RESEARCH BRIEF

T-cell Immunoglobulin and Mucin-domain-containing Molecule-1 in Peripheral Blood Mononuclear Cells in Henoch-Schönlein Purpura

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The T-cell immunoglobulin- and mucin-domain-containing molecules (Tim) have been implicated in the pathogenesis of immune diseases. In this study, we used quantitative real-time reverse transcription-polymerase chain reaction to examine the Tim-1 mRNA expression in peripheral blood mononuclear cells from Henoch-Schönlein purpura patients. The results showed that Tim-1 mRNA expression was significantly higher in patients, which was closely correlated with serum TNF-α, IL-4 levels, IgA1 levels.

Key words: *Henoch-Schönlein purpura, IgA1, Mononuclear cell, Tim-1.*

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Cellular and humoral immune abnormalities with T-cell dysfunction and imbalance of Th1/Th2 cytokines are proposed to play an important role in the development of HSP [1]. The T-cell immunoglobulin- and mucin-domain-containing molecules-1 (Tim-1) is expressed on T helper type 2 (Th2) and plays an important role in the activation of T cells and Th2-mediated immune responses [2]. Recently, Wang, *et al.* [3] showed the expression of Tim-1 and relevant cytokines in peripheral blood mononuclear cells in patients with lupus erythematosus, suggesting that the molecule may play an important role in the pathogenesis of autoimmune diseases.

HSP has been proved to be initiated and mediated by autoreactive T cells triggered by uncertain etiology. However, it has not been determined whether the Tim gene-family plays a role in the development of HSP. In the present study, we examined the expression of Tim-1 and relevant cytokines in peripheral PBMC in HSP and healthy children to investigate the role of Tim-1 and its modulating the balance of Th1/Th2 cells in the disease.

METHODS

Twenty Chinese children (mean age 8.75 ± 2.20 years old, range 6-13 years old) with acute onset and/or active presentation of HSP at this hospital during January 2007 to June 2009 were included. The diagnosis of HSP was based on standard classification criteria [4].

Fifteen healthy subjects (mean age 9.35 ± 2.30 years, range 6-13 years old) were recruited as normal controls. Informed consent and institutional approval were obtained for the study. The activity of HSP was scored [5]. The mean clinical score was 4.50 ± 1.15.

PBMC were isolated from peripheral blood following standard protocols. PBMC were harvested and the proportion of viable cells assessed by trypan blue exclusion. More than 95% of the cells were viable. Real time quantitative polymerase chain reaction (PCR) was performed to determine on RNA expression for Tim-1.Total RNA were isolated from PBMCs using Trizol reagent (Invitrogen, Shanghai, China). Total RNA (1 μg) was reverse transcribed into cDNA using AMV reverse transcriptase (Fermentas,). Primers for Tim-1 and -actin were as follows:Tim-1, Forward: 5'-CCAGTAG CCACTTC ACCATCTT-3'; Reverse: 5'-TGTTATTC CAAA GGCC ATCTGA-3'(160bp); ²-actin, Forward: 5'-TGACGTGGACATCCGCAAAG-3'; Reverse: 5'-CTGGAAGGTGGACAGCGAGG - 3'(205bp). Conditions for the PCR were as follows: 95ºC for 4 minutes, followed by 35 cycles for Tim-1 or 30 cycles for β-actin. The PCR products were run on an agarose gel and were in all cases confined to a single band of the expected size (data not shown). $2^{-\Delta CT}$ was used to figure the expression value of Tim-1. Blood levels of tumor microsibfactor α (TNF-α), IL-4 and IgA1 were estimated by ELISA [6].

Differences in relative mRNA levels of Tim-1 and cytokines were tested for significance using Mann–Whitney test. Correlations between Tim-1 and cytokine

TABLE I TIM-1 EXPRESSION AND SERUM TNF-A, IL-4 AND IGA1 LEVELS IN THE TWO GROUPS

Group	*n*	*IgA1 (mg/mL)*	*TNF-α (pg/mL)*	*IL-4 (ng/mL)*	*Tim-1 expression**
Controls	15	0.43 ± 0.13	5.19 ± 2.35	24.29 ± 4.37	0.67 ± 0.09
Henoch-Schonlein Purpura	20	1.48 ± 0.40	33.66 ± 2.96	77.42 ± 12.21	2.05 ± 0.83**

**Measured in peripheral blood mononuclear cells using quantitative real-time reverse transcription-polymerase chain reaction; Data are expressed as mean ± SD; P<0.01 compared with the controls for all measurements; Tim-1; T-cell immunoglobulin – and mucin-domain – containing molecule -1; TNF-α : Tumour necrosis factor – α; IL-4: Interlactin – 4; IgA 1: Immunoglobulin A1.*

levels were analyzed with Spearman's rank test; *P* value<0.05 was considered significant.

