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Expression of Programmed Death Ligand-1 (PD-L1) on B Cells Regulates IL-17 Production of Activated CD4+ T Cells

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= 국문초록 =

PD-L1 발현 B세포를 통한 활성 T세포의 IL-17 분비 조절

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목적: 최근 들어 면역조절능을 가지는 B세포의 존재에 관한 연구가 보고되고 있다. B세포의 특성 중 면역조절기능을 세포상호작용을 통해 연구하고자 본 실험에서는 B세포를 B세포 수용체(anti IgM)와 CD40에 대한 단클론항체(agonist anti mouse CD40 monoclonal antibody)로 자극하여 관찰하였다.

방법: 마우스의 비장에서 분리한 B세포를 lipopolysaccharide (LPS), B세포 수용체 또는 CD40에 대한 단클론항체로 자극하여 24시간 동안 배양하여 B세포에서 발현하는 공동신호분자(CD40, CD80, CD86, MHC-II, PD-1, PD-L1)를 유세포 분석기로 조사하였다. 그리고 활성화된 B세포를 anti CD3로 자극한 T세포와 공조 배양하여 T세포에서 발현하는 사이토카인을 중합효소연쇄반응(PCR, polymerase chain reaction)과 효소결합 면역흡수 분석법(ELSIA, enzyme-linked immunosorbent assay)으로 조사하였다.

결과: B세포에서의 공동신호분자는 자극(LPS, B세포 수용체 또는 CD40에 대한 단클론항체)에 대해 반응하여 모두 증가하는 경향을 나타냈으며 특히 B세포 수용체와 CD40에 대한 단클론항체로 자극하였을 경우 음성신호분자로 알려져 있는 PD-L1의 발현이 가장 의미 있게 증가되었다. B세포를 T세포와 공조배양 하면, T세포에서 분비되는 IL-17이 의미 있게 감소(nil: 1932±386 pg/mL vs anti IgM+agonist CD40mAb: 1193±334 pg/mL, $p<0.01$)하였다. 그러나 IFN-γ (nil: 4658±752 pg/mL,

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anti IgM+agonist CD40mAb: 5525±315 pg/mL)와 TNF-α (nil: 1445±178 pg/mL, anti IgM+agonist CD40mAb: 1,469±281 pg/mL)의 형성에는 변화가 없었다. 또한 공조배양을 통한 IL-17 생성 억제는 PD-L1 중성화 항체 처리로 회복되는 경향을 보였다.

결론: 이와 같은 연구로 B세포는 B세포 수용체와 CD40에 대한 단클론항체로 자극하면 PD-L1의 발현이 증가되고, T세포와 공조배양 시 IL-17분비를 뚜렷이 감소시킨다는 결과를 얻을 수 있었다. 이는 면역조절능을 가진 B세포는 PD-L1발현을 통해 결과적으로 IL-17의 생성을 억제시킨다는 것을 시사한다.

Key Words: PD-L1, Regulatory B cells, IL-17, Th17, BCR and CD40 pathway

INTRODUCTION

The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. The immune system also maintains tolerance and prevents the body from attacking itself. B cells, T cells, dendritic cells (DCs) and NKT cells are immune cells that control effector cells, the production of antibodies and antigen presentation (1).

The principal function of B cells is to make antibodies against soluble antigens, to present antigens and to activate naive T cells (2). Recent studies indicate that regulatory B cells (Bregs) develop in several murine models of chronic inflammation (3).

B cells that produce IL-10 appear after stimulation with antigen and agonistic anti-mouse CD40 monoclonal antibody (mAb) in arthritogenic splenocytes (4). When transferred to syngeneic mice, these IL-10-secreting B cells control the pathogenic T helper cell type 1 (Th1) response, which inhibit the onset of collagen induced arthritis (CIA), and counteract disease progression (4). B cells also play a key role in recovery from experimental autoimmune encephalomyelitis (EAE) by producing IL-10 and consequently regulating type 1 autoreactivity (5). At different stages of development, B cells play different roles in the immunoregulation of CIA. During the induction phase of CIA, the transfer of transitional 2-marginal zone precursor (T2-MZP) B cells, but not mature B cell subsets, inhibits the pathogenic Th1 response in vivo and the delayed-type hypersensitivity response, and prevents the development of arthritis through the production of IL-10 (6).

B cells are second-line antigen-presenting cells (APCs) that can support an ongoing T cell response initiated by DCs (7). Downregulation of T Cell Receptor (TCR) is mediated primarily by B cells and may serve as a mechanism of tolerance maintenance (8).

