Biochemical and Biophysical Research Communications 425 (2012) 918–923

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

IgA plasma cells express the negative regulatory co-stimulatory molecule programmed cell death 1 ligand and have a potential tolerogenic role in the intestine

Tomomitsu Doi [1], Takanori Kanai *, Yohei Mikami, Tomohisa Sujino, Li Jun, Yuichi Ono, Atsushi Hayashi, Toshifumi Hibi *

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo 160-8582, Japan

ARTICLE INFO

Article history:
Received 3 August 2012
Available online 12 August 2012

Keywords:
Plasma cells
Intestine
PD-L1
Tolerance
FoxP3

ABSTRACT

To maintain immune homeostasis in the intestine, the intestinal immune system has evolved several tolerogenic mechanisms toward intestinal microflora and food antigens. Although programmed cell death-1 (PD-1) protein has been implicated in immunological tolerance in the intestine and gut-associated lymphoid tissues (GALTs), distribution of its ligands PD-L1 and PD-L2 in the small intestine lamina propria (LP) are unknown. We investigated PD-L1 expression in intestinal LP and found that IgA plasma cells (PCs) were major PD-L1 expressing cells. PD-L1 expression levels on IgA PCs were higher than that on IgG PCs in peripheral lymphoid tissues. IgA PCs expressed antigen-presenting molecule MHC class II and co-stimulatory molecules CD80, CD86, and PD-L2. IgA PCs isolated from intestinal LP exhibited antigen presentation activity, and in the presence of TGF-β induced FoxP3$^+$ regulatory T cells, but not IFN-γ$^+$ Th1 cells, from naïve T cells. Thus, IgA PCs in the intestine may be involved in an immune regulatory role in the intestinal immune system.

1. Introduction

The intestinal immune system is constantly exposed to intestinal microbiota and food antigens; thus, the intestinal immune system can induce and maintain tolerance toward these harmless antigens [1]. CD4$^+$CD25$^+$FoxP3$^+$ regulatory T (Treg) cells play an important role in intestinal immune system homeostasis. Although most Treg cells develop in the thymus as naturally-occurring Treg cells [2], recent studies suggest that FoxP3$^+$ Treg cells can be induced from naïve T cells during tolerance induction toward newly encountered antigens in the periphery [3–5].

Oral tolerance is an antigen-specific immune suppression mechanism induced by prior antigen administration through the oral route [6]. Treg cells are thought to be involved in oral tolerance; oral administration of ovalbumin (OVA) to OVA-specific TCR transgenic mice induces oral tolerance towards OVA and concomitantly induces FoxP3 expression in OVA-specific CD4$^+$ T cells [3,7]. Mesenteric lymph nodes (MLNs) have an essential role in inducing oral tolerance [8]. Tolerance induction in MLNs is mediated by a specialized dendritic cell (DC) subset: CD103$^+$ DCs [9]. However, the intestinal immune system produces immunoglobulin A (IgA) antibodies against commensal bacteria and maintains a symbiotic relationship between a host and commensal bacteria [10,11].

Programmed cell death-1 (PD-1) was originally identified as a gene that was upregulated in T cell lymphoma cells upon cell death. PD-1 is a member of the immunoglobulin superfamily and has an immunoreceptor tyrosine based inhibitory motif (ITIM) in its cytoplasmic domain [12]. PD-1 is induced during the later phase of T cell activation and terminates T cell activation to prevent excessive, prolonged activation [12]. PD-1 deficient mice develop spontaneous late-onset lupus-like arthritis and glomerulonephritis in a C57BL/6 background and autoimmune dilated cardiomyopathy in a BALB/c background [13,14]. Recently, several reports showed that PD-1 was involved in inducing and maintaining tolerance and unresponsiveness toward chronic viral infections [12,15]. Consistently, PD-L1 coated beads induced cells with phenotypic features of Treg (iTreg) cells that maintained suppressive functions of iTreg cells in vitro [16].

PD-1 has two ligands: PD-L1 and PD-L2. PD-L1 is preferentially expressed on B cells, DCs, macrophages, and T cells and is further upregulated on cell activation [12]. PD-L1 is expressed on

Abbreviations: PD-1, programmed cell death-1; GALTs, gut-associated lymphoid tissues; MLN, mesenteric lymph node; PCs, plasma cells; SI, small intestine; LP, lamina propria; DCs, dendritic cells.

* Corresponding authors. Fax: +81 3 3341 3631.
E-mail addresses: takagast@sc.itc.keio.ac.jp (T. Kanai), thibi@sc.itc.keio.ac.jp (T. Hibi).
[1] Present address: Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.08.010

non-hematopoietic cells, such as vascular endothelial cells, pancreatic islet cells, and placenta to maintain immune tolerance and protect these tissues from untoward immune attacks [12]. Conversely, PD-L2 expression is more restricted to hematopoietic cells and is induced by B cell, DC, macrophage, and T cell activation [12]. PD-L2 has a threefold higher affinity for PD-1 as compared to PD-L1 [12]. For oral tolerance induction, $CD103^+$ DCs induce unresponsiveness toward orally administered antigens and induce $FoxP3^+$ regulatory T cells through PD-1 and its interactions with its ligands [17].

