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TIM-1 signaling in B cells regulates antibody production

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ABSTRACT

Members of the T cell Ig and mucin (TIM) family have recently been implicated in the control of T cell-mediated immune responses. In this study, we found TIM-1 expression on anti-IgM- or anti-CD40-stimulated splenic B cells, which was further up-regulated by the combination of anti-IgM and anti-CD40 Abs. On the other hand, TIM-1 ligand was constitutively expressed on B cells and inducible on anti-CD3$^+$ anti-CD28-stimulated CD4$^+$ T cells. In vitro stimulation of activated B cells by anti-TIM-1 mAb enhanced proliferation and expression of a plasma cell marker syndecan-1 (CD138). We further examined the effect of TIM-1 signaling on antibody production in vitro and in vivo. Higher levels of IgG2b and IgG3 secretion were detected in the culture supernatants of the anti-TIM-1-stimulated B cells as compared with the control IgG-stimulated B cells. When immunized with T-independent antigen TNP-Ficoll, TNP-specific IgG1, IgG2b, and IgG3 Abs were slightly increased in the anti-TIM-1-treated mice. When immunized with T-dependent antigen OVA, serum levels of OVA-specific IgG2b, IgG3, and IgE Abs were significantly increased in the anti-TIM-1-treated mice as compared with the control IgG-treated mice. These results suggest that TIM-1 signaling in B cells augments antibody production by enhancing B cell proliferation and differentiation.

1. Introduction

The T cell immunoglobulin and mucin domain (TIM) family has recently been implicated in the regulation of T cell activation and immune responses [1,2]. The genes of this family were found within the *Tapr* (T cell and airway phenotype regulator) locus on mouse chromosome 11B1.1, which is syntenic to human chromosome 5q33.2, a region that has been linked to allergic and autoimmune diseases [3]. To date, four proteins (TIM-1, -2, -3, and -4) have been identified in mice and three proteins (TIM-1, -3, and -4) have been found in humans [2]. In the mouse, TIM-1 and TIM-3 have polymorphism at the protein level, represented by BALB/c-type and B6-type [3]. The polymorphism of mouse TIM-1 is strongly associated with Th2 differentiation and the expression of airway hyperreactivity [3]. No human orthologue for mouse TIM-2 has not been identified although, given its close sequence homology, TIM-1 may share some of functions [1–3]. All proteins are type I transmembrane protein with common structural motifs including extracellular IgV and mucin domains, and intracellular domain. TIM-1, TIM-2, and TIM-3, but not TIM-4, contain a conserved intracellular tyrosine phosphorylation motif that is involved in transmenbrane signaling [2,3].

The expression of TIM-1 has been found on Th2 cells, mast cells, NKT cells, and tubular epithelial cells of kidney [1,3]. Some studies have indicated that mouse TIM-1 can bind to TIM-1, TIM-3, and TIM-4 [4,5]. TIM-1 also induces phagocytosis by recognizing phosphatidylserine (PtdSer) on apoptotic cells [6–8]. It has been shown that TIM-1 and TIM-4 can bind to separate sites on the surface of exosome [8]. Therefore, it is possible that TIM-1 interacts with other TIM molecules through binding to PtdSer on the surface of exosome or membrane fragments. Some studies have indicated that TIM-1 has a costimulatory effect on T cell responses. Cross-linking of TIM-1 with an agonistic anti-TIM-1 mAb increased the proliferation of CD4$^+$ T cells and enhanced the production of IL-4 by Th2 cells [9]. Administration of agonistic anti-TIM-1 mAb in vivo increased antigen-specific T cell proliferation and production of IL-4 and IFN-γ [9]. It has been shown that TIM-1 costimulation prevents allogenic transplant tolerance by reducing Foxp3 expression and thereby preventing regulatory T cell development, while enhancing development of Th1 and Th17 responses [10]. Moreover, administration of agonistic anti-TIM-1 mAb during the induction phage of experimental autoimmune encephalomyelitis

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enhanced pathogenic Th1 and Th17 responses and increased the severity [11].

To further characterize the expression and the role of TIM-1 in the immune response, we newly generated an mAb against mouse TIM-1 and found that TIM-1 was highly expressed on splenic B cells after anti-IgM and anti-CD40 stimulation. On the other hand, TIM-1 ligand (TIM-1L) was constitutively expressed on B cells and also inducible on anti-$CD3^+$ anti-CD28-stimulated $CD4^+$ T cells. In vitro stimulation of activated B cells by the anti-TIM-1 mAb induced expression of syndecan-1 (CD138), which is a plasma cell marker. Moreover, in vivo administration of the anti-TIM-1 mAb increased serum levels of antigen-specific antibodies in response to T-independent 2 (TI-2) and T-dependent (TD) antigens. These results suggest that the TIM-1 signaling in B cells may be a novel target for manipulating antibody responses.

2. Materials and methods

2.1. Animals and cells

Female BALB/c mice and Sprague Dawley rats were purchased from Charles River Laboratories (Kanagawa, Japan). FcRγ-deficient mice were kindly supplied by Y. Suzuki (Department of Nephrology, Juntendo University, Tokyo, Japan) [12]. All mice were 6–8 weeks old at the start of experiments and kept under specific pathogen-free conditions during the experiments. All animal experiments were approved by Juntendo University Animal Experimental Ethics Committee.