Here we focused on the interaction of B and T cells through by costimulatory and coinhibitory molecules. Costimulatory and coinhibitory accessory molecules expressed on the surface of T cells play a key role in modulating immune responses to pathogens or autoantigens regardless of the particular antigen, MHC, or TCR engaged (9). Consequently, interference of costimulatory signals or increases of coinhibitory signals is able to regulate immune responses. PD-1 is an immunoinhibitory receptor that belongs to the CD28/CTLA-4 family. B7-H1 (PD-L1) and B7-DC (PD-L2), which belong to the B7 family, are ligands for PD-1. Paradoxically, both B7-H1 and B7-DC costimulate or inhibit T cell proliferation and cytokine production (10). Consistent with this, PD-$1^{-/-}$ mice develop spontaneous autoimmune diseases that target particular organs depending on the background strain (11). PD-L1 is expressed on activated T cells, B cells, monocytes, and DCs, as well as on nonhematopoetic cells such as keratinocytes, endothelial cells, pancreatic islets, and tumor cells. This expression pattern suggests that PD-1-PD-L1 interaction has the role in constraining immune cell function in the periphery (12).

We investigated the effect on T cells proliferation and cytokine production of B cells stimulation through

the B Cell Receptor (BCR) and the CD40 pathway using anti-IgM and agonist CD40 mAb. Furthermore we studied the effects of expression of costimulatory and coinhibitory molecules on activated B cells. B cells stimulated with anti-IgM plus agonist CD40 mAb increased the expression of PD-L1. PD-L1 was highly expressed in B cells that inhibited IL-17 secretion by activated T cells but had no effect on IFN-γ or TNF-α production. Blocking the PD-1-PD-L1 interaction recovered IL-17 secretion by activated T cells cocultured with B cells stimulated with anti-IgM plus agonist CD40 mAb and PD-L1 neutralizing Ab.

MATERIALS AND METHODS

1. Mice

Male DBA/1J mice (SLC, Inc., Shizuoka, Japan) 5～8 weeks of age were maintained in a specific pathogen- free environment and fed standard laboratory mouse chow (Ralston Purina, St. Louis, MO) and water ad libitum. The animals were treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea, which conforms to all USA National Institutes of Health guidelines.

2. B cell and T cell purification and culture

The spleen was washed twice with phosphate- buffered saline (PBS). Spleens from mice were pooled and minced in RPMI 1640 containing 10% fetal calf serum. Cells were filtered through a cell strainer (40 μm) with PBS and centrifuged at 1300 rpm at 4°C for 5 min. The cell pellet was resuspended and washed with MACs buffer. Mononuclear cells were incubated with a B cell isolation kit (Miltenyi Biotec, Auburn, CA) and subjected to negative selection through MACs® magnetic cell sorting separation columns. Cells selected according to their $CD19^+$ expression routinely showed 98% viable B cells. Isolated $CD19^+$ B cells (5×10^5/ well) were cultured in 24-well plates with 10 μg/mL LPS (Sigma, LPS from *Escherichia coli* 0111:B4), 10 μg/mL anti-IgM (Pierce, Cat No. 31172), 5 μg/mL mAb to CD40 (mouse) (Alexis Biochemicals, FGK45, ALX-805-046), and 5 μg/mL functional grade purified Rat IgG2a isotype control (eBiosciences, San Diego, CA; 16-4321). The plates were incubated at 37°C in 5% CO_2 for 24 h. Mononuclear cells were incubated with CD4 (L3T4) microbeads (Miltenyi Biotec) and subjected to positive selection through MACs magnetic cell sorting separation columns. Cells selected according to their $CD4^+$ expression routinely showed 90% viable $CD4^+$ T cells. Isolated $CD4^+$ T cells (5×10^5/ well) were cultured with preactivated $CD19^+$ B cells in CD3-coated (2 μg/mL, BD Pharmingen, San Diego, CA) 24-well plates for 1 d. The plates were incubated at 37°C in 5% CO_2 for 72 h.

3. Flow cytometric analysis of costimulatory molecules on B cells

Isolated $CD19^+$ B cells (2×10^5 cells/well) were stimulated and double-stained with allophycocyanin (APC)-labeled anti-mouse CD19 and phycoerythrin (PE)-labeled anti-mouse CD40, CD80, CD86 (BD Pharmingen), I-A/I-E (Biolegend, San Diego, CA), PD-1, or PD-L1 (eBiosciences). The cells were washed with FACS staining buffer. Expression of each costimulatory molecule on $CD19^+$ B cells was analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA).

4. Measurement of cytokine mRNA by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

mRNA was extracted using RNAzol B (Biotex Laboratories, Houston, TX) in accordance with the manufacturer's instructions. Reverse transcription of 2 μg total mRNA was carried out at 42°C using the superscript reverse transcription system (TaKara, Shiga, Japan).