B lymphocytes (B cells) play an important role in humoral immune responses. In response to antigen receptor stimulation, B cells differentiate into PCs and produce antibodies in conjunction with the aid of helper $CD4^+$ T cells [18]. B cells can influence T cell differentiation toward Th2 type responses [19]. B cells attenuated inflammatory disorders, such as T cell-mediated colitis, and played a role in experimental autoimmune encephalomyelitis remission [20–22]. A subset of B cells, designated regulatory B cells, produce IL-10 and suppress the onset of inflammatory diseases [23–25].

Although IgA-producing PCs are a major immune cell type in the intestinal lamina propria (LP), other than antibody production, the roles of these cells have not been fully investigated. In this study, we found that IgA PCs were major PD-L1-expressing cells in the intestine that could induce $FoxP3^+$ Treg cells from naïve T cells in vitro.

2. Materials and methods

2.1. Mice

C57BL/6J mice were purchased from Clea Japan (Tokyo). DO11.10 mice [26], lymphotoxin α-deficient (LT-$\alpha^{-/-}$) mice [27], were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed in specific pathogen-free (SPF) conditions in the Animal Care Facility of Keio University School of Medicine. All experiments were approved by the regional animal study committees and followed institutional guidelines and Home Office regulations.

2.2. Cell preparations and flow cytometry

Intestinal lamina propria mononuclear cells (LPMCs) from small intestine (SI) were prepared as described previously [28]. Cell surfaces were stained with anti-B220, IgA, PD-L1, PD-L2, CD80, CD86, $I\text{-}A^b$, CD103, CD11b, and CD11c antibodies. For intracellular cytokine staining, cells were incubated for 12 h with 50 ng/ml of phorbol 12-myristrate 13-acetate (PMA; Sigma, St. Louis, MO), 1000 ng/ml of ionomycin (Sigma), and 1 μl/ml of GolgiPlug (BD Pharmingen, San Diego, CA) in a CO_2 incubator at 37 °C. After staining for cell surface molecules, the cells were resuspended in Fixation/Permeabilization solution (BD Pharmingen, San Diego, CA), and intracellular staining was done with an anti-IFN-γ antibody. For intracellular FoxP3 staining, cells were fixed in Fixation/Permeabilization solution (eBioscience). The cells were analyzed using a FACSCanto™ II (Becton Dickinson, NJ, USA) and FlowJo software (Tree Star, Inc., Ashland, OR). The following mAbs were used for flow cytometric analysis: biotinylated anti-IgA (RMA-1, BioLegend); PE-Cy7 anti-B220 (RA3-6B2, BD Bioscience); PE-Cy7 anti-CD11c (HL3, BD Bioscience); PE anti-CD80 (16-0A1, BD Bioscience); PE anti-CD86 (GL1, BD Bioscience); PE anti-PD-L1 (MIH5, eBioscience); PE anti-PD-L2 (122, eBioscience); PE anti-I-Ab (AF6-120.1 BD Bioscience); PE anti-CD103 (2E7 eBioscience); FITC anti-IgG1 (A85-1, BD Bioscience); APC anti-CD138 (281-2, BD Bioscience); PE anti-FoxP3 (eBioscience); FITC anti-B220 (RA3-6B2, BioLegend); APC-Cy7 streptavidin (BD Bioscience); APC streptavidin (BD Bioscience); and APC-Cy7 CD11b (M1/70, BD Bioscience).

2.3. In vitro T cell differentiation

Naïve OVA-specific $CD4^+$ T cells were isolated from spleens of DO11.10 or OT-II TCR transgenic mice using a Naïve $CD4^+$ T cell isolation Kit II (Miltenyi). $CD11c^+$ DCs were purified from collagenase A digested spleen tissues and MLNs with CD11c microbeads (Miltenyi). IgA plasma cells were purified by depleting $B220^+$ cells with B220 microbeads (Miltenyi) followed by positive selection with biotin-anti-IgA antibody (BioLegend) and anti-biotin microbeads (Miltenyi). Naïve T cells (2×10^5) were co-cultured with either splenic $CD11c^+$ DCs (2×10^4), MLN $CD11c^+$ DCs (2×10^4), or small intestinal IgA^+ PCs (2×10^5), with or without 0.1 μM OVA peptide and 1 ng/ml TGF-β, for 3 days in 96-well flat-bottom plates. For blocking experiments, anti-PD-L1 (MIH6) (Abcam), anti-PD-L2 (TY25) (Abcam), and Rat IgG2a, kappa monoclonal (aRTK2758) (Abcam) antibodies were added.

2.4. Statistical analysis

Results are given as means ± standard errors of the mean (SEMs). Statistical comparisons were made by unpaired Student's *t* tests using Prism software (Graphpad).

3. Results

3.1. PD-L1 is expressed on IgA PCs in the intestine

Intestinal LP is an effector site of immune reactions and contains numerous immune cells, including T and B cells. To determine PD-L1 expression distributions in the mucosal immune system of the intestine, we first examined PD-L1 expression in LP mononuclear cells (LPMCs) obtained from SIs of normal mice by flow cytometry. PD-L1 was expressed by 29% of total LPMCs (Fig. 1A). Next, LPMCs were stained for B220, IgA, and CD11c in addition to PD-L1. Among $PD\text{-}L1^+$ cells, 81% were IgA PCs, whereas $CD11c^+$ DCs constituted only 6.9% of $PD\text{-}L1^+$cells in LP of SI (Fig. 1B). Next, we examined cell surface molecules related to antigen-presenting activity, including MHC class II and co-stimulatory molecules on IgA PCs, B cells, and DCs isolated from the spleen and MLNs. IgA PCs expressed MHC class II molecules and the negative regulatory co-stimulatory molecules PD-L2 and PD-L1, whereas expression levels of the positive co-stimulatory molecules CD80 and CD86 on IgA PCs were lower than those on dendritic cells (Fig. 1C). CD103 was detected only on some DCs in MLNs, as reported previously [29]. PD-L1 was expressed on both IgA PCs and DCs; PCs were the major cell type that expressed PD-L1 in the small intestine LP.

