

The correlations between Th1 and Th2 cytokines in human alveolar echinococcosis

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Abstract

Background: Alveolar echinococcosis (AE) is a cosmopolitan zoonotic parasitic disease caused by *Echinococcus multilocularis* larval tapeworm infections in humans that severely impairs the health of affected patients.

Methods: The expression levels of 20 cytokines associated with AE infection were measured by enzyme-linked immunosorbent assay, and the correlations between these cytokines were analysed in the R programming language.

Results: Serum cytokine levels differed among individuals in both the AE patient and healthy control groups. Related studies have shown that different cytokines are associated with AE. Therefore, we investigated the correlations among the cytokines; these correlations were simple in the healthy control group but complex in the AE patient group. Th2 cytokines, such as interferon (IFN)- γ , interleukin (IL)-1 β and monocyte chemoattractant protein (MCP)-1, had high betweenness centrality in AE patients, whereas Th1 cytokines, such as growth-regulated oncogene (GRO)- α , eotaxin and IL-5, had high betweenness centrality in the healthy control group.

Conclusions: The altered correlations between Th1 and Th2 cytokines may be closely associated with AE progression.

Background

Alveolar echinococcosis (AE) is a severe parasitic disease caused by *Echinococcus multilocularis* larval tapeworm infection in humans that is fatal if left untreated [1, 2]. The liver is the primary target of the disease and is affected in nearly 95% of cases; this disease can also spread and affect other organs, including the lungs, brain and bone [1, 3]. AE causes severe damage or dysfunction of target organs. The clinical symptoms include tapeworm infiltration into liver tissue and the major vasculature and granuloma formation [4, 5]. This disease is restricted to the Northern Hemisphere, principally in rural areas of western, northern and eastern Europe; the highest disease prevalence is in central Asia, China and Kyrgyzstan [6, 7]. The World Health Organization (WHO) has listed echinococcosis as one of the 17 neglected diseases targeted for control or elimination by 2050 (http://whqlibdoc.who.int/hq/2012/WHO_HTM_NTD_2012.1_eng.pdf). To date, surgery is the only potentially curative option for the treatment of AE; however, AE recurrence after hepatectomy is high, and many patients present with inoperable disease [8]. Recently, immunotherapy has been used to complement anti-infective drug approaches, and this approach was suggested to be highly effective in treating echinococcosis; however, there is no accepted immunotherapy against AE infection due to the complicated interactions between the parasites and host immunity.

The type of immune response impacts disease development, and T helper (Th) cells can selectively differentiate into the Th1 or Th2 subtype in response to an *E. multilocularis* antigen. A Th1/Th2 imbalance has been suggested to play an important role in controlling the immunological response to AE

infection [9, 10]. AE patients with Th1-oriented immunity are more likely to harbour fewer parasites or even aborted parasites, whereas AE patients with Th2-oriented immunity are more likely to develop chronic AE [11]. In mice, the Th1 response was shown to dominate at the early stage of AE, and the immune response gradually shifted towards a Th2-dominated response at the late stage of AE to prevent Th1-mediated damage [11, 12]. The imbalance between Th1-type cytokines and Th2-type cytokines in AE is not completely understood due to the limited number of studies, regional differences and complex interactions between parasites and host immunological and genetic factors [9, 12]. Epidemiological investigations showed that pastoral regions on the Tibetan Plateau appear to be high-risk areas for AE disease due to specific landscape features and husbandry practices. Specifically, a range of different wildlife hosts, especially small mammals, are involved in the transmission of *E. multilocularis* in a pastoral region of Qinghai province[5–7].

In this study, 20 cytokines, including Th1 and Th2 cytokines, were selected according to the related literature [13–15]. The expression levels of these cytokines were compared and analysed by bioinformatics and statistical analysis methods to explore the correlations among Th1- and Th2-type cytokines in AE patients and healthy controls from Qinghai Province in China.

