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• BRIEF REPORTS •

Serum sIL-2R, TNF-α and IFN-γ in alveolar echinococcosis

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Abstract

AIM: To approach the relationship between alveolar echinococcosis (AE) pathology and level of sIL-2R,TNF-α and IFN-γ in sera and the significance of cytokines in development of AE.

METHODS: After 23 patients with AE were confirmed by ELISA and ultrasound, their sera were collected and the concentrations of sIL-2R,TNF-α and IFN-γ were detected by double antibody sandwich. Twelve healthy adults served as controls. According to the status of livers of AE patients by ultrasound scanning, they were divided into 4 groups: P_2, P_3, P_4 groups and C group (control). Average of concentrations of sIL-2R,TNF-α and IFN-γ in homologous group was statistically analyzed by both ANOV and Newman-Keuls, respectively.

RESULTS: The mean of sIL-2R in P_2 group was 97±29, P_3: 226±80, P_4: 194±23 and control group (111±30)×10^3 u/L (P<0.01). The mean of TNF-α in P_2 group was 1.12±0.20, P_3: 3.67±1.96 , P_4: 1.30±0.25 and control group 0.40±0.19 μg/L (P<0.01). The mean of IFN-γ in P_2 group was 360±20, P_3: 486±15, P_4: 259±19 and control group: 16±2 ng/L (P<0.01). Judged by ANOV and Newman-Keuls, the mean concentrations of sIL-2R, TNF-α and IFN-γ had a significant difference among groups. Except for P_2 group, the mean sIL-2R between other groups of AE patients had a significant difference (P<0.05). The mean of TNF-α concentration in P_3 group was the highest (P<0.01). The mean of IFN-γ concentration in all patients was higher than that in control group (P<0.01), but there was no difference between AE groups (P>0.05).

CONCLUSION: Low sIL-2R level indicates an early stage of AE or stable status, per contra, a progression stage. Higher level of TNF-α might be related to the lesion of liver. The role of single IFN-γ is limited in immunological defense against AE and it can not fully block pathological progression.

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INTRODUCTION

Alveolar echinococcosis (AE) is a rare and potentially fatal parasitic disease[1,2]. B ultrasound and immunological tests are the most useful diagnostic methods for AE. In keeping with the popularity of B-ultrasound in diagnosis and epidemiological survey of AE, it is of important clinical and theoretical significance to understand the relationship between AE clinical types or pathological process and cytokines from patients with AE. B ultrasound has the characteristics of rapidity, accurate location and direct viewing for AE diagnosis and could reflect the pathological process of AE.

Since the early 1990 s, the study on cell mediated immunity of AE has rapidly progressed[3,4], but studies on AE clinical types coupled cytokines are rare. During the epidemiological survey in Gansu Province, China, cytokines in patients with AE were detected. According to the pathological lesions obtained by B-ultrasound, the patients were divided into different stages, and the relationship between clinical types and cytokines in sera was discussed.

MATERIALS AND METHODS

Twenty-three cases of AE were confirmed by ELISA and ultrasound and their sera were collected. Absorbency values of sIL-2R, TNF-α and IFN-γ were detected by double antibody sandwich. Twelve healthy adults served as controls. The standard curve was drawn and the concentrations of above cytokines were measured respectively. In reference to the standardization of TNM classification system for hepatocellular carcinomas, all subjects were divided into 4 groups: P_2, P_3, P_4 groups and C group (control group). The average of concentrations of sIL-2R,TNF-α and IFN-γ was statistically analyzed by both ANOV[5] and Newman-Keuls[6] respectively.