3.2. Intestinal IgA PCs induce $FoxP3^+$ T cells from naive T cells in vitro

Because IgA PCs expressed antigen-presenting molecules, such as MHC class II molecules and PD-L1, we next examined whether IgA PCs could induce $FoxP3^+$ Treg cells from naïve T cells, as it had been previously reported that MLN DCs could induce $FoxP3^+$ Treg cells from naïve T cells in vitro through the interaction between PD-L1 on DCs and PD-1 on T cells in the presence of TGF-β [17]. To test this, naïve T cells ($CD4^+CD62^+$ T cells) from OVA-specific TCR transgenic mice were co-cultured with either IgA^+ intestinal PCs, splenic DCs, or MLN DCs in the presence or absence of TGF-β and examined for intracellular FoxP3 and IFN-γ expressions.

Splenic and MLN DCs induced IFN-γ^+ Th1 cells (Fig. 2B). MLN DCs but not splenic DCs induced $FoxP3^+$ T cells in the presence of TGF-β, as described previously [17] (Fig. 2A). Although IgA PCs could not induce IFN-γ^+ Th1 cells, IgA PCs induced $FoxP3^+$ Treg cells in the presence of TGF-β as did MLN DCs (Fig. 2A). To examine

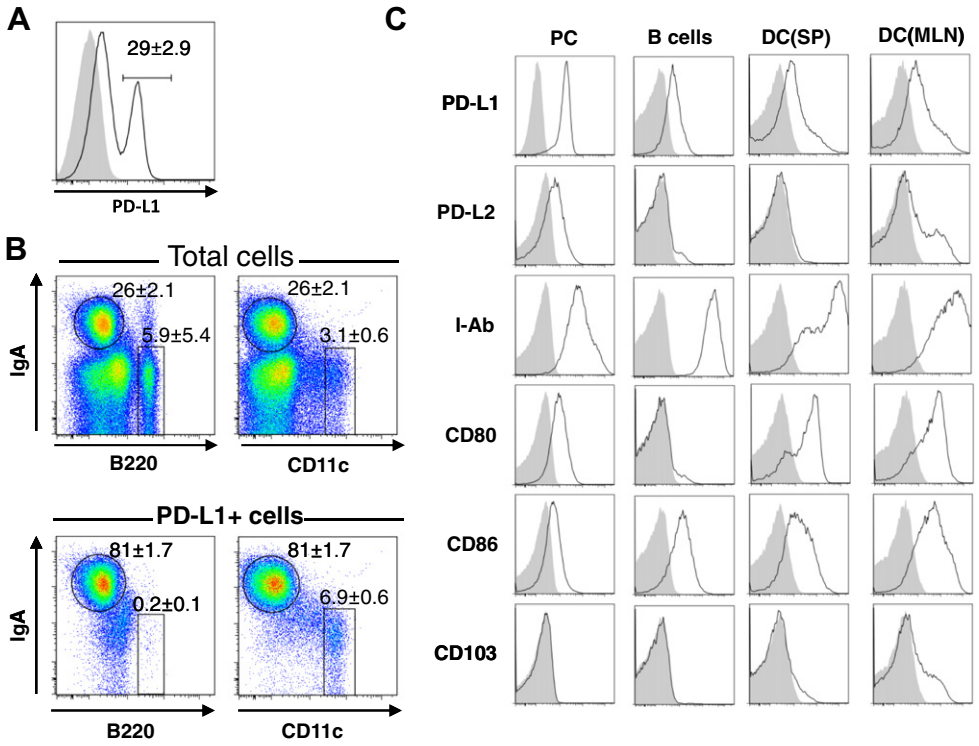

Fig. 1. IgA plasma cells are major PD-L1 expressing cells in the intestine. (A) Black lines indicate PD-L1 stained total LPMCs among 7AAD$^-$ gated cells. Filled histogram indicates isotype control antibody staining. (B) Upper panels show IgA and B220 or CD11c expressions among 7AAD$^-$ total LPMCs. Bottom panels show IgA and B220 or CD11c expressions among 7AAD$^-$PD-L1$^+$ gated cells. Numbers for each gate indicate means ± SDs; $n = 3$. (C) Surface staining of purified IgA plasma cells, B cells, splenic DCs, and mesenteric DCs. Black lines indicate staining with indicated antibodies. Filled histograms indicate isotype control antibody staining. Results are representative of three independent experiments.

the roles of PD-L1/L2 and PD-1 interactions in antigen presentation by IgA PCs, neutralizing antibodies against PD-L1 and PD-L2 were added during in vitro differentiation. IgA PCs did not induce IFN-γ ± Th1 cells even in the presence of neutralizing antibodies against PD-L1 and PD-L2 (data not shown). Although neither anti-PD-L1 nor PD-L2 antibody alone suppressed induction of FoxP3 ± T cells, a combination of anti-PD-L1 and PD-L2 antibodies significantly suppressed induction of FoxP3 ± T cells (Fig. 2C).