Methods

Sample collection

Forty-five AE patients and 45 healthy controls were recruited from Guoluo Tibetan Autonomous Prefecture of Qinghai Province and diagnosed at Qinghai Provincial People's Hospital. All participants signed informed consent forms, and this study was approved by the Ethics Committee of Qinghai Institute of Endemic Disease Control and Prevention. The diagnosis of AE was based on clinical findings, epidemiological data, imaging techniques, histopathology and/or nucleic acid detection, and serology according to the People's Republic of China Health Industry Standard—Diagnostic Criteria for Hydatid Disease (WS257-2006). The mean age of the AE patients was 38 years (range, 21–52 years), and 64% of the AE patients were female. The mean age of the healthy controls was 39 years (range, 26–53 years), and 60% of the healthy controls were female. All the recruited participants were Tibetan, and 91% were herdsmen. None of the AE patients had any other disease nor had received any antiparasitic drugs before blood collection. Five millilitres of peripheral venous blood was harvested from each participant after an 8–12 h fast, and 1 mL of serum was immediately separated from the blood and preserved at -20 °C.

Serum analysis

The serum levels of 20 cytokines in AE patients and healthy controls were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Scientific) according to the manufacturer's protocols. The level of each cytokine was measured three times, and the mean value was taken for analysis. The 20 analysed cytokines are listed in Table 1.

Table 1.The Th1 and Th2 cytokines analysed in the study.

Cytokine type	Specific cytokines
Th1 cytokines	IL-8, IL-2, IL-12, IL-1 β , IFN- γ -inducible protein 10 (IP-10), MIP-1 β , MCP-1 α , and IFN- γ .
Th2 cytokines	IL-4, IL-5, IL-6, IL-13, IL-18, GRO- α , and eotaxin.
Both Th1 and Th2 cytokines	Stromal cell-derived factor (SDF-1 α), TNF- α , GM-CSF, MIP-1 α , and regulated on activation, normal T cell expressed and secreted (RANTES).

Statistical methods

A principal component analysis (PCA) was performed based on the Bray-Curtis distance matrix across the samples using the vegan package in R (version 3.4.4). Student's t-test was performed to compare PC1/PC2 between groups using the stats package in R. Spearman's correlations between cytokines were calculated using the Hmisc package in R. The correlation networks between cytokines were constructed using the GeneNet package in R and further visualized using Cytoscape 3.4.0 [16].

Results

Comparison of cytokine expression levels between the AE patient group and the healthy control group

To better understand the role of cytokines in AE, we compared the expression levels of 20 cytokines between the AE patient group and the healthy control group. The ELISA results showed that cytokine expression levels differed among individuals in both the AE patient and healthy control groups. Thus, cytokine levels alone could not explain the occurrence of AE. Moreover, we searched related studies in the NCBI database using the keywords "cytokine" and "human alveolar echinococcosis" to explore data on cytokine expression in previous studies associated with AE. A total of 56 studies were identified, 17 of which reported cytokine levels in human AE (Table 2). Compared to other cytokines, the Th2-type cytokines IL-10 and TGF- β were more frequently reported to be highly expressed, but the results were not always consistent. This finding confirms our hypothesis that cytokine levels alone cannot explain the occurrence of AE.

Table 2

The expression levels of cytokines in *E. multilocularis*-infected humans or mice.