PCR amplification of cDNA aliquots was performed by adding 2.5 mM dNTP mix (TaKara), 10×Taq buffer (iNtRON Biotech, Seoul, Korea), 2.5 U Taq polymerase (iNtRON), and 10 pmol each of sense and antisense primers. The following sense and antisense primers were used: mouse beta-actin, 5'-GAA ATC GTG CGT GAC

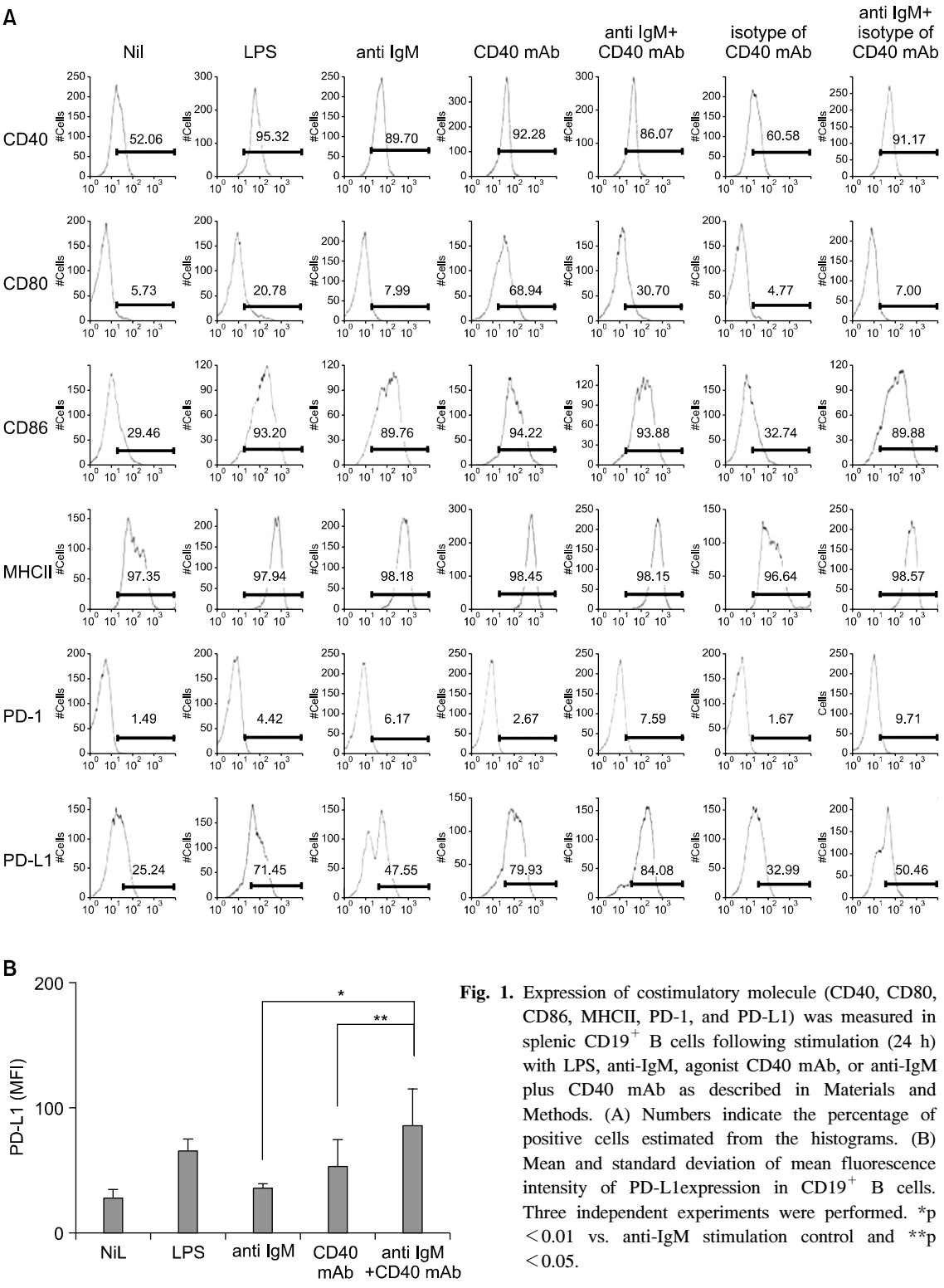

Fig. 1. Expression of costimulatory molecule (CD40, CD80, CD86, MHCII, PD-1, and PD-L1) was measured in splenic $CD19^+$ B cells following stimulation (24 h) with LPS, anti-IgM, agonist CD40 mAb, or anti-IgM plus CD40 mAb as described in Materials and Methods. (A) Numbers indicate the percentage of positive cells estimated from the histograms. (B) Mean and standard deviation of mean fluorescence intensity of PD-L1expression in $CD19^+$ B cells. Three independent experiments were performed. *$p < 0.01$ vs. anti-IgM stimulation control and **$p < 0.05$.