RESULTS

A total of 23 patients with AE were divided into P_2 group: 7 cases, P_3 group: 9 cases, P_4 group: 7 cases. The mean of sIL-2R in P_2 group was 97±29, 226±80 in P_3 group, 194±23 in P_4 group and (111±30)×10^3 u/L in control group (F: 9.19; P<0.01). The mean of TNF-α in P_2 group was 1.12±0.2, 3.67±1.96 in P_3 group, 1.30±0.25 in P_4 group and (0.40±0.19) μg/L in control group (F: 13.56; P<0.01). The mean of IFN-γ was 360±20 in P_2 group, 486±15 in P_3 group, 259±19 in P_4 group and (16±2) ng/L in control group (F: 17.25, P<0.01). This indicated that the mean concentrations of sIL-2R, TNF-α and IFN-γ had a significant difference between different groups (P<0.01). Except in P_2 group, the mean concentration of sIL-2R between other groups of AE patients and controls had a significant difference (P<0.05), so was between P_2 and other 2 groups of AE. The mean concentration of TNF-α in P_3 group was the highest and had a significant difference from the other 3 groups (P<0.01). The mean concentration of IFN-γ in all patients was higher than that in control group (P<0.01), but there was no difference between AE groups (P>0.05). Table 1 summaries the comparison of the Q value of mean sIL-2R, TNF-α, IFN-γ between each two groups.

DISCUSSION

Ninety-five percent of primary foci of AE locate in the liver. They proliferate through exogenous budding and metastasize from the primary location to distant sites. At the early stage of AE,

Table 1 Comparison of Q value of mean sIL-2R,TNF-α, IFN-γ between each two groups

	P_2 and P_3	P_2 and P_4	P_3 and P_4	P_2 and C	P_3 and C	P_4 and C
sIL-2R	6.19[b]	4.64[a]	1.56	0.64	5.56[b]	4.00[a]
TNF-α	5.99[b]	0.41	5.58[b]	1.7	7.69[b]	2.11
IFN-γ	0.28	1.72	2	7.98[b]	8.26[b]	6.26[b]

[a]$P<0.05$, P_2 *vs* P_4, P_4 *vs* C for sIL-2R; [b]$P< 0.01$, P_2 *vs* P_3, P_3 *vs* C for sIL-2R; P_2 *vs* P_3, P_3 *vs* P_4, P_3 *vs* C for TNF-α; P_2 *vs* C, P_3 *vs* C, P_4 *vs* C, for IFN-γ.

the main pathologic manifestation was limited to vesicles with a few millimeters in diameter, while hepatic ultrasound scanning showed limited nodes. Next AE lesions infiltrated, without well-defined limits, and tended to extend to a large area of the liver. The infiltration, which is similar to some malignant hepatic neoplasms, could bring about stenoses of intrahepatic bile ducts, the hepatic veins and portal branches. Following parasite reproduction, necrosis would occur and gave rise to a large central cavity containing gelatinous effusion with debris, bile and sometimes pus. Although TNM-system has some defect for the classification of liver cancers[7], it is still considered authoritative, because it could reflect the size of tumor, growing pattern, encapsulation of tumor, daughter nodules (including microscopic nodules), vascular invasion, or biliary involvement and metastases[8]. AE is similar to a malignant hepatic tumor in growth and pathologic process, however there has been no standard classification of AE by now. In reference to the standardization of liver cancer in the present study, AE was divided into 3 types, namely parasite location in the liver (type P), involvement of adjacent organs (type I), and metastasis (type M). Type P was further divided into 5 stages: P_0: no detectable tumor in the liver; P_1: single lesion involving <2 segments without intra-hepatic vascular or biliary involvement; P_2: single lesion involving 2 segments with intra-hepatic vascular or biliary involvement or single lesion involving 3 or 4 segments without intra-hepatic vascular or biliary involvement; P_3: single lesion involving 3 to 5 segments with intra-hepatic vascular or biliary involvement or multiple lesions without intra-hepatic vascular or biliary involvement; P_4: single lesion involving 6 to 8 segments or multiple lesions with intra-hepatic vascular or biliary involvement. Type I was also divided into 3 stages: I_0: no regional involvement; I_1: regional involvement of only one contiguous organ or tissue; I_2: regional involvement of several organs or tissues (> 1). There were a single lesion involving 2 segments in P_2 and P_1 groups with ultrasound image locating multiple nodule lesions, a single lesion involving 3-5 segments with heterogeneous hyper-reflective image in P_3 group; there were multiple lesions involving 3-5 segments and intra-hepatic vascular or biliary involvement with pseudocystic sonogram of central necrosis in P_4 group. Besides involvement of portal vein and gallbladder, the pathological process of liver involved 3-5 segments with pseudo-cystic sonogram of central necrosis in type I, which belonged to mid- and late stages of the disease. Since the late 1 970 s, ultrasound has been used for detecting pathological lesions due to a number of parasitic infections including cystic echinococcosis. The manifestations of B ultrasound of AE were local multiple echogenic nodules in early stage and large non-heterogeneous hyperreflective lesion or pseudo-cystic image in mid- or late stage. B ultrasound could also reflect the pathological process of AE[9].

