis (without egg excretion in the stool) presenting with

hepatic pain and fever, revealing eosinophilia and diagnosed serologically by positive indirect hameagglutination test (Fumouse-France Kit). (2) Fourteen patients with chronic fascioliasis as evidenced by the presence of *Fasciola* ova in repeated stool samples by Kato-Katz technique (Katz et al., 1970). They were serologically examined by IHA test. Ten apparently healthy free parasitic subjects of matched age and sex were enrolled as a control group.

Preparation of serum: Under sterile conditions, 2 ml blood were withdrawn and then allowed to clot. Serum was collected and stored at -40°C until used.

Assay of cytokines: Human serum IL-10, IFN-γ and TNF-α levels were detected using commercial available Enzyme lined immunosorbent assay (ELISA) kit according to the manufacturer's specifications (Genzyme). Samples were incubated with solid phase monoclonal antibodies to IL-10, IFN-γ and TNF-α which were used as capture antibodies. Second polyclonal antibodies biotinylated rabbit anti-IL-10, anti-IFN-γ and anti-TNF-α were used. The resulting immune complexes were then incubated with a streptavidin peroxidase conjugate. The substrate and the chromogen were added. Resultant colour development was stopped, it was directly proportional to the concentration of IL-10, IFN-γ and TNF-α respectively.

RESULTS

Indirect haemagglutination titres in acute and chronic fascioliasis (Table 1) showed high specific *Fasciola* antibody levels with titres ranging from 1/640 to 1/2560 in all cases of acute fascioliasis. However, in chronic cases, antibodies were either absent or showed low positive values in only two cases.

IL-10, IFN-γ and TNF-α cytokine levels in acute and chronic fascioliasis (Table 2) during the acute stage, showed a significant increase in IL-10, IFN-γ as well as TNF-α as compared to the controls. In chronic phase, IL-10 showed a significant increase, IFN-γ showed a lower level as compared to the acute stage; TNF-α was still high.

Table (1): IHA titre in cases of acute and chronic fascioliasis.

Reciprocal* IHA titres	Acute Fascioliasis		Chronic Fascioliasis	
	No.	%	No.	%
80	-	-	9	64.3
160	-	-	3	21.4
320	-	-	2	14.3
640	2	16.7	-	-
1280	4	33.3	-	-
2560	6	50.0	-	-
Total	12	100.0	14	100/0

Positive titre ≥ 320

Table (2): IL-10, IFN-γ and TNF-α cytokine levels in acute and chronic fascioliasis.

Group studied	Cytokine level		
	IL-10 (pg/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)
Controls (n=10)			
Range	0.9-2.9	1.1-3.8	35-85
Mean	2.04	1.92	48.7
S.D	0.56	0.797	13.138
Acute fascioliasis (n=12)			
Range	25-75	38-105	75-175
Mean	42.25*@	67.75*@	125.0*@
S.D	15.21	21.939	27.763
Chronic fascioliasis (n=14)			
Range	78-125	14-56	105-230
Mean	105.071*	25.571*	158.785*
S.D	14.542	11.938	42.307

* Significant difference compared to control

@ Significant difference compared to the other stage of the disease.

DISCUSSION

In the present study, during the acute stage of infection, a significant increase was observed in IL-10, IFN-γ as well as TNF-α and in specific antibody level. This can be explained by the fact that in early infection, the immature *Fasciola* worms wander in the liver parenchyma releasing metabolites, E/S antigens and toxins and inducing severe tissue damage (Beaver et al., 1984). These parasite products initiate a complex immune response including macrophage activation and proliferation of cells, Th1 and Th2. In controlling the acute infection, non specific host defense mechanisms together with the development of specific cells mediated and humoral responses are both important. Activation of marophage is a general feature of the early stage of infection. These cells secrete many cytotoxic factors enabling them to kill parasites without ingesting them. They secrete TNF-α and interact with Th1 leading to production of IFN-γ. Elevated level of IL-10 (a Th2 product) was observed as well at this stage. This leads to activation of B cells with release of high specific antibody level which helps the macrophage in its action as killer cell through antibody dependent cell mediated cytotoxicity (Roitt' et al., 1998). TNF-α is both protective and pathogenic. Protection versus pathologic role of TNF-α depends on the quantity of TNF-α released, the time period over which its production is sustained, the site of its expression and also the presence of other cytokines regulating its production (Jacobs et al., 1996). Early during infection, TNF-α has a protective role which involves more than the inhibition of parasite growth. It has no direct parasiticidal effect but has a crucial role in modulating the initial phase of the cell mediated immune response (Bromberg et al., 1992). TNF-α can activate endothelial cells and thus promote neutrophil margination and migration into the inflammed sites (Peakman and Vergani 1997). Phagocytosis and intracellular killing by neutrophils was reported a prominent feature of the acute phase of fascioliasis (Osman et al., 1995). IFN-γ has an important role in the up-regulation of TNF-α production and has a crucial role in development of immunity. It allows the host to maintain control over the acute infection by enhancing the activity of Th1 cells and therefore augments macrophage reaction (Smythies et al., 1992). The role of IL-10 (a Th2 product) in preventing infection induced immune hyperactivity may be a general principle related to infections. IL-10

may be anti-inflammatory and immuno-suppressive. It is only one, of perphaps several immuno-suppressive molecules, involved in the down regulatory response during actue infection (Mossmann 1994; Neyer et al., 1997; Roitt et al., 1998).

In the chronic phase of fascioliasis IL-10 showed a significant increase while antibodies were either absent or low, INF-γ showed a lower level as compared to the acute stage but TNF-α was still high. In this stage the adult flukes are located inside the bile ducts and the gall bladder; most of their eggs, excretions and metabolites pass with the stools away from the sites of systemic immune response (Fawzy et al., 1992). Pathologically, biopsy studies of the human liver have demonstrated granulomatous reactions with foci of degeneration and necrosis of the liver parenchyma with ductular hyperplasia (Abou Basha et al., 1990). In chronic stage, the anti-inflammatory and immunosuppressive role of IL-10 probably predominates over its function as B cell activator. In fact, low antibody level was observed in the present work, as well as in several other studies (Osman et al., 1992). IL-10 is known to inhibit macrophage secretion of mediators such as TNF-α resulting in a decrease in recruitment, activation and expansion of cells in granulomatous foci (Amiri et al., 1992). Morevoer, IL-10 is a potent antagonist of IFN-γ (Fiorentino et al, 1991). Unchecked production of IFN-γ and TNF-α was reported responsible for inducing immunopathology to the host (Beutler and Grau, 1993). Thus, this increase of IL-10 observed in the present work is expected to be beneficial to the host, limiting the pathology during the chronic phase. In fact, TNF-α which is known to cause local coagulation with blockage of the blood supply leading to infarcts and necrosis (Oppenheim and Ruscetti, 1997) may be partially responsible for the great magnitude of parenchymal destruction described during parasite development.

In conclusion, in fascioliasis in the early phase of infection B cells, macrophages, Th1 and Th2 cells were all activated. They cooperate in overcoming the parasite and work to the benefit of the host. With time and after maturation of the evading worms, Th2 action predominates. IL-10 which is antagonistic to IFN-γ and consequently to TNF-α limits the immunopathology that may be caused by the latter.

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