ATC AAA G-3' (sense) and 5'- TGT AGT TTC ATG GAT GCC ACA G-3' (antisense); mouse IL-17, 5'-TCT CAT CCA GCA AGA GAT CC-3', (sense) and 5'-AGT TTG GGA CCC CTT TAC AC-3' (antisense); mouse IFN-γ, 5'-GAA AAT CCT GCA GAG CCA GA-3' (sense) and 5'-TGA GCT CAT TGA ATG CTT GG-3' (antisense); mouse TNF-α, 5'- ATG AGC ACA GAA AGC ATG ATC-3' (sense) and 5'-TAC AGG CTT GTC ACT CGA ATT-3' (antisense). The reaction was done in a total volume of 25 μL using in a dual-bay thermal cycler system (MJ Research, Waltham, MA) for 23 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s for β-actin; 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s for IL-17; 29 cycles of 94°C for 30 s, 60°C of 90 s, and 72°C for 30s for IFN-γ; 29 cycles of 94°C for 30 s, 60°C for 60 s, and 72°C for 30 s for TNF-α. The PCR products were run on a 2% agarose gel and stained with ethidium bromide. The results are expressed as the ratio of target PCR product relative to β-actin product.

5. Cytokine measurement

$CD19^+$ B cells were preactivated for 24 h and cocultured with $CD4^+$ T cells from the spleens of DBA/1J mice in the presence of anti-CD3 Ab for 72 h. The culture supernatants were collected and the concentrations of IL-17, IFN-γ, and TNF-α were measured using a sandwich ELISA (R&D system).

RESULTS

1. Expression of costimulatory molecules in $CD19^+$ B cells stimulated with LPS, anti-IgM or/and CD40 mAb

For an effective T cell response, recognition of antigen in the form of peptide-MHC complexes expressed by APCs is not sufficient. Additional signals from costimulatory receptors are required for many T cell activities. Isolated $CD19^+$ B cells from DBA/1J mice were stimulated for 24 h with LPS, anti-IgM, agonist CD40 mAb (hereafter CD40 mAb), anti-IgM plus CD40 mAb, with or without isotype control of CD40 mAb. CD40, CD80, CD86, and I-A/I-E expression increased in all stimulations compared with the isotype control. In contrast, PD-1 expressions were similar in all stimuli conditions (Fig. 1A). Interestingly, when the $CD19^+$ B cells were stimulated with anti-IgM plus CD40 mAb, PD-L1 expression was significantly increased (Fig. 1B). Increase PD-L1, coinhibitory molecule, showed in a time-dependent manner (Fig. 2).

2. Expression of cytokines in CD3 mAb-activated $CD4^+$T cells

Isolated $CD4^+$ T cells from the spleen of DBA/1J mice were stimulated with CD3mAb for 72 h. IL-17, IFN-γ and TNF-α expression was measured by RT-PCR (Fig. 3A) and concentration by ELISA (Fig. 3B). The concentrations of IL-17, IFN-γ, and TNF-α were significantly higher in $CD4^+$ T cells stimulated through TCR.

3. Effect of activated $CD19^+$ B cells on cytokine production by $CD4^+$ T cells stimulated with CD3 mAb

We investigated the influence of $CD19^+$ B cells in vitro $CD4^+$ T cells proliferative response. Splenic $CD19^+$ B cells were stimulated for 24 h with LPS, anti-IgM, CD40 mAb or/and anti-IgM plus CD40 mAb. $CD19^+$ B cells cocultured for 72 h with $CD4^+$ T cells from syngeneic mice stimulated with CD3 mAb. Stimulated $CD19^+$ B cells had no effects on the proliferative responses of $CD4^+$ T cells to CD3 mAb (data not shown). Next, we examined the profile of cytokines secreted by the coculture supernatants of $CD19^+$ B cells and $CD4^+$ T cells. The concentrations of IL-17, IFN-γ, and TNF-α in the culture supernatants were measured by sandwich ELISA. IL-17 production was significantly lower in TCR stimulated $CD4^+$ T cells cocultured with $CD19^+$ B cells that had been stimulated with anti-IgM plus CD40 mAb than TCR stimulated $CD4^+$ T cells cocultured with $CD19^+$ B cells stimulated with another conditions. IFN-γ and