3.3. PD-L1 is expressed on IgG1$^+$ PCs in systemic lymphoid organs

IgM-negative PCs in peripheral lymphoid tissues had antigen-presenting activities and induced a series of helper T cell-derived cytokines by naïve T cells, including IFN-γ [30]. To compare PD-L1 expression on IgA PCs derived from gut-associated lymphoid tissues (GALTs) and IgG1 PCs in peripheral lymph nodes induced by systemic immunization, we immunized mice with alum-precipitated chicken gamma globulin (CGG) and analyzed PD-L1 expressions on CD138$^+$IgG1$^+$ PCs and IgA PCs from the spleen and MLNs.

CD138$^+$IgA$^+$ PCs were observed in the spleen and MLNs prior to immunization and were not induced by immunization to the same extent as IgG1$^+$ PCs (Fig. 3A). IgA$^+$ PCs are thought to be induced by continuous stimulation by intestinal microbiota in GALT and are not induced by systemic immunizations [31]. CD138$^+$IgG1$^+$ PCs were induced in the spleen and MLNs by immunization. Although PD-L1 was expressed on IgG1$^+$ PCs, PD-L1 expression level on IgG1$^+$ PCs was significantly lower than that on IgA$^+$ PCs (Fig. 3B and C).

3.4. PD-L1 expression is enhanced on IgA plasma cells from LT-α deficient mouse intestine

To examine the effect of PC deficiency on PD-L1 expression in the intestine, we compared PD-L1 expressions on intestinal IgA PCs of wild type mice and LT-α deficient littermate mice, which have fewer PCs in the intestine [32] (Fig. 4A and B). PD-L1 expression level on intestinal IgA PCs of LT-α deficient mice were higher than those on IgA PCs of wild type mice (Fig. 4C). These results suggest that a reduction in intestinal PCs is compensated by augmented PD-L1 expression to maintain intestinal immune homeostasis.

4. Discussion

The intestinal immune system has evolved mechanisms to maintain immunological quiescence in the presence of harmless food antigens and the intestinal microflora. One of these mechanisms is oral tolerance induced in intestinal LP and GALT. Interactions between PD-1 and its ligands, PD-L1 and PD-L2, have been implicated in immunological tolerance, including oral tolerance, in the intestine. In this study, we demonstrated that IgA PCs were the major cell type that expressed PD-L1 in SI LPMCs.

The expressions of molecules that are required for antigen presentation functions, such as MHC class II and CD80, CD86, and PD-L1, suggest that IgA PCs function as antigen-presenting cells (APCs) in the intestinal LP. Indeed, we found that IgA PCs isolated from SI LPMCs could induce FoxP3$^+$ iTreg cells but not IFN-γ$^+$ Th1 cells from naïve T cells in vitro (Fig. 2). FoxP3 ± iTreg cell induction was suppressed by the addition of both anti-PD-L1 and PL-L2 antibodies, but not by either antibody alone. Therefore, PD-L1 and PD-L2 on IgA PCs have redundant roles during the induction of FoxP3 ± iTreg cells.

In a previous report [30], isotype switched PCs induced by subcutaneous immunization with NP-CGG retained their antigen presentation apparatus and induced Th1, Th2, and Th17 (except follicular helper T cells; T_{FH}) associated cytokine production by

