Short Communication

Iran J Pediatr
Jun 2013; Vol 23 (No 3), Pp: 345-348

Expression of T-cell Immunoglobulin- and Mucin-domain-containing Molecule-3 on Lymphocytes from Henoch-Schoenlein Purpura Patients

Li-ping Yuan[1], PhD; Yan Bo[2], MD; Lu Ling[1], BS; Hu Bo[1], BS

1. Department of Pediatrics, First Affiliated Hospital of Anhui Medical University, Hefei, China
2. Department of Medical Technology, Anhui Medical College, Hefei, China

Received: *May 16, 2012*; Accepted: *Jan 23, 2013;* First Online Available: *Feb 06, 2013*

Abstract

Objective: To investigate T-cell immunoglobulin domain and mucin domain-containing molecule-3 (Tim-3) and its ligand galectin-9 mRNA expression in peripheral blood mononuclear cells (PBMCs) from Henoch-Schoenlein Purpura (HSP) patients.

Methods: Quantitative real-time reverse transcription-polymerase chain reaction (PCR) was used to examine the mRNA expression of Tim-3 and its ligand galectin-9 in PBMCs from HSP patients. ELISA methods were used to examine the levels of serum IFN-γ and immunoglobulin A1 (IgA1). The Spearman rank test was used for correlation analysis between Tim-3, galectin-9 mRNA expression and serum IFN-γ and IgA1 levels, respectively.

Findings: The results showed that Tim-3 and galectin-9 mRNA expression was obviously higher in patients, which was closely correlated with serum IFN-γ and IgA1, respectively.

Conclusion: The results suggested that Tim-3/Tim-3L may be related to the pathogenesis of HSP.

Iranian Journal of Pediatrics, Volume 23 (Number 3), June 2013, Pages: 345-348

Key Words: Henoch-Schoenlein Purpura; T-cell Immunoglobulin; Mucin domain containing 3 protein; TIM-3 protein; Immunoglobulin A

Introduction

T-cell immunoglobulin domain and mucin domain-containing molecule-3 (Tim-3) was first found to be expressed on Th1 (T helper type 1) but not Th2 cells[1]. Tim-3 negatively regulates Th1 response and induces tolerance through the Tim-3/Galectin-9 pathway in autoimmune diseases[2]. More recently, engagement of Tim-3 with Tim-3 ligand has been shown to regulate both the function of Th1 cells and the ability to induce tolerance, as blockade of the Tim-3 pathway accelerates diabetes in the non-obese diabetes (NOD) mice model of diabetes and prevents the acquisition of transplantation tolerance induced by costimulatory blockade[3]. Furthermore, Tim-3-deficient mice are refractory to induction of high dose tolerance in experimental autoimmune encephalomyelitis (EAE)[1].

Henoch-Schoenlein Purpura (HSP) is a kind of systemic small vessel vasculitis that was initiated and mediated by autoreactive T cells triggered by uncertain etiology. It has been proved that T-cell dysfunction and imbalance of Th1/Th2 cytokines contribute to the pathogenesis of HSP[4]. However, the role of Tim-3 in HSP has not been clarified. Therefore we investigated the expression of Tim-3 on peripheral T cells in HSP patients and tested

*** Corresponding Author;**
Address: Department of Pediatrics, the First Affiliated Hospital of Anhui Medical University, Hefei, China 230022
E-mail: yuanliping3986@sina.com

whether the expression level of Tim-3 correlates with disease progression.

Subjects and Methods

HSP patients with acute onset and/or active at this hospital during January 2007 to June 2009 were included. The diagnosis of HSP was based on standard classification criteria[5]. Normal healthy children were also recruited. The approval and fully informed counseled consent were obtained from the ethical committee of the first affiliated hospital of Anhui Medical University and the children's parents, respectively.