Cytokine levels	Citation
IL-10 levels were increased in CD8 + lymphocytes from AE patients.	Kilwinski et al. [17]
Peripheral blood mononuclear cells (PBMCs) from abortive AE patients secreted significantly less IL-10 than those isolated from progressive AE patients.	Godot et al. [18]
Serum TGF-β levels were high, and TGF-β was expressed by most of the infiltrating lymphocytes in patients with progressive AE.	Zhang et al. [19]
The level of the Th9-related cytokine IL-9 was elevated in hepatic lesions and perilesional tissues in AE patients.	Tuxun et al. [10]
IL-10 and TGF-β levels are high in the course of AE progression in humans.	Gottstein et al. [20]
IL-12, IL-23, IL-17A, IL-17F, and IL-10 levels were higher in patients with hepatic AE.	Tuxun, et al. [21]
IL-23 levels were significantly higher in the AE group than in the healthy control group.	Tuxun et al. [22]
Viable proliferating <i>E. multilocularis</i> metacestode vesicles induced significant IL-8 and MCP-1 production by peripheral blood cells from AE patients.	Dreweck et al. [23]
Increased IL-17 and TGF-β levels in PBMC supernatants from healthy blood donors were observed after exposure to <i>E. multilocularis</i> vesicular fluid.	Bellanger et al. [24]
A significant increase in TGF-β production was induced in PBMCs from healthy blood donors after exposure to <i>E. multilocularis</i> vesicular fluid and Toll-like receptor agonists.	Bellanger et al. [25]
In patients with AE, the plasma levels of the proinflammatory cytokine IL-17B and its soluble receptor sIL-17RB were significantly elevated, and IL-17F and sIL-17RA levels were reduced.	Lechner et al. [26]
IL-10 secretion by circulating mononuclear cells are associated with the progressive form of human AE.	Harraga et al. [27]
Serum TNF-α levels were higher in the AE patient group than in the control group; the increased level of TNF-α might be related to the AE lesion.	Shi et al. [28]
The serum levels of the proinflammatory cytokines IL-31 and IL-33 were clearly reduced in AE patients, whereas the levels of the regulatory cytokine IL-27, the anti-inflammatory cytokine SDF-1, and eotaxin cytokines increased with disease progression.	Huang et al. [29]
The production of MIP-1α, MIP-1β, RANTES and GRO-α by peripheral blood cells cultured with <i>E. multilocularis</i> antigens was constitutively higher in AE patients than in controls.	Kocherscheidt et al. [30]
Serum IL-10 levels were significantly higher in AE patients than in healthy controls, with a tendency towards increased concentrations in advanced-stage cases.	Wellinghausen et al. [31]

Cytokine levels	Citation
IL-5 production was particularly increased in PBMCs from patients with advanced AE upon stimulation with <i>E. multilocularis</i> antigenic preparations.	Jenne et al. [32]

PCA of AE patients and healthy controls

As the comparison of cytokine levels did not clarify the differences between the AE patient group and the healthy control group, we investigated the relationships between the cytokine levels in the two groups by PCA based on the Bray-Curtis distance to explore the pathogenesis of AE. Although the PCA showed that some samples in the healthy control group had similar cytokine composition values as some in the AE patient group, most samples in the AE patient group had obviously different cytokine compositions than those in the healthy control group (Fig. 1). The differences in cytokine composition between the two groups were further analysed by comparing their PC1 and PC2 values (Fig. 2); these values were significantly different between the two groups ($P < 0.001$), which suggests that there were some kinds of differences of cytokines between the two groups.

Correlations between the cytokines

To further understand the differences in cytokines between the two groups, Spearman's correlations were used to explore correlations among cytokines in both groups (Fig. 3a and b). The majority of the cytokines showed similar correlations between the two groups, but some slight differences were detected; the correlations among some cytokines were different between the two groups. In the healthy control group, there was no correlation between macrophage inflammatory protein (MIP)-1 α and IL-13 or among monocyte chemoattractant protein (MCP)-1, MCP-1 α and MIP-1 β (Fig. 3a). These cytokines had weak correlations in the AE patient group (Fig. 3b). This finding suggests that the correlations among cytokines were more complex in the AE patient group than in the healthy control group.

A partial correlation network analysis of the cytokines confirmed the presence of more complex cytokine interactions in the AE patient group than in the healthy control group (Fig. 4a and b). In the healthy control group, the interactions among the cytokines were simple: IL-5 interacted with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-13, and Th2 cytokines such as eotaxin and growth-regulated oncogene (GRO)- α interacted with Th1 cytokines such as interferon (IFN)- γ , MCP-1 α , and IL-8 (Fig. 4a). In the AE patient group, Th1 and Th2 cytokines formed a complex network dominated by Th1 cytokines such as IFN- γ and IL-18; however, Th2 cytokines such as eotaxin and GRO- α cytokines showed weak correlations with Th1 cytokines (Fig. 4b).