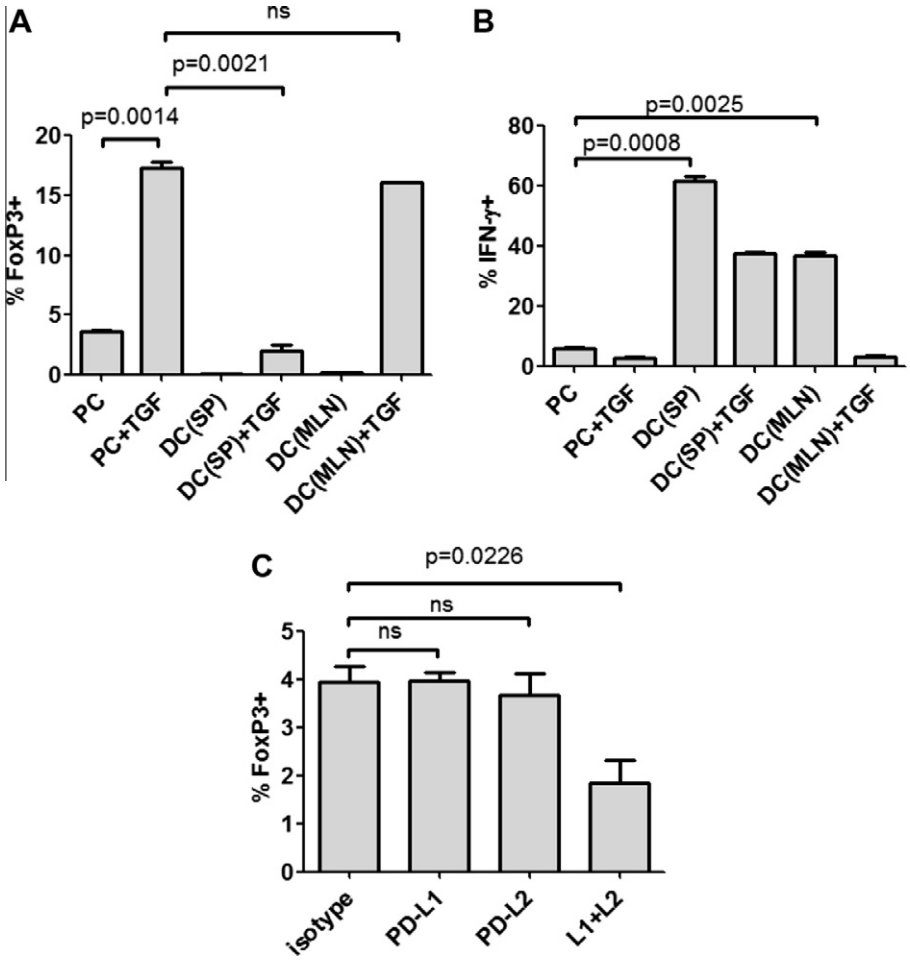

Fig. 2. IgA$^+$ plasma cells induce FoxP3 expression in naïve T cells in the presence of TGF-β in vitro. IgA$^+$ plasma cells were purified from intestinal LPMCs and CD11c$^+$ DCs were purified from spleens and MLNs. Naïve T cells from OVA-specific TCR transgenic mice were cultured with either plasma cells, splenic DCs, or MLN DCs in the presence of 0.1 μM OVA peptide with or without 1 ng/ml of TGF-β in triplicate. (A) Percentages of FoxP3 positive cells among CD3$^+$CD4$^+$ cells. (B) Percentages of IFN-γ positive cells among CD3$^+$CD4$^+$ cells. Experiments were repeated 3 times. (C) Naïve T cells and IgA ± plasma cells were co-cultured as described above with 5 μg/ml of either anti-PD-L1 (L1), anti-PD-L2 (L2), or isotype control antibody in the presence of 0.1 μM OVA peptide and 1 ng/ml of TGF-β in triplicate. Total antibody amounts were adjusted to 10 μg/ml with isotype control antibody. Mean percentages of FoxP3 ± cells are shown. Error bars are SEMs. *P*-values derived from Student's *t*-tests are indicated on the graph (ns; not significant).

naïve CD4 T cells. This contradictory result may be explained by the lower expressions of CD80 and CD86 on IgA PCs than those on DCs and the different PD-L1 expression levels on intestinal IgA PCs and peripheral IgG1 PCs, as we showed that PD-L1 expression level on IgA PCs was higher than that on IgG1 PCs. Peripheral switched PCs expressed levels of CD80 and CD86 that were comparable to those on DCs [30].

The fate of T cell differentiation is determined by the relative expression levels between positive co-stimulatory molecules (CD80 and CD86) and negative co-stimulatory molecules (PD-L1 and PD-L2) in addition to the cytokines produced by antigen presenting and surrounding cells [33]. Therefore, PD-L1 higher expression levels and the lower levels of CD80 and CD86 on IgA PCs may reasonably explain why IgA PCs induced FoxP3$^+$ Treg cells rather than IFN-γ$^+$ Th1 cells in contrast to PCs induced in peripheral lymphoid tissues by systemic immunization.

Furthermore, mice that have fewer IgA PCs in the intestine, such as lymphotoxin-α (LT-α) deficient mice, do not exhibit any inflammatory reactions in the intestine [34]. We found that PD-L1 expression levels on IgA PCs from LT-α deficient mice were higher than on these cells from wild type mice (Fig. 4). This suggested that the lower numbers of IgA PCs in LT-α deficient mice could be compensated by augmented PD-L1 expressions on IgA PCs in the intestine. However, we cannot exclude the contribution of PD-L1 on DCs and macrophages in the intestine and GALT for maintaining immunological homeostasis. Further studies will be needed to address this issue.

Although intestinal PCs almost exclusively produce IgA antibodies in the normal intestine, antibody production in inflammatory sites associated with inflammatory bowel diseases (IBDs), particularly ulcerative colitis (UC), is significantly skewed toward IgG production [35]. IgG PCs that infiltrate into inflammatory sites reportedly express inflammatory cytokines and matrix metalloproteinases and secrete IgG that can activate inflammatory macrophages through complement activation [36–38]. Furthermore, IgG PCs may function as stimulatory antigen presenting cells, and thus abrogate tolerance as opposed to tolerogenic IgA PCs in IBD inflammatory sites. Previous reports suggest that FoxP3$^+$ Treg cells preferentially differentiated into T_{FH} cells, formed germinal centers in Peyer's patches, and supported IgA class switching and the generation of IgA PCs in the intestine [39]. However, we showed that IgA plasma cells could induce FoxP3$^+$ Treg cells. Antigen administration through the oral route induces IgA antibody production and the subsequent immune tolerance in the intestine.