PBMCs were isolated from peripheral blood following standard protocols. PBMCs 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. Total RNA was 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-3, galectin-9 and β-actin were as follows: Tim-3 forward: 5′GGCTAAATGGGGATTCCG 3′, and reverse, 5′GACCTTGGCTGGTTTGATGAC 3′; β-actin forward: 5′TGACGTGGACATCCGCAAAG3′, and reverse,5′CTGGAAGGTGGACAGCGAGG3′; galectin-9 forward: 5′CCATCCTCCTGTCAGGCACT 3′, and reverse, 5′TTTTCGGGGCAGACTTCG 3′. Conditions for the PCR were as follows: 95°C for 4 minutes, followed by 35 cycles for Tim-3, 40 cycles for galectin-9 or 30 cycles for β-actin. The PCR products were run on 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-3 and galection-9. Blood levels of IFN-γ and IgA1 were estimated by ELISA[6].

Differences in relative mRNA levels of Tim-3, galectin-9 and cytokines were tested for significance using Mann–Whitney test. Correlations between Tim-3, galectin-9 and cytokine levels were analyzed with Spearman's rank test; *P.* value 0.05 was considered significant.

Findings

20 HSP patients (mean age 8.75±2.20 years, range 6~13 years) and 15 healthy subjects (mean age 9.35 ± 2.30 years, range 6~13 years), recruited as normal controls, were included in the experiment. The mean clinical score[7], activity of HSP, was 4.50±1.15. Tim-3 and galectin-9 levels were detected in HSP patients and 15 age-matched healthy children using real-time RT-PCR. The expression of Tim-3 and its ligand, galectin-9 in HSP was significantly higher than in the controls (Table 1).

Moreover we correlated the expression of Tim-3 and galectin-9 with the clinical score of HSP patients. The results showed that there was a significant positive correlation between Tim-3, galectin-9 expression and active HSP, respectively (r=0.50, P=0.02; r=0.612, P=0.02).

The serum IFN-γ level was significantly decreased in HSP patients compared with the controls (14.68±5.73 vs 27.87±5.90ng/ml), and there was a significant increase in serum IgA1 level from HSP patients compared with the controls (1.48±0.40 vs 0.43±0.13 mg/ml). The IFN-γ and IgA1 level was next correlated with Tim-3 and its ligand, galectin-9, respectively (Fig 1A and 1B).

Table 1. Tim-3, galetin-9 expression and serum IFN-γ, IgA1 levels in groups

Group	n	Tim-3 expression	Galectin-9 expression	IFN-γ (ng/ml)	IgA1 (mg/ml)
Controls	15	0.47 (0.09)	0.27 (0.07)	27.87 (5.90)	0.43 **(**0.13**)**
HSP	20	0.71 (0.06)*	0.80 (0.10)*	14.67 (5.73)*	1.48 (0.40)*

*P<0.01, compared with controls.

The expression of Tim-3 and galectin-9 was measured in peripheral blood mononuclear cells from HSP patients using quantitative real-time reverse transcription-polymerase chain reaction. The serum IFN-γ and IgA1 levels were determined with ELISA methods. Data are expressed as mean ±SD.