In the early 1 990 s, it was found that Th_1 cytokines had a role against AE infection and a relation with the slow growth stage of tumor or parasites. IL-2 secreted by CD_4^+ T lymphocytes induced and activated by its antigens, reinforced host immunity and had anti-tumor activities or restrained growth of parasites[10]. Its activity is dependent on expression of mIL-2R (membrane interleukin-2 receptor), which can block IL-2. Due to the action of protein lyase at special sites, mIL-2R is partly incised and chopped off in blood, forming the so-called sIL-2R. It also has activity of blocking IL-2R and plays a negative regulation role in immune response of tumor and parasites, thus promoting the growth of tumor and parasites. Therefore sIL-2R level could also act as an index of tumor stage[11]. As shown in Table 1 except for P_2 group, the mean of sIL-2R between other 2 groups of AE and controls had a significant difference ($P<0.05$), so was the difference between P_2 and other 2 groups of AE ($P<0.05$, $P<0.01$). It could be rationally explained that IL-2 played a role in inhibiting worms in early stage or in stable status of AE, while low level of sIL-2R occurred in blood. Per contra, high-level of sIL-2R in blood means AE in progression stage.

TNF is secreted by macrophages and B lymphocytes and the former is called TNF-α and the latter TNF-β. Both have similar structures and functions, and act on the same acceptor. They could strengthen phagocytic ability of neutrophils. TNF could inhibit reproduction of some protozoa and decrease their density in blood. It could activate phagocytes, depending on nitric oxide that acts on parasites. However, the pathological process of brain tissue of patients with encephalic-malaria, omphalos lesion and vascular hemorrhagic necrosis, was related to the high level of TNF in blood[12]. The mean concentration of TNF-α in P_3 group was the highest ($P<0.01$) and had a significant difference from other 3 groups ($P<0.01$). P_3 stage was the key time of AE from early stage to mid and late stages and the tissues appeared severe damage at this stage. It is obvious that TNF could participate in and exacerbate the pathological process of AE. But accompanying serious tissue necrosis, serum TNF concentration was also decreased in cases of P_4 group. This seems to be related with TNF location, in other words, it depends more on TNF concentration in local tissue. It was reported that TNF-mRNA was expressed in cells of the periparasitic granuloma in AE patients, and this particular expression was observed only in those patients with severe fertile lesions and associated with centro-granulomatous necrosis. No cytokine mRNA expression was observed in patients with an abortive disease[13]. It was proved that TNF-α could inhibit growth of alveolar echinococcosis in experimental mice. In fact, TNF-α plays a complex role in patients with AE. But in cases of P_4 group, accompanying serious tissue necrosis, TNF value was also decreased and its mechanism still remains to be elucidated.

IFN-γ is induced by antigen and it could inhibit Th_2 cells to secrete in defence against parasites. It could fortify phagocytic function of macrophages and restrict multiplication of metacestodes in mice. In this study, the mean concentration of IFN-γ in any group of AE patients was higher than that in control group ($P<0.01$), but there was no difference between AE groups ($P>0.05$). This indicated the efficacy of IFN-γ was limited on inhibiting AE growth in humans. Even though IFN-γ inhibited metacestode growth in mice with AE at a low dose[14], when it was used in combination with mebendazole or nitric oxide which plays a role in host defense mechanisms in human hydatidosis, it was effective for patients with AE[15,16]. So IFN-γ depends on the synergism of chemical medicines or other factors to produce curative effects on AE.

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