Because IgA PCs still express antigen receptors on their cell surfaces, it is possible that IgA PCs acquire specific antigens through

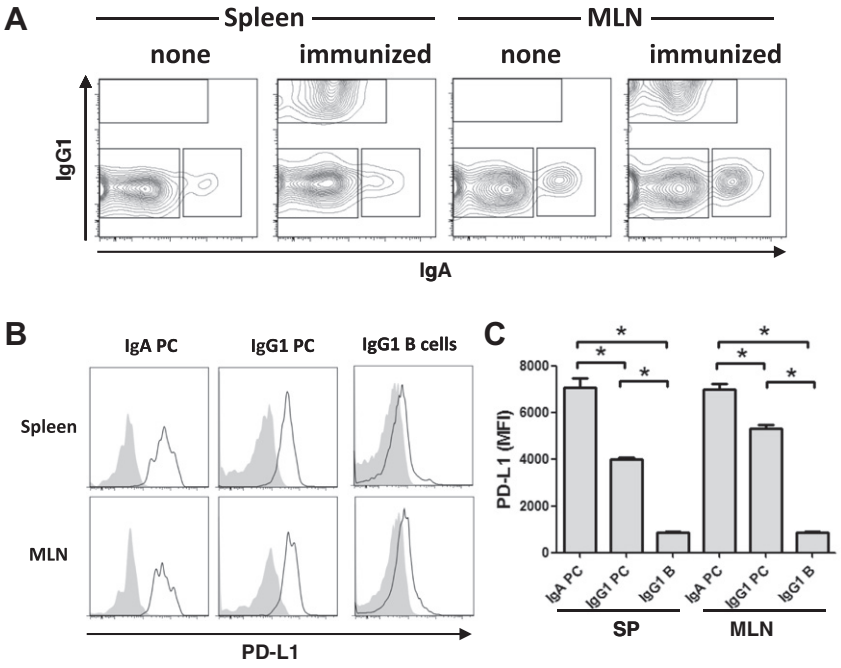

Fig. 3. Peripheral IgG1$^+$ plasma cells express PD-L1 at a level lower than that of IgA plasma cells. Mice were immunized with Alum-precipitated NP-CGG. After 7 days, splenocytes and MLN cells were analyzed by flow cytometry. (A) Intracellular IgA and IgG1 expressions in 7AAD$^-$CD138$^+$ gated splenocytes and MLN cells from immunized and non-immunized mice. (B) Histograms show PD-L1 expressions by IgA$^+$ plasma cells, IgG1$^+$ plasma cells, and IgG1$^+$B cells in the spleen and MLNs. Filled histograms show isotype control antibody staining. IgA PCs, IgG1 PCs, and IgG1 B cells are CD138$^+$IgA$^+$, CD138$^+$IgG1$^+$, and CD138$^-$IgG1$^+$, respectively. (C) Mean fluorescence intensities of IgA PCs, IgG1 PCs, and IgG1 B cells are shown ($n = 5$; $^*P < 0.0001$).

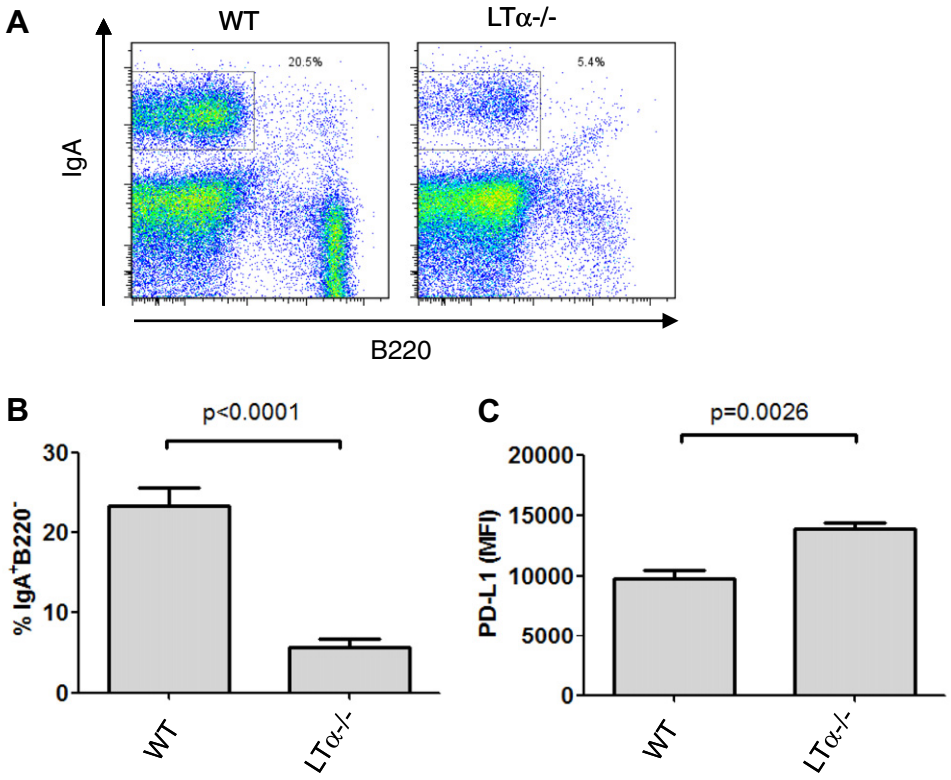

Fig. 4. PD-L1 expression levels on IgA plasma cells from LTα$^{-/-}$ mouse intestines are higher than those of cells from wild type mouse intestines. (A) IgA and B220 expressions by LPMCs from wild type (WT) and LTα$^{-/-}$ mice. (B) Mean percentages of IgA$^+$B220$^-$ cells among LPMCs from WT and LTα$^{-/-}$ mice are shown. (C) Mean fluorescence intensities of PD-L1 expression by IgA$^+$ plasma cells from WT and LTα$^{-/-}$ mice are shown ($n = 3$. Numbers in upper graph are P-values derived from Student's t-tests).

their antigen receptors and induce antigen-specific Treg cells. However, immunological homeostasis between Treg cells and IgA PCs must be maintained by some kind of positive feedback circuit, which needs to be addressed in future studies. From our current

results, IgA PCs in the intestine may possibly be involved in an immune regulatory role in the intestinal immune system to suppress the development of IBDs.