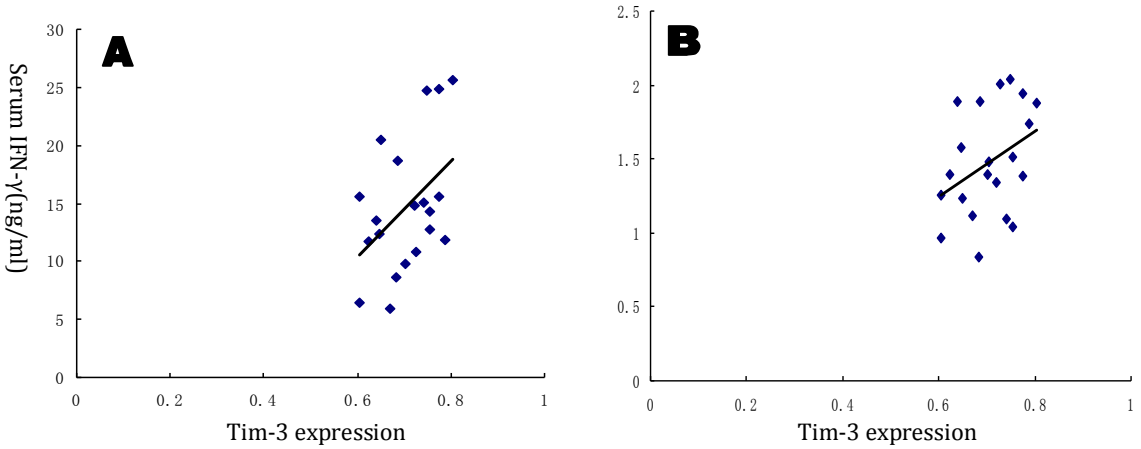

Fig. 1. Correlation between IFN-γ (left) and IgA1 (right) level with Tim-3 and its ligand, galectin-9

Discussion

Tim molecules, constitute a family of molecules expressed on T cells, is associated with the regulation of Th2 immune responses[1]. In the present study we found that Tim-3 and galectin-9 mRNA expression in HSP children increased significantly, compared with healthy controls. Moreover Tim-3 and galectin-9 expression was correlated with disease activities as determined by the clinical score of HSP, respectively.

The immuno-inflammatory response induced by IgA1-containing immune complexes is considered important in the pathogenesis of HSP[6]. In our studies we also found the serum IgA1 levels were significantly increased in HSP patients. Moreover the high IgA1 was closely related with Tim-3 and galectin-9 expression.

Accumulating evidence indicates that that Tim-3 could negatively regulate adaptive immune response. Upon interaction with its ligand, Galectin-9, Tim-3 induces Th1 cell death and suppresses IFN-gamma production[2]. Further, IFN-γ secretion from $CD4^+$ and $CD8^+$ T cells could be restored by blockade of the Tim-3/Galectin-9 pathway or by Tim-3 knockdown using specific shRNAs[8]. In this study, we demonstrated higher expression of Tim-3/Galectin-9 mRNA and lower levels of serum IFN-gamma in HSP patients, suggesting an impaired Th1/Th2 response in HSP patients. We further found an inverse correlation between Tim-3, Galectin-9 expression and serum IFN-gamma in HSP patients, respectively. These results provided suggestive evidence that Tim-3/Galectin-9 pathway down-regulates the Th1 response, which might contribute to the development of HSP. These results will be verified with a big sample size.

Conclusion

Tim-3 and galectin-9 mRNA expression was obviously higher in HSP patients. Moreover their expression was closely correlated with serum IFN-γand IgA1, respectively.

Acknowledgement

This research was supported by the Project supported by the National Natural Science Foundation of China (NO 81001339). Authors thank for authors' Institutional Review Board for their permission for human studies.

Conflict of Interest: None

References

1. Sabatos CA, Chakravarti S, Cha E, et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat Immunol* 2003;4(11):1102-10.
2. Zhu C, Anderson AC, Schubart A, er al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005;6(12):1245-52.
3. Sánchez-Fueyo A, Tian J, Picarella D, et al. Tim-3 inhibits T helper type 1-mediated auto- and

alloimmune responses and promotes immunological tolerance. *Nat Immunol* 2003;4(11):1093-101.

4. Xu ZC, Zeng XQ, Dang XQ, et al. Th l/Th2 polarization in Hench-Schonlein purpura nephritis. *Int J Pathol Clin Med* 2007;27(1):64-67.
5. Ozen S, Ruperto N, Dillon MJ, et al. EULAR/PReS endorsed consensus criteria for the classification of childhood vasculitides. *Ann Rheum Dis* 2006; 65(7):936-41.
6. Zhang Q, Lu L, Lin D. Levels and significance of IgA1 in serum and skin lesions of pediatric patients with anaphylactoid purpura. *Chinese J Dermatol* 2008;41: 55-6. [In Cjinease]
7. Foster BJ, Bernard C, Drummond KN, Sharma AK. Effective therapy for severe Henoch-Schonlein purpura nephritis with prednisone and azathioprine: a clinical and histopathologic study. *J Pediatr* 2000;136(3): 370-5.
8. Jones RB, Ndhlovu LC, Barbour JD, et al. Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J Exp Med* 2008;205(12):2763-79.