2.2. Generation of anti-mouse TIM-1 mAb

The anti-mouse TIM-1 mAb was generated by immunizing Sprague Dawley rats with TIM-1-Ig, consisting of the extracellular domains (aa 1-236 of TIM-1 BALB) [3] and the Fc portion of mouse IgG2a, emulsified in CFA (Difco Laboratories, Detroit, MI, USA). Three days after the final immunization, lymph node (LN) cells were fused with P3U1 myeloma cells (American Type Culture Collection, Manassas, VA, USA). After hypoxanthine–aminopterin–thymidine selection, a hybridoma producing anti-TIM-1 mAb (RMT1-17, rat IgG1/κ) was selected by its reactivity to mouse TIM-1-transfected cells, but not to parental cells by flow cytometry and then cloned by limiting dilution. Anti-TIM-2 (RMT2-14), anti-TIM-3 (RMT3-23), and anti-TIM-4 (RMT4-54) mAbs were also generated in our laboratory [13].

2.3. Preparation of activated CD4 T cells and B cells

CD4 T cells were purified from the spleen of BALB/c mice by passage through nylon wool column (Wako Biochemicals, Tokyo, Japan) and by using an auto-MACS column with CD4 T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purified CD4 T cells (3×10^6/ml; >95% $CD4^+$) were stimulated with immobilized anti-CD3 mAb (145-2C11; 5 μg/ml) in the presence or absence of anti-CD28 mAb (PV-1; 5 μg/ml) for 24–72 h. Small resting B cells were purified from the spleen of BALB/c mice as previously described [14]. Purified B cells (3×10^6/ml; >95% $B220^+$) were stimulated with goat anti-mouse IgM F(ab')2 Ab (5 μg/ml) and/or anti-CD40 mAb (HM40-3; 5 μg/ml), or LPS (5 μg/ml) for 24–72 h.

2.4. Flow cytometric analysis

Spleen cells were digested with 400 U/ml collagenase (Wako Biochemicals) and 5 mM EDTA. Cells were first preincubated with unlabeled anti-CD16/32 mAb to avoid non-specific binding of Abs to FcγR and then incubated with FITC- or APC-labeled mAb, or biotinylated mAb followed by PE-labeled streptavidin. The stained cells (live gated on the basis of forward and side scatter profiles and propidium iodide exclusion) were analyzed on a FACSCalibur (BD Biosciences), and data were processed using the CellQuest program (BD Biosciences).

2.5. In vitro B cell proliferation and Ig production assays

Purified B cells (1×10^5/well) from BALB/c or FcRγ-deficient mice were cultured with anti-IgM Ab (5 μg/ml), anti-CD40 mAb (5 μg/ml), and/or recombinant mouse IL-4 (20 ng/ml; BD Biosciences) in 96-well flat-bottomed plates. Anti-CD16/32 mAb (5 μg/ml) and 10 μg/ml of anti-TIM-1 mAb or control rat IgG were also added at the start of culture. To assess proliferative responses, the cultures were pulsed with ^{3}H-thymidine (0.5 μCi/well; PerkinElmer, MA, USA) for the last 6 h of a 48 h culture and harvested on a Micro96 Cell Harvester (Molecular Devices, Sunnyvale, CA USA). Incorporated radioactivity was measured on a microplate beta counter (Micro β Plus; PerkinElmer). For analysis of Ig secretion, 50 μl of day 7 culture supernatants were subjected to the cytometric bead array (CBA) using Mouse Immunoglobulin Isotyping Kit (BD Biosciences) according to the manufacturer's instructions.

2.6. ELISA for antigen-specific serum Abs

To measure the production of Abs against a T-independent 2 (TI-2) antigen, BALB/c mice ($n = 8$) were i.p. immunized with 10 μg of TNP-Ficoll (Biosearch technologies, Novato, CA, USA) on days 0 and administrated with 300 μg of anti-TIM-1 mAb or control rat IgG (Sigma–Aldrich) on days 1 and 3. Sera were collected on day 7 and the titers of anti-TNP Abs were measured by ELISA against TNP-BSA (Biosearch technologies). To examine the response against a T-dependent (TD) antigen, BALB/c mice ($n = 8$) were i.p. immunized with 50 μg of OVA (Sigma–Aldrich) in 4 mg of alum (Thermo Scientific, Rockford, IL, USA) on days 0 and 14 and administrated with 300 μg of anti-TIM-1 mAb or control rat IgG every 3 days from day 0 to day 18. Sera were collected on day 21 and the titers of anti-OVA Abs were measured by ELISA.

2.7. Statistical analysis

Significant differences between two experimental groups were analyzed by the unpaired Student's *t*-test. The results are expressed as the mean ± SEM. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Expression of TIM-1 and TIM-1L on spleen cells

Newly generated an anti-TIM-1 mAb (RMT1-17) reacted with TIM-1 transfectants (TIM-1 BALB/NRK, TIM-1 B6/NRK, TIM-1 BALB/L5178Y, and TIM-1 B6/L5178Y) but not with parental NRK and L5178Y cells (Fig. 1A). Anti-TIM-1 mAb did not bind to the other TIM family-transfected cells (TIM2/NRK, TIM-3 BALB/NRK, TIM-3 B6/NRK, and TIM-4/NRK) (Fig. 1B), indicating that anti-TIM-1 mAb is specific for TIM-1. We examined the expression of TIM-1 and TIM-1L on freshly isolated spleen cells by flow cytometric analysis using this anti-TIM-1 mAb and TIM-1-Ig. As shown in Fig. 1C, a marginal expression of TIM-1 was detected on $B220^+$ $CD19^+$ B cells. On the other hand, a substantial level of TIM-1L expression was found on antigen-presenting cells including B cells, $CD11b^+$ $F4/80^+$ macrophages, and both $CD8^-$ $CD11c^+$ and $CD8^+$