Discussion

In the present study, we firstly searched the related literatures about cytokines associated with AE and summarized that the results are always inconsistent, we speculated that analysis of cytokine expression

levels is not always an effective method to research the pathogenesis of AE due to complex factors that affect AE development. Our results confirmed our speculation, we investigated 20 cytokines expression levels between 45 healthy people and 45 AE patients, the results are inconsistent, that is the cytokines expression levels cannot distinguish AE patients from healthy people. Thus we tried to explain the disease through the interrelationship among the cytokines, which seems more convincing.

E. multilocularis metacestodes can modulate the secretion of Th1 and Th2 cytokines by Th lymphocytes in AE patients [33], and the cytokine orientation depends on the host immune response induced by *E. multilocularis* antigens. Th1 cell activation induces considerable protective immunity, which involves the initiating cytokines IFN- α and IL-12 and the effector cytokines IFN- γ and tumour necrosis factor (TNF)- α , to defend against intracellular parasitic infections [34, 35]. Th2 cytokines allow parasites to proliferate at low rates by producing high levels of IL-4, IL-5 and IL-10 [13, 14]. *E. multilocularis* antigenic preparations have been reported to induce increased IL-5 production due to the activation of CD4 $^{+}$ T lymphocytes in patients with progressive AE [33]. In the present study, the stronger correlation between the Th2-type cytokine IL-5 and the Th1-type cytokines IFN- γ and IL-1 β in AE patients may be associated with the enhanced immunological response induced by parasite infection, which indicates that a strong inflammatory reaction is induced in AE patients. In addition, the Th2 cytokines GRO- α and eotaxin were well controlled by the Th1 cytokines MCP-1, IFN- γ and IL-8 in the healthy control group, whereas GRO- α and eotaxin levels were poorly controlled by Th1 cytokines in the AE patient group, confirming the strong inflammatory response in AE patients. The altered correlations among the cytokines may explain the essential differences between the AE patients and the healthy controls.

Furthermore, this parasite can secrete proteins to regulate the host immune response and survive in humans for a long time, and different secreted protein profiles at different stages of AE progression may explain the complex interactions between the parasite and the host [36]. A proteomic analysis of cyst vesicular fluids in AE patients contributed to the identification of potential molecular markers for diagnostic and follow-up tools, but the mechanism underlying the interplay between secreted proteins and cytokines requires further exploration.

Conclusions

The correlations between Th1 and Th2 cytokines play an important role in the pathogenesis of AE, the correlations were simple in the healthy control group but complex in the AE patient group. Th2 cytokines, such as IFN- γ , IL-1 β and MCP-1, had high betweenness centrality in AE patients, whereas Th1 cytokines, such as GRO- α , eotaxin and IL-5, had high betweenness centrality in the healthy control group. These findings may be of great clinical significance and value for detecting AE and improving the clinical manifestations of AE.

Abbreviations

alveolar echinococcosis	AE
enzyme-linked immunosorbent assay	ELISA
granulocyte-macrophage colony-stimulating factor	GM-CSF
growth-regulated oncogene-alpha	GRO- α
interferon-gamma (IFN- γ)-inducible protein 10	IP-10
interleukin	IL
macrophage inflammatory protein-1 α	MIP-1 α
macrophage inflammatory protein-1 β	MIP-1 β
monocyte chemoattractant protein	MCP-1 α
regulated on activation, normal T cell expressed and secreted	RANTES
principal component analysis	PCA
stromal cell-derived factor-1 α	SDF-1 α
T helper cells	Th cells
tumour necrosis factor α	TNF- α
World Health Organization	WHO

Declarations

Ethics declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Qinghai Institute of Endemic Disease Control and Prevention, and all participants signed informed consent forms.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

Data supporting the conclusions are included within the article.

Competing interest

The authors declare that no conflicts of interest exist in this study.