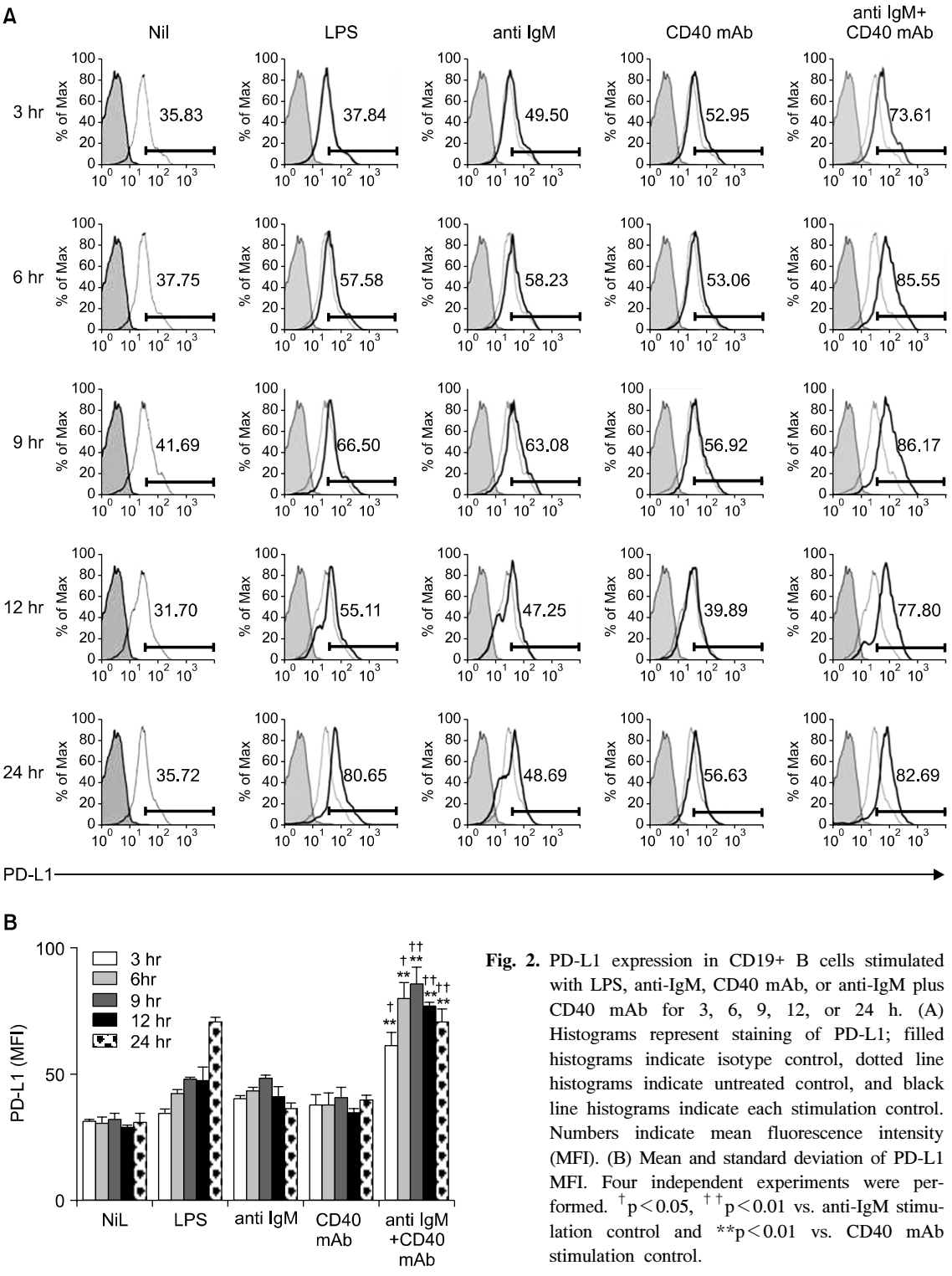

Fig. 2. PD-L1 expression in CD19+ B cells stimulated with LPS, anti-IgM, CD40 mAb, or anti-IgM plus CD40 mAb for 3, 6, 9, 12, or 24 h. (A) Histograms represent staining of PD-L1; filled histograms indicate isotype control, dotted line histograms indicate untreated control, and black line histograms indicate each stimulation control. Numbers indicate mean fluorescence intensity (MFI). (B) Mean and standard deviation of PD-L1 MFI. Four independent experiments were performed. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. anti-IgM stimulation control and $^{**}p<0.01$ vs. CD40 mAb stimulation control.