RESULTS

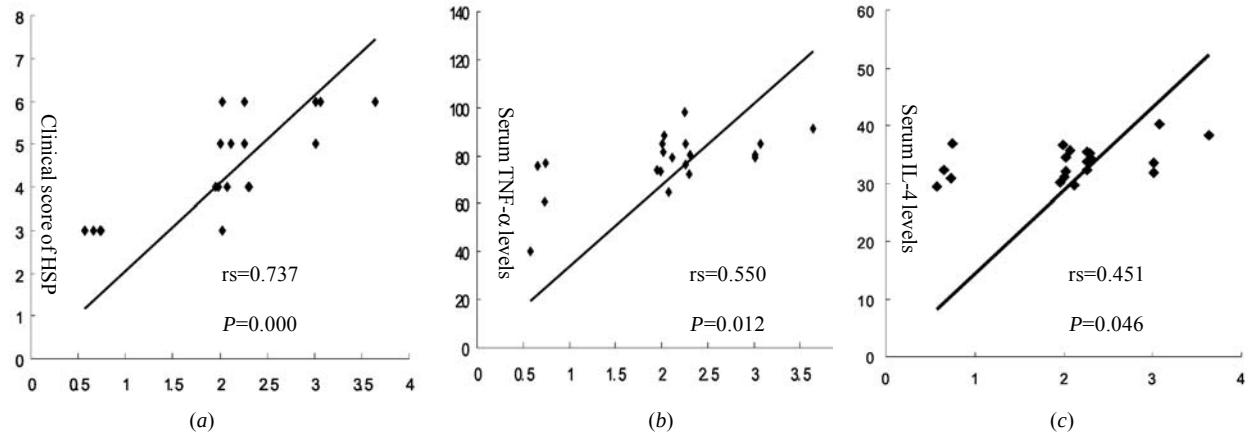
The expression of Tim-1 in HSP patients was significantly higher than the controls (2.05 ± 0.83 *vs* 0.67 ± 0.09) (***Table* I**). There was a significant positive correlation between Tim-1 expression and active HSP (***Fig.* 1*a***). In addition, there was a significant increase in expression of TNF-α and IL-4 in HSP patients compared with the controls (33.66 ± 2.96 *vs* 5.19 ± 2.35 pg/mL; and 77.42 ± 12.21 *vs* 24.29 ± 4.37 ng/mL, respectively). The levels of TNF-α and IL-4 correlated with Tim-1 expression in HSP patients (***Fig.* 1*b*** and ***c***). The serum IgA1 levels were elevated in HSP patients with compared with healthy controls (1.48 ± 0.40 *vs* 0.43 ± 0.13 mg/mL) (*P* <0.01) and there was significant correlation between the serum IgA1 and Tim-1mRNA in HSP patients.

DISCUSSION

Tim molecules, constitute a family of molecules expressed on T cells, and are associated with the regulation of Th2 immune responses [2]. In the present study, we found that Tim-1 expression was upregulated on PBMC from patients with HSP, correlated with the clinical score.

Two studies recently demonstrated that Tim-1 engagement can promote allograft acceptance or rejection, effects that are dependent on regulatory T cells [7]. *In vitro* T cell stimulation with an agonistic anti-Tim-1 Ab increased the number of interleukin (IL-17)- and interferon (IFN-α)-producing cells [8]. Mice treated with an anti–Tim-1 Ab or a Tim-1 extracellular domain protein showed attenuated development of antigen-induced airway inflam-mation and of contact or delayed-type hyper-sensitivity responses [9]. The immuno-inflammatory response induced by IgA1-containing immune complexes is considered important in the pathogenesis of HSP [1]. We also found the serum IgA1 levels were significantly increased in HSP patients. Moreover the high IgA1 was closely related with Tim-1 expression, indicating that upregulation in Tim-1 expression may a cause of triggering the activation of B cells and IgA1 secretion.

There was a significant increase in the expression of serum TNF-α and IL-4 in HSP patients. The expression TNF-α of and IL-4 correlated well with Tim-1. Taken together, our results indicate an increased Th2 response in HSP patients associated with a upregulation of Tim-1 expression. Our results suggest that Tim-1 may act as a potent regulator of Th2 cells by regulating their cytokines.



(*a*) (*b*) (*c*)

FIG. 1 *Correlation between Tim-1 expression and (a) clinical score of Henoch-Schonlein purpura (b) Interleukin-4, and (c) Tumour necrosis factor α.*

WHAT THIS STUDY ADDS?

- High Tim-1 expression was correlated with blood levels of TNF-α, IL-4 and IgA1 in patients with HSP, which may be involved in the pathogenesis of HSP.

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