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Authors' contributions

Xiao Ma, Huixia Cai, Haining Fan and Jianye Zhou conceived the study and Xiao Ma, Jia Liu and Huixia Cai conducted the study. Wen Lei, Na Liu, Jingxiao Zhang, Yongshun Wang, Wei Wang and Peizhen Zhan collected the samples. Xiongying Zhang, Qing Zhang, Kemei Shi and Peiyun Liu conducted the data analysis. Xiao Ma, Xuefei Zhang and Jia Liu wrote the draft, and Xiao Ma, Yufang Liu and Cunzhe Zhao wrote and approved the final manuscript. All authors read and approved the final manuscript.

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Figures

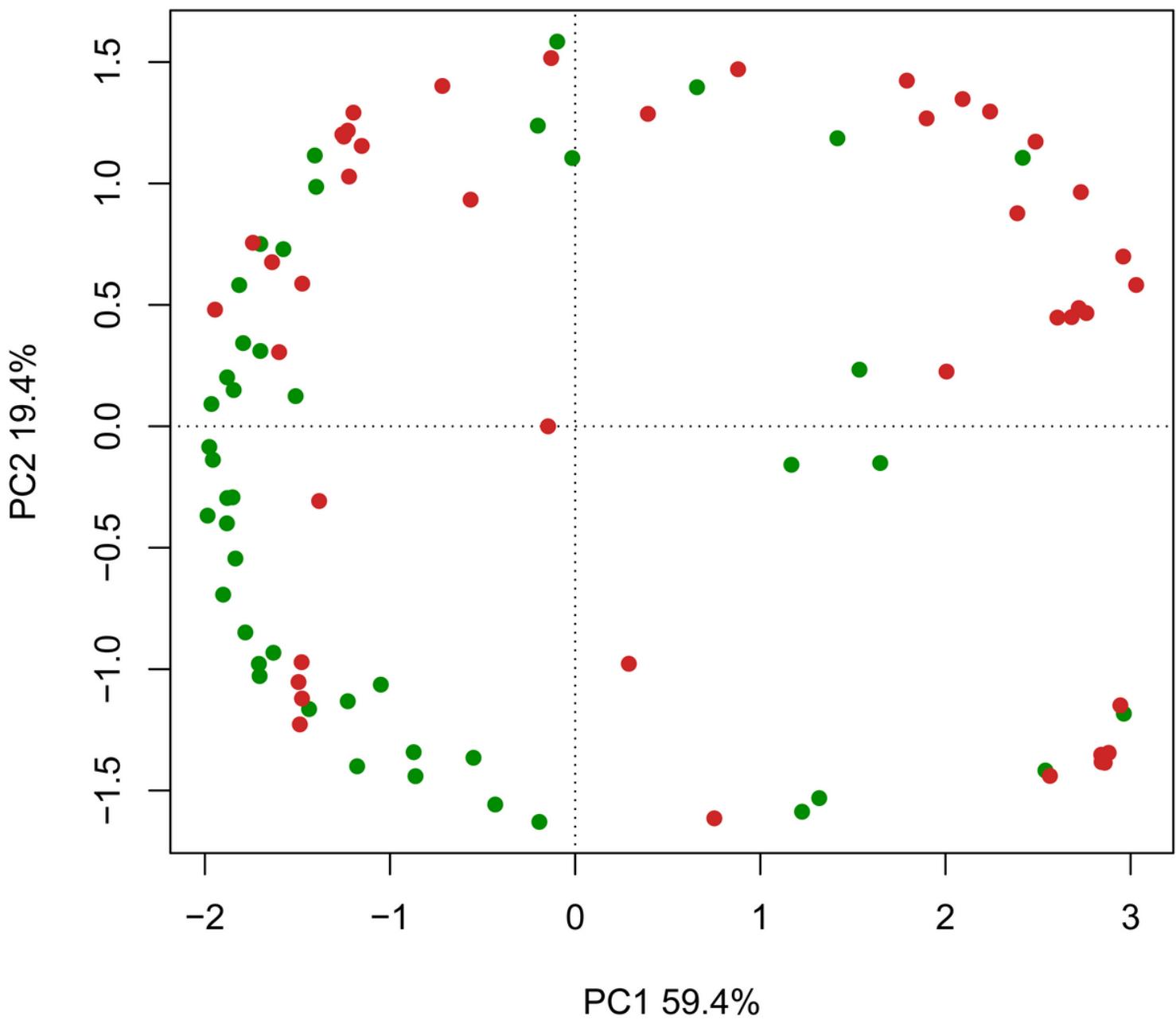


Figure 1

PCA of AE patients (orange) and healthy controls (green) based on the Bray-Curtis distance. The percentages of variance explained by PC1 and PC2 are 59.4% and 19.4%, respectively.

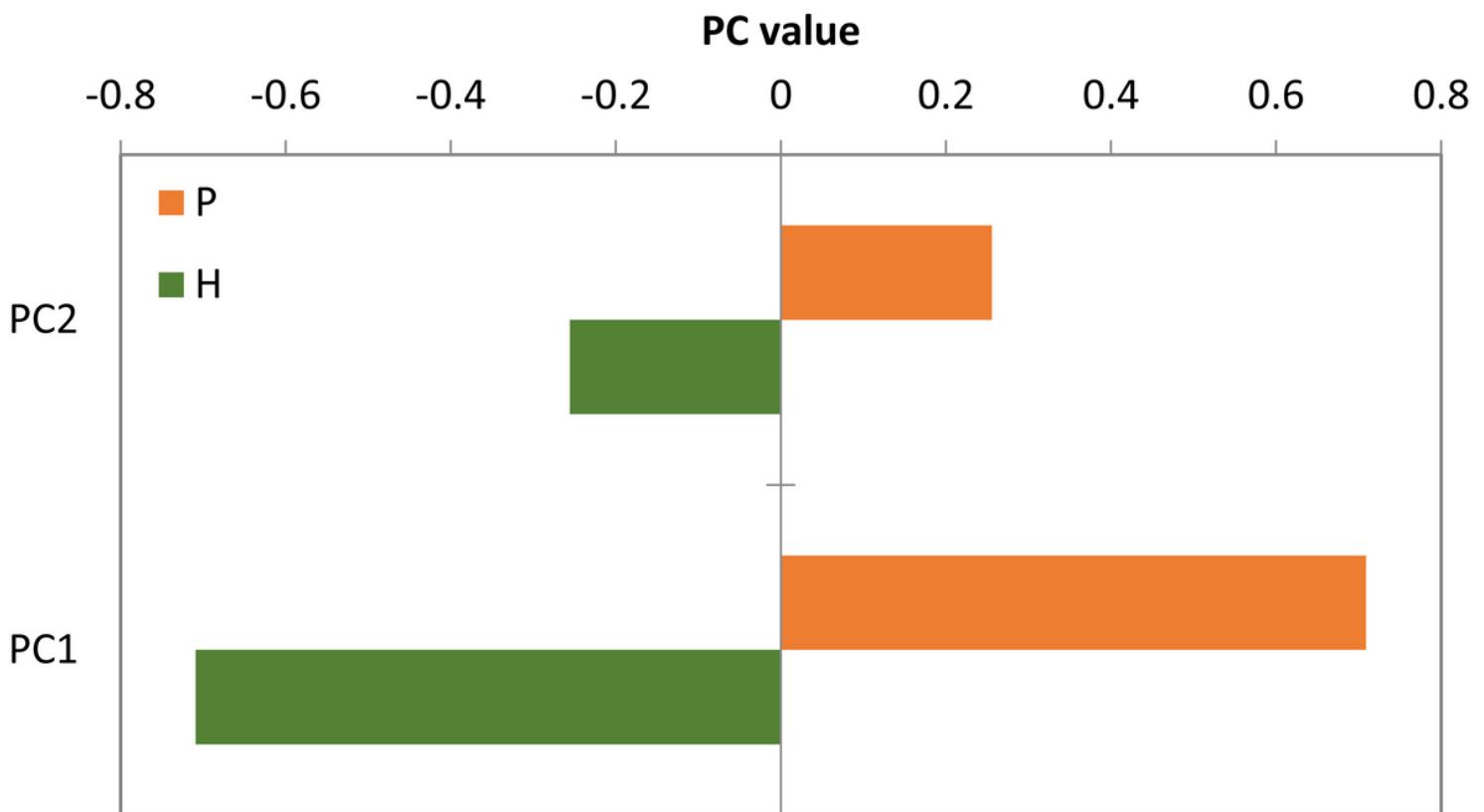


Figure 2

Comparison of PC1 and PC2 values between AE patients and healthy controls.