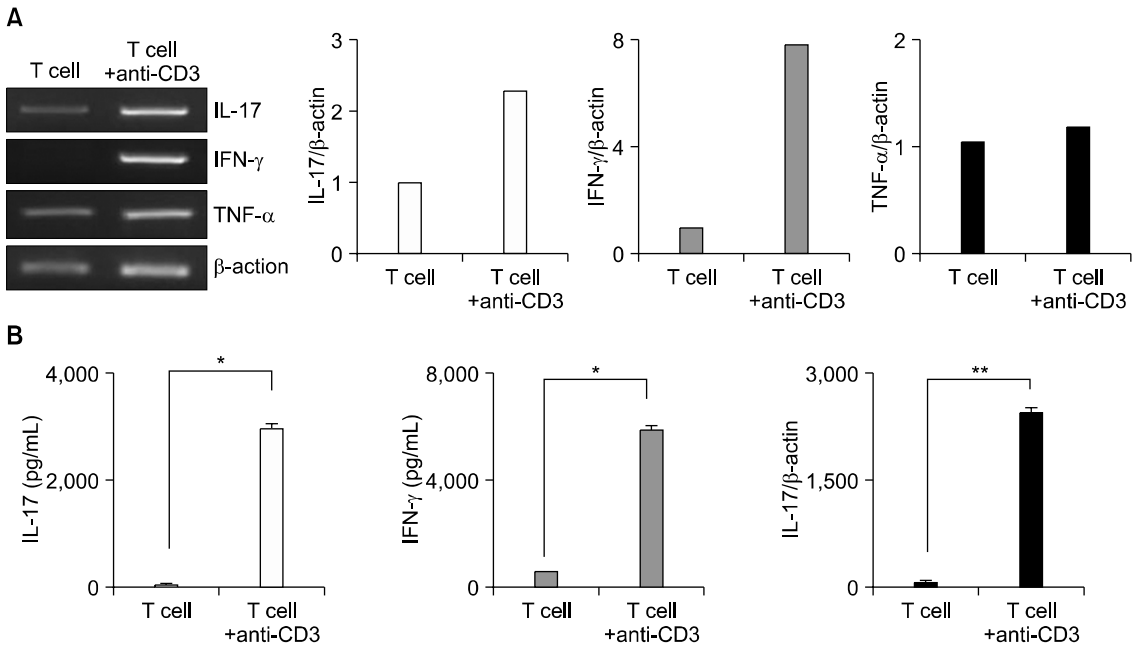

Fig. 3. Increased expression of IL-17, IFN-γ and TNF-α on the CD3mAb stimulated $CD4^+$ T cells. (A) IL-17, IFN-γ, and TNF-α mRNA expression induced by CD3mAb in $CD4^+$ T cells. Cells were cultured in CD3mAb coated 24-well plates at a concentration of 5×10^5/well for 72 h. The expression of IL-17, IFN-γ, and TNF-α mRNA was evaluated by RT-PCR. Optical densities were normalized to the band for β-actin. (B) The concentrations of IL-17, IFN-γ and TNF-α in the culture supernatant were measured by sandwich ELISA analysis. The data represent the means from three independent. *$p<0.05$, **$p<0.01$ vs. nil.

TNF-α concentration did not differ between TCR stimulated $CD4^+$ T cells cocultured with $CD19^+$ B cells and TCR stimulated $CD4^+$ T cells and coculture with $CD19^+$ B cells that had been stimulated with anti-IgM plus CD40mAb (Fig. 4).

4. Effect of PD-L1 neutralizing Ab on cytokine production by $CD4^+$ T cells

To define the role of PD-L1 on the production of IL-17 by $CD4^+$ T cells stimulated with CD3 mAb, $CD19^+$ B cells were stimulated with anti-IgM plus CD40 mAb and/or in the presence of PD-L1 neutralizing Ab. After 24 h, $CD4^+$ T cells isolated from syngeneic mice were stimulated with CD3 mAb and added preactivated $CD19^+$ B cells. And the cells were re-stimulated with PD-L1 neutralizing Ab and/or isotype control for 72 h. The production ability of IL-17 on $CD4^+$ T cells was significantly recovered, when the activated $CD19^+$ B cells were added with anti-IgM plus CD40 mAb plus PD-L1 neutralizing Ab compare with anti-IgM plus CD40 mAb (Fig. 5).

Our data suggests that stimulation of anti-IgM plus CD40 mAb on $CD19^+$ B cells upregulate the expression of PD-L1, and this increase can suppress the production of IL-17 on CD3 mAb stimulated $CD4^+$ T cells. However, comparable to IL-17, the secretions of IFN-γ or TNF-α were not changed by PD-L1 neutralizing Ab in the coculture system.

DISCUSSION

B cells play a critical role in the immune response to inhaled allergens and are required for the development of allergen-specific T cell unresponsiveness induced by respiratory allergen (13). Several limited reports have stressed that matured B cells could be in-