Acknowledgments

We thank K. Kinoshita and R. Shinkura for critical reading of this paper and M. Takabe for excellent animal care. This study was supported by a Grant-in-Aid for Young Scientists (B).

References

[1] M. Murai, P. Krause, H. Cheroutre, M. Kronenberg, Regulatory T-cell stability and plasticity in mucosal and systemic immune systems, Mucosal Immunol. 3 (2010) 443–449.
[2] C.C. Picca, J. Larkin 3rd, A. Boesteanu, M.A. Lerman, A.L. Rankin, A.J. Caton, Role of TCR specificity in CD4$^+$ CD25$^+$ regulatory T-cell selection, Immunol. Rev. 212 (2006) 74–85.
[3] J.B. Sun, S. Raghavan, A. Sjoling, S. Lundin, J. Holmgren, Oral tolerance induction with antigen conjugated to cholera toxin B subunit generates both Foxp3$^+$CD25$^+$ and Foxp3$^-$CD25$^-$ CD4$^+$ regulatory T cells, J. Immunol. 177 (2006) 7634–7644.
[4] K. Kretschmer, I. Apostolou, D. Hawiger, K. Khazaie, M.C. Nussenzweig, H. von Boehmer, Inducing and expanding regulatory T cell populations by foreign antigen, Nat. Immunol. 6 (2005) 1219–1227.
[5] K.M. Thorstenson, A. Khoruts, Generation of anergic and potentially immunoregulatory CD25$^+$CD4 T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen, J. Immunol. 167 (2001) 188–195.
[6] A.M. Mowat, Anatomical basis of tolerance and immunity to intestinal antigens, Nat. Rev. Immunol. 3 (2003) 331–341.
[7] X. Zhang, L. Izikson, L. Liu, H.L. Weiner, Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration, J. Immunol. 167 (2001) 4245–4253.
[8] T. Worbs, U. Bode, S. Yan, M.W. Hoffmann, G. Hintzen, G. Bernhardt, R. Forster, O. Pabst, Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells, J. Exp. Med. 203 (2006) 519–527.
[9] J.L. Coombes, K.R. Siddiqui, C.V. Arancibia-Cárcamo, J. Hall, C.M. Sun, Y. Belkaid, F. Powrie, A functionally specialized population of mucosal CD103$^+$ DCs induces Foxp3$^+$ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism, J. Exp. Med. 204 (2007) 1757–1764.
[10] S. Fagarasan, M. Muramatsu, K. Suzuki, H. Nagaoka, H. Hiai, T. Honjo, Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora, Science 298 (2002) 1424–1427.
[11] M. Wei, R. Shinkura, Y. Doi, M. Maruya, S. Fagarasan, T. Honjo, Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense, Nat. Immunol. 12 (2011) 264–270.
[12] L.M. Francisco, P.T. Sage, A.H. Sharpe, The PD-1 pathway in tolerance and autoimmunity, Immunol. Rev. 236 (2010) 219–242.
[13] H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor, Immunity 11 (1999) 141–151.
[14] H. Nishimura, T. Okazaki, Y. Tanaka, K. Nakatani, M. Hara, A. Matsumori, S. Sasayama, A. Mizoguchi, H. Hiai, N. Minato, T. Honjo, Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice, Science 291 (2001) 319–322.
[15] M.E. Keir, S.C. Liang, I. Guleria, Y.E. Latchman, A. Qipo, L.A. Albacker, M. Koulmanda, G.J. Freeman, M.H. Sayegh, A.H. Sharpe, Tissue expression of PD-L1 mediates peripheral T cell tolerance, J. Exp. Med. 203 (2006) 883–895.
[16] L.M. Francisco, V.H. Salinas, K.E. Brown, V.K. Vanguri, G.J. Freeman, V.K. Kuchroo, A.H. Sharpe, PD-L1 regulates the development, maintenance, and function of induced regulatory T cells, J. Exp. Med. 206 (2009) 3015–3029.
[17] T. Fukaya, H. Takagi, Y. Sato, K. Sato, K. Eizumi, H. Taya, T. Shin, L. Chen, C. Dong, M. Azuma, H. Yagita, B. Malissen, Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific CD4$^+$Foxp3$^+$ regulatory T cells in the establishment of oral tolerance, Blood 116 (2010) 2266–2276.
[18] S. Crotty, Follicular helper CD4 T cells (TFH), Annu. Rev. Immunol. 29 (2011) 621–663.
[19] D.M. Lindell, A.A. Berlin, M.A. Schaller, N.W. Lukacs, B cell antigen presentation promotes Th2 responses and immunopathology during chronic allergic lung disease, PLoS One 3 (2008) e3129.
[20] A. Mizoguchi, E. Mizoguchi, R.N. Smith, F.I. Preffer, A.K. Bhan, Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice, J. Exp. Med. 186 (1997) 1749–1756.
