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Upregulation of T-bet expression in peripheral blood mononuclear cells during Vogt-Koyanagi-Harada disease

B Li, P Yang, H Zhou, X Huang, H Jin, L Chu, Y Gao, L Zhu, A Kijlstra

Aim: To test the hypothesis that T-bet expression is altered in patients with Vogt-Koyanagi-Harada (VKH) disease.

Methods: Peripheral blood was withdrawn from 16 VKH patients before and after immunosuppressive treatment and from 16 healthy individuals. IFN-γ, IL-2, and IL-4 in the serum and the supernatants of peripheral blood mononuclear cells (PBMC) cultured with or without phytohaemagglutinin (PHA) were measured by ELISA. T-bet mRNA and protein expression in PBMC cultured with or without PHA was detected by RT-PCR and western blot, respectively.

Results: The level of IFN-γ, but not IL-2 and IL-4, was significantly higher in the supernatants of stimulated PBMC in patients than in controls. A significantly increased T-bet mRNA was found in VKH patients during an active uveitis episode, but not in quiescent patients, compared to controls. T-bet protein was detectable in VKH patients during an active uveitis episode, but not in quiescent patients nor in the healthy controls. Stimulation of PBMC with PHA resulted in a marked upregulation of T-bet mRNA and protein expression for both patients and controls with no significant difference between the two groups.

Conclusions: Upregulation of T-bet may be associated with the development of a Th1 mediated immune response in VKH disease.
pg/ml, respectively, and showed no significant difference (p>0.05). They were both significantly higher than concentrations in controls (28.94 (6.76) pg/ml) (p<0.001). IL-2 and IL-4 contents were both below the detection level in the supernatants of PHA stimulated PBMC from the patients and controls.

Expression of T-bet mRNA in patients and controls
The sequenced polymerase chain reaction (PCR) products showed a 99.6% homology with the known T-bet sequence. T-bet mRNA transcripts were observed in all the tested samples (fig 1). T-bet mRNA band intensity was normalised by the respective β actin mRNA band and was found to be 0.74 in patients with active uveitis. This T-bet mRNA level was significantly higher than that in patients with inactive uveitis (0.18) as well as in controls (0.17) (p<0.001). However, there was no significant correlation between T-bet mRNA expression and the severity of the intraocular inflammation in patients with active uveitis. No difference was found between quiescent patients and controls concerning this ratio. Moreover, there was no difference in this ratio between VKH patients with (0.81) or without (0.84) active uveitis and controls (0.82) after PHA stimulation (fig 2).

Expression of T-bet protein in patients and controls
T-bet protein was assayed using western blot. Before PHA treatment, a protein band of approximately 62 kDa could only be detected in the PBMC from patients with active uveitis, but not in those from patients with inactive uveitis or the controls (fig 3). In contrast, after PHA stimulation, all the tested samples showed the 62 kDa band. Furthermore, quantitative analysis indicated that the expression of T-bet protein after stimulation was essentially identical in patients and controls (fig 4).

DISCUSSION
Our study revealed an upregulated T-bet expression at both mRNA and protein levels, in association with a significantly increased IFN-γ in the supernatants of PHA stimulated PBMC, in VKH patients with active uveitis. These results suggest that T cells in PBMC in VKH disease are apt to deviate towards the Th1 type and that T-bet may have a role in the pathogenesis of VKH disease.
T-bet expression has been shown to be increased in activated T cells and is the key transcriptional factor for Th1 cell activation and cytokine production. Our findings are in agreement with studies on T-bet expression in other autoimmune diseases, such as Crohn’s disease, and Behçet’s disease (BD). The observation that T-bet expression is increases in VKH patients during an active uveitis episode, but not during inactive uveitis supports a role of activated T cells during the pathogenesis of this disease. In addition, our study also showed that a significantly upregulated T-bet expression was readily detected in the quiescent patients and controls after PHA stimulation. However, no marked difference was found in the expression of T-bet in patients with active uveitis before and after stimulation. These results may suggest that a large number of T-bet+ cells already exist in the patients with active uveitis, and only few T-bet+ cells are present in patients with inactive uveitis. Quiescent patients in that respect are similar to the controls. Therefore, T-bet may be closely associated with activity of VKH disease and may serve as an immunomodulatory target.

The increased T-bet expression in VKH patients with active uveitis in this study is consistent with the results in BD patients with active uveitis. It is interesting to note that there are marked differences in clinical and pathological features between these two uveitis entities. The similar results in T-bet expression observed in VKH patients and BD patients, however, suggest a common pathway involved in their pathogenesis.

Given the fact that T-bet can be expressed in both CD4+ T cells and B cells, future studies should address the exact cellular origin of T-bet+ cells using multicolour FACS co-localisation studies. The fact that T-bet upregulation is associated with a concomitant IFN-γ expression points the T cell as the most likely source. Whether T-bet is an associated with a concomitant IFN-γ is already exist in the patients with active uveitis, and only few T-bet+ cells are present in patients with inactive uveitis. Quiescent patients in that respect are similar to the controls. Therefore, T-bet may be closely associated with activity of VKH disease and may serve as an immunomodulatory target.

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