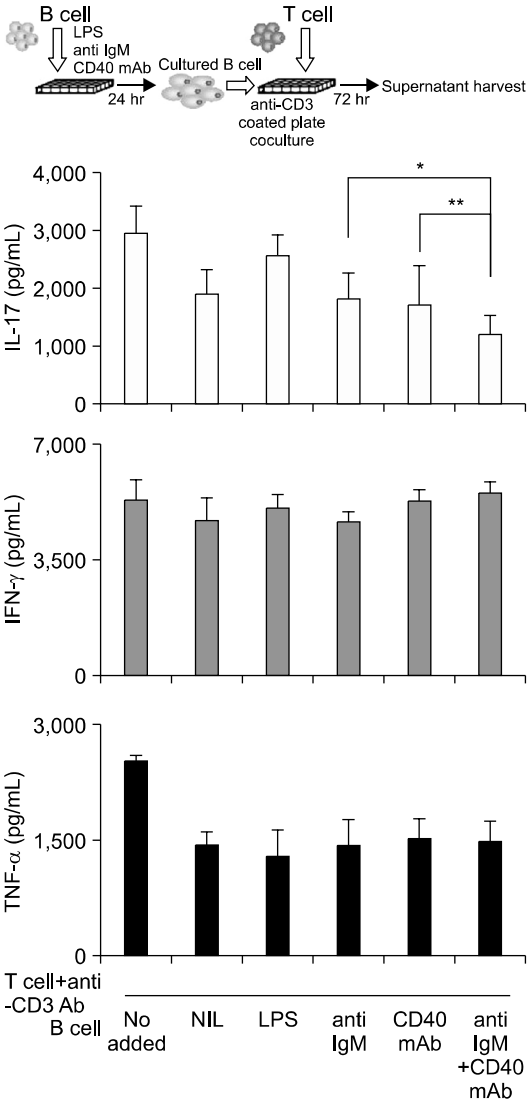

Fig. 4. Cytokines production in the supernatants from $CD4^+$ T cells stimulated with CD3 mAb cocultured with $CD19^+$ B cells that had been stimulated with LPS, anti-IgM, CD40 mAb and anti-IgM plus CD40 mAb. IL-17, IFN-γ and TNF-α expression were measured by ELISA. Three independent experiments were performed. *$p<0.01$ vs. anti-IgM stimulation control and **$p<0.01$ vs. CD40 mAb stimulation control.

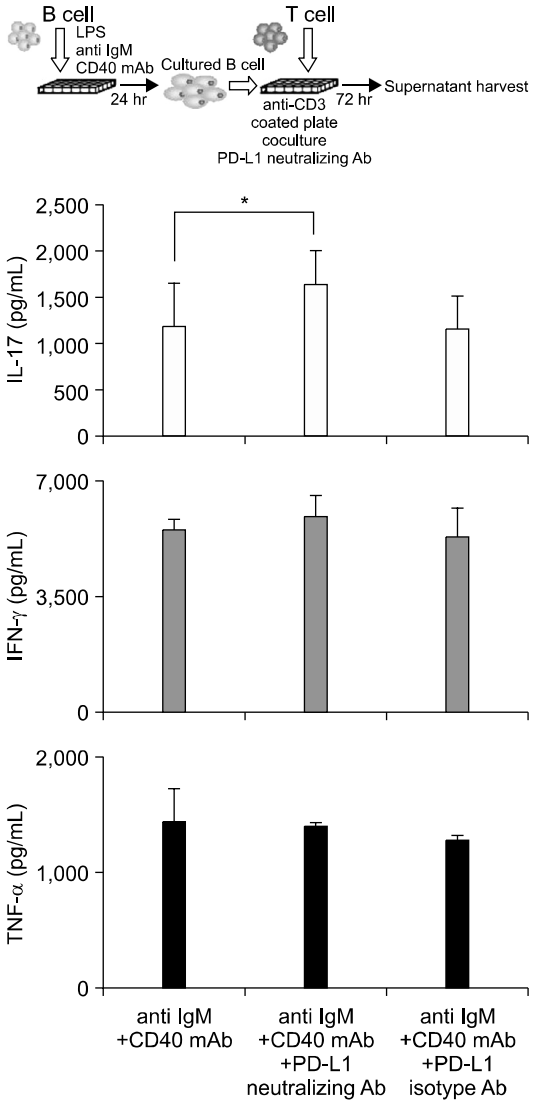

Fig. 5. Cytokines production in the supernatants from $CD4^+$ T cells stimulated with CD3 mAb cocultured with $CD19^+$ B cells that had been stimulated with anti-IgM plus CD40 mAb and anti-IgM plus CD40 mAb plus PD-L1-blocking Ab. IL-17, IFN-γ and TNF-α expression were measured by ELISA. Three independent experiments were performed. *$p<0.05$, vs. anti-IgM plus CD40 mAb stimulation control.

volved in direct regulation of pathogenic T cells through the CD40 and B7-2 pathways (14). These regulatory functions of B cells (Bregs) may be explained either by secreted antibodies or, more directly, through T cell-B cell interactions or suppressive cytokines. In acquired immunity-mediated diseases, Bregs can be developed from activated follicular B cells following further activation through the CD40 pathway or BCR ligation with self-Ags. In innate immune system, one Breg subset from messentric lymphnode (MLN) cells is derived from splenic MZB cells following activation of toll like receptor (TLR) pathways (3).

CD40 is a member of the TNF superfamily and is expressed mainly on B cells and DCs as well as on endothelial cells, macrophages, T cells, and fibroblasts (15,16). Its ligand, CD40 ligand (CD40L), is a member of the TNF ligand family and is expressed mainly on activated T cells. CD40-CD40L system plays a pivotal role in T cell and B cell interaction. In B cells, interaction with CD40L promotes proliferation and survival of B cells, Ig isotype switching, and germinal center reaction (17).