[21] B. Wei, P. Velazquez, O. Turovskaya, K. Spricher, R. Aranda, M. Kronenberg, L. Birnbaumer, J. Braun, Mesenteric B cells centrally inhibit CD4$^+$ T cell colitis through interaction with regulatory T cell subsets, Proc. Natl. Acad. Sci. USA 102 (2005) 2010–2015.
[22] P. Reichardt, B. Dornbach, S. Rong, S. Beissert, F. Gueler, K. Loser, M. Gunzer, Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse, Blood 110 (2007) 1519–1529.
[23] A. Mizoguchi, E. Mizoguchi, H. Takedatsu, R.S. Blumberg, A.K. Bhan, Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation, Immunity 16 (2002) 219–230.
[24] C. Mauri, D. Gray, N. Mushtaq, M. Londei, Prevention of arthritis by interleukin 10-producing B cells, J. Exp. Med. 197 (2003) 489–501.
[25] S. Fillatreau, C.H. Sweenie, M.J. McGeachy, D. Gray, S.M. Anderton, B cells regulate autoimmunity by provision of IL-10, Nat. Immunol. 3 (2002) 944–950.
[26] K.M. Murphy, A.B. Heimberger, D.Y. Loh, Induction by antigen of intrathymic apoptosis of CD4$^+$CD8$^+$TCRlo thymocytes in vivo, Science 250 (1990) 1720–1723.
[27] P. De Togni, J. Goellner, N.H. Ruddle, P.R. Streeter, A. Fick, S. Mariathasan, S.C. Smith, R. Carlson, L.P. Shornick, J. Strauss-Schoenberger, et al., Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin, Science 264 (1994) 703–707.
[28] T. Sujino, T. Kanai, Y. Ono, Y. Mikami, A. Hayashi, T. Doi, K. Matsuoka, T. Hisamatsu, H. Takaishi, H. Ogata, A. Yoshimura, D.R. Littman, T. Hibi, Regulatory T cells suppress development of colitis, blocking differentiation of T-helper 17 into alternative T-helper 1 cells, Gastroenterology 141 (2011) 1014–1023.
[29] O. Annacker, J.L. Coombes, V. Malmstrom, H.H. Uhlig, T. Bourne, B. Johansson-Lindbom, W.W. Agace, C.M. Parker, F. Powrie, Essential role for CD103 in the T cell-mediated regulation of experimental colitis, J. Exp. Med. 202 (2005) 1051–1061.
[30] N. Pelletier, L.J. McHeyzer-Williams, K.A. Wong, E. Urich, N. Fazilleau, M.G. McHeyzer-Williams, Plasma cells negatively regulate the follicular helper T cell program, Nat. Immunol. 11 (2010) 1110–1118.
[31] H.E. Mei, T. Yoshida, W. Sime, F. Hiepe, K. Thiele, R.A. Manz, A. Radbruch, T. Dorner, Blood-borne human plasma cells in steady state are derived from mucosal immune responses, Blood 113 (2009) 2461–2469.
[32] H.S. Kang, R.K. Chin, Y. Wang, P. Yu, J. Wang, K.A. Newell, Y.X. Fu, Signaling via LTbetaR on the lamina propria stromal cells of the gut is required for IgA production, Nat. Immunol. 3 (2002) 576–582.
[33] R.J. Greenwald, G.J. Freeman, A.H. Sharpe, The B7 family revisited, Annu. Rev. Immunol. 23 (2005) 515–548.
[34] H.P. Eugster, M. Muller, U. Karrer, B.D. Car, B. Schnyder, V.M. Eng, G. Woerly, M. Le Hir, F. di Padova, M. Aguet, R. Zinkernagel, H. Bluethmann, B. Ryffel, Multiple immune abnormalities in tumor necrosis factor and lymphotoxin-alpha double-deficient mice, Int. Immunol. 8 (1996) 23–36.
[35] K. Kett, T.O. Rognum, P. Brandtzaeg, Mucosal subclass distribution of immunoglobulin G-producing cells is different in ulcerative colitis and Crohn's disease of the colon, Gastroenterology 93 (1987) 919–924.
[36] J.N. Gordon, K.M. Pickard, A. Di Sabatino, J.D. Prothero, S.L. Pender, P.M. Goggin, T.T. MacDonald, Matrix metalloproteinase-3 production by gut IgG plasma cells in chronic inflammatory bowel disease, Inflamm. Bowel Dis. 14 (2008) 195–203.
[37] E.K. Onuma, P.S. Amenta, K. Ramaswamy, J.J. Lin, K.M. Das, Autoimmunity in ulcerative colitis (UC): a predominant colonic mucosal B cell response against human tropomyosin isoform 5, Clin. Exp. Immunol. 121 (2000) 466–471.
[38] T.S. Halstensen, K.M. Das, P. Brandtzaeg, Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kDa putative autoantigen in ulcerative colitis, Gut. 34 (1993) 650–657.
[39] M. Tsuji, N. Komatsu, S. Kawamoto, K. Suzuki, O. Kanagawa, T. Honjo, S. Hori, S. Fagarasan, Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches, Science 323 (2009) 1488–1492.