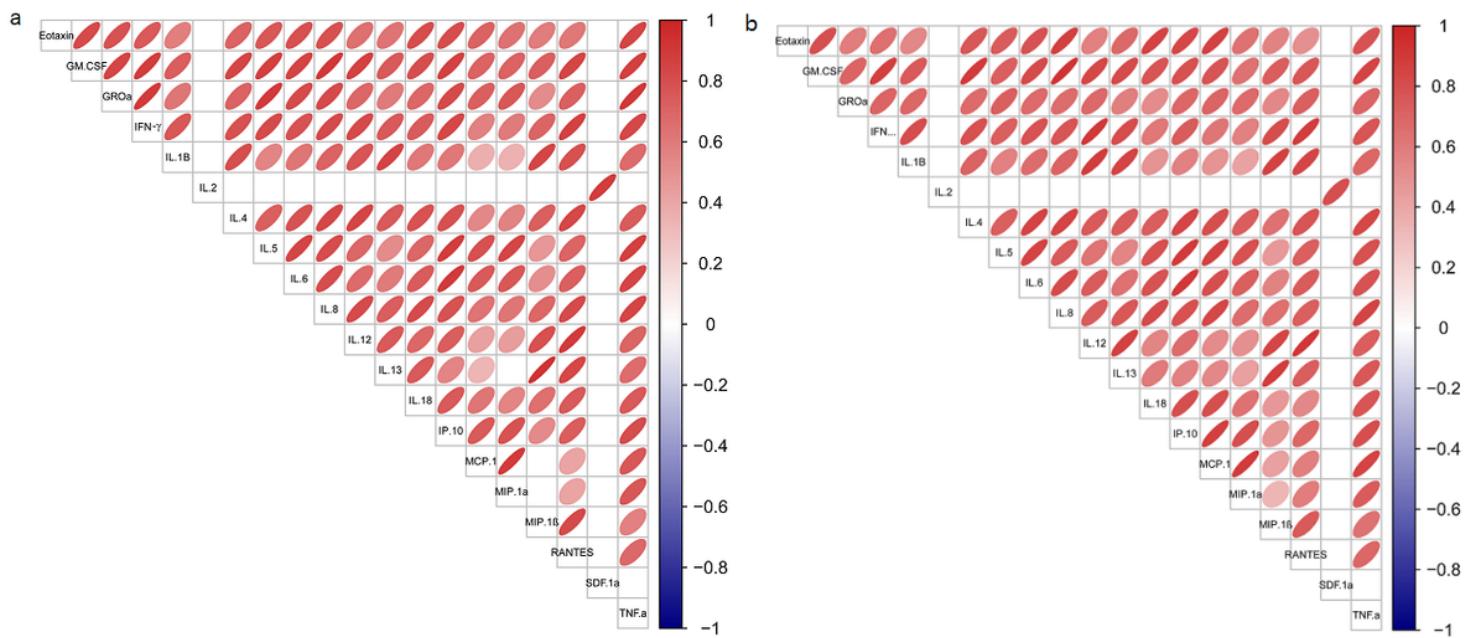


Figure 3

Spearman's correlations among cytokines in the healthy controls (a) and the AE patients (b).