In our experiments, we examined the expression profiles of costimulatory molecules (CD40, CD80, CD86, and MHCII) and coinhibitory molecules (PD-1 and PD-L1). Expressions of the costimulatory molecules on $CD19^+$ B cells are increased after stimulation of LPS, anti-IgM, CD40 mAb, or anti-IgM plus CD40 mAb (Fig. 1). Notably, one coinhibitory molecule PD-L1 was significantly increased on $CD19^+$ B cells, stimulated with anti-IgM and CD40 mAb (Fig. 1, 2). We observed the cytokine production ability of $CD4^+$ T cells, which influenced by PD-L1 expression on $CD19^+$ B cells. CD3 mAb treated naive $CD4^+$ T cells up-regulated the production of IL-17, IFN-γ and TNF-α (Fig. 3). When the CD3 mAb treated $CD4^+$ T cells were coculture with activated $CD19^+$ B cells, the increase in production of IFN-γ and TNF-α was not significantly suppressed. Interestingly, IL-17 secretion from CD3mAb activated T cells was significantly inhibited after coculture with $CD19^+$ B cells that had treated with anti-IgM plus CD40 mAb (Fig. 4). This result is quite comparable to IFN-γ and TNF-α production in the same coculture conditions. IL-17 can activate the expression of a variety of proinflammatory cytokines, chemokines and cell adhesion molecules in a wide range of cell types, including macrophages, dendritic cells, T cells, synovial cells and endothelial cells (18). IL-17, proinflammatory cytokine, is overproduced in the rheumatoid arthritis (RA) synovium and synovial fluids, where this cytokine plays a pathogenic role because of its association with bone destruction (19-21). IFN-γ exerts its protective effect by suppressing the expansion of activated $CD4^+$ T cells (22) or by upregulating regulatory T cell activity (23,24). IFN-γ -deficient knockout (KO) mice show an accelerated and more severe form of CIA than their wild-type counterparts (25-28). TNF-α is implicated in autoimmune diseases and may play an indirect role in activation of pain pathways (29). TNF-α is an inflammatory cytokine released by activated monocytes, macrophages, and T lymphocytes, and promotes inflammatory responses that are important in the pathogenesis of RA (30-32). Especially IL-17 and TNF-α also play an important role in arthritis models (33,34).

The fact that highly expressed PD-L1 on $CD19^+$ B cells may down-regulate the production ability of IL-17 from anti-CD3mAb treated $CD4^+$ T cells. As shown in Fig 4, 5 the IL-17 production of $CD4^+$ T cells was decreased followed by an increase in the expression of PD-L1 on $CD19^+$ B cells. The production ability of IL-17 was recovered after treatment of PD-L1 neutralizing Ab, suggests that the PD-1-PD-L1 interaction between $CD4^+$ T cells and $CD19^+$ B cells is important in regulation of IL-17 production.

The immune system must respond to low concentrations of antigen for efficient elimination of infectious agents. The growth and differentiation of lymphocytes are mediated by antigen receptors that have low affinity for their ligands because they are products of recombinatorial gene rearrangements that take place in the absence of selection by antigen. Therefore, lym-

phocytes must have mechanisms that enable them to be stimulated when relatively few receptors have bound antigens (35). Depending upon their stage of maturation, cross-linking of the BCR may result in activation or apoptosis. Mature B lymphocytes proliferate and secrete Ig in response to antigen (Ag) receptor cross-linking (36). In contrast, Ag receptor cross-linking on immature B lymphocytes causes cell death, which is thought to be an important mechanism in the maintenance of immune tolerance (37,38). BCR transduces either proapoptotic or antiapoptotic signals in mature B cells depending on the nature of the stimuli. Mature B cells also undergo apoptosis by signaling through CD95. The apoptotic signals through BCR or CD95 are blocked by various transmembrane signals such as those occurring through CD40, BCR, CD21, and the IL-4 receptor, which are generated presumably by interaction with T helper cells or the components of innate immunity such as complements (39).

The PD-L1-PD-1 pathway plays an important role in maintaining peripheral T cell tolerance under normal physiological conditions and certain pathological processes (40,41). Under the normal conditions, PD-L1 expressed in the tissues and cells can downregulate the effector functions of T cells and thus prevent T cells from overreacting to tissues and cells, which may lead to autoimmune diseases (41).

In conclusion, $CD19^+$ B cells stimulated with anti-IgM plus CD40 mAb upregulated the production of the co-inhibitory molecule PD-L1. $CD4^+$ T cells stimulated with CD3 mAb upregulate the production of IL-17, IFN-γ and TNF-α. When the $CD19^+$ B cells stimulated with anti-IgM plus CD40 mAb, the production of IL-17 was down-regulated via PD-1-PD-L1 interaction between activated $CD4^+$ T cells and B cells. Blocking PD-L1 with neutralizing Ab recover IL-17 production of CD3 mAb activated $CD4^+$ T cells. These findings suggest that the regulatory activity of activated B cells via over expression of PD-L1 give us another therapeutic insight of IL-17 mediated chronic inflammatory diseases such as rheumatoid arthritis.

CONCLUSION

Our results suggest that B cell stimulation by anti-IgM and agonist CD40 mAb increases PD-L1 expression and decreases IL-17 production by activated T cells. PD-L1 may play an important role in regulatory activity of B cells under the influence of autoimmune diseases and inflammatory condition.

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