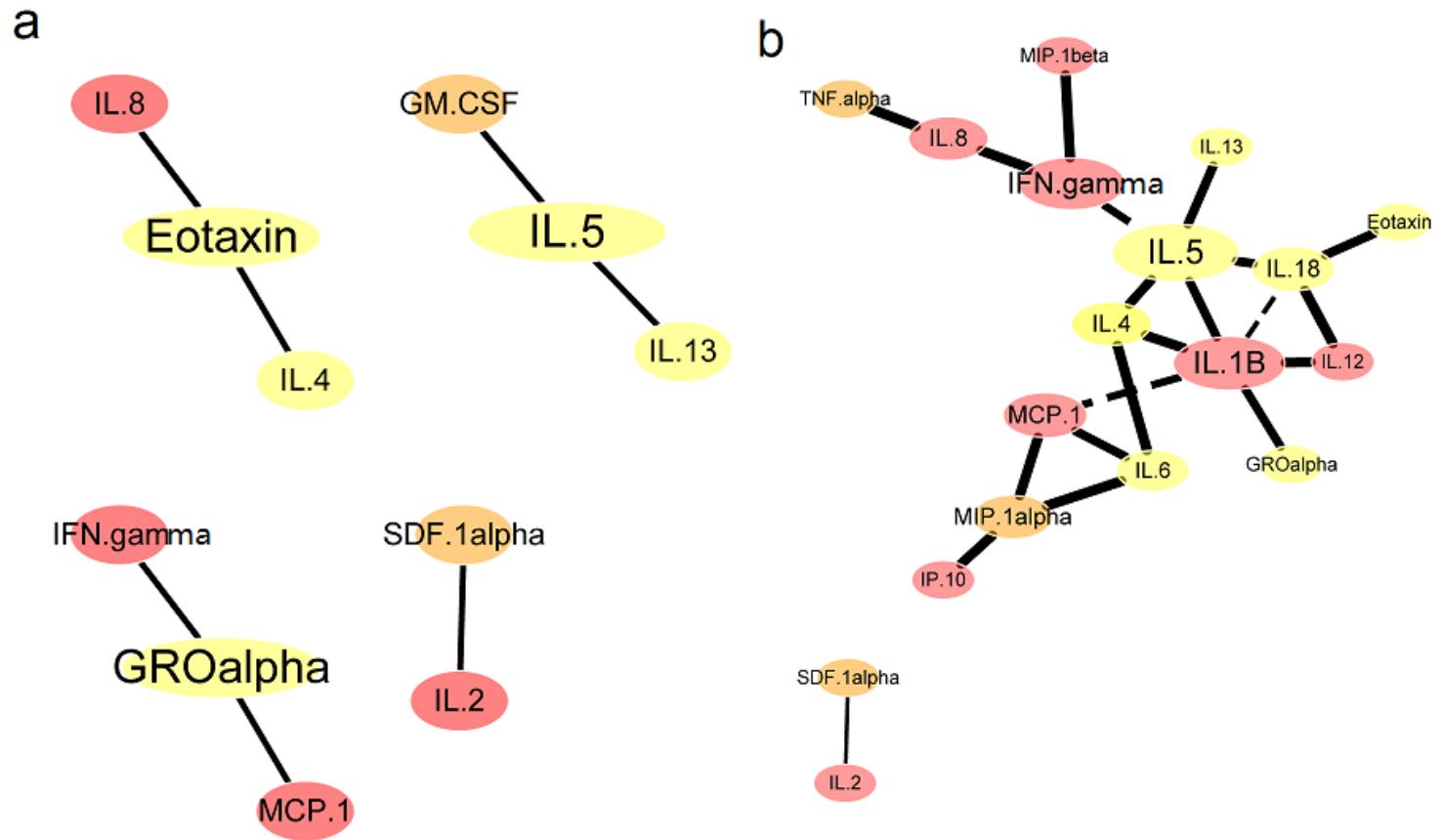


Figure 4

Partial correlation networks among cytokines in the healthy controls (a) and AE patients (b). Note: The colour of the node indicates the origin of the cytokine: red, Th1; yellow, Th2; and orange, both Th1 and Th2. The size of the node indicates the betweenness centrality;a node with increased betweenness centrality has increased control over the network. The solid and dotted lines indicate positive and negative relationships, respectively.

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