Published in final edited form as:
J Immunol. 2009 April 15; 182(8): 4516–4520. doi:10.4049/jimmunol.0803161.

Cutting Edge: A Critical Role of B and T Lymphocyte Attenuator in Peripheral T Cell Tolerance Induction[1]

Xikui Liu[*], **Maria Alexiou**[‡], **Natalia Martin-Orozco**[*], **Yeonseok Chung**[*], **Roza I. Nurieva**[*], **Li Ma**[§], **Qiang Tian**[§], **George Kollias**[‡], **Sijie Lu**[†], **Daniel Graf**[‡,2], and **Chen Dong**[*,2]

[*] Department of Immunology, M.D. Anderson Cancer Center, Houston, TX 77030

[†] Department of Stem Cell Transplantation and Cellular Therapy, M.D. Anderson Cancer Center, Houston, TX 77030

[‡] Institute of Immunology, Biomedical Sciences Research Center "Alexander Fleming," Vari, Greece

[§] Institute for Systems Biology, Seattle, WA 98103

Abstract

T cell activation and tolerance are delicately regulated by costimulatory molecules. Although B and T lymphocyte attenuator (BTLA) has been shown as a negative regulator for T cell activation, its role in peripheral T cell tolerance induction in vivo has not been addressed. In this study, we generated a novel strain of BTLA-deficient mice and used three different models to characterize the function of BTLA in controlling T cell tolerance. In an oral tolerance model, BTLA-deficient mice were found resistant to the induction of T cell tolerance to an oral Ag. Moreover, compared with wild-type OT-II cells, $BTLA^{-/-}$ OT-II cells were less susceptible to tolerance induction by a high-dose OVA peptide administered i.v. Finally, $BTLA^{-/-}$ OT-I cells caused auto-immune diabetes in RIP-mOVA recipient mice. Our results thus demonstrate an important role for BTLA in the induction of peripheral tolerance of both $CD4^{+}$and $CD8^{+}$ T cells in vivo.

T cell activation and tolerance are tightly regulated by costimulatory molecules, especially those in the B7 and CD28 superfamilies (1,2). Some of these costimulatory molecules deliver critical negative signals that control the extent of T cell responses. For example, CTLA-4 inhibits T cell responses and regulates peripheral T cell tolerance (3). Also, PD-1 (programmed cell death 1), possibly by interacting with its ligands PD-L1 and PD-L2, has been shown to be a negative regulator of T cell activation and is crucial for maintaining immune tolerance (2).

[1]This work is supported in part by National Institutes of Health Grant AI054912 (to C.D.), a Specialized Center of Research sub-grant from the Leukemia and Lymphoma Society (to C.D.), and a grant from the Center for Target Therapy of M. D. Anderson Cancer Center (to C.D.). D.G. was a recipient of a Marie Curie fellowship, and D.G., G.K., and M.A. received support from MUGEN Network of Excellence 6FP (LSHG-CT-2005-005203). X.L. received a postdoctoral fellowship from the Arthritis Foundation, R.I.N. received an American Heart Association Scientist Development award, and C.D. received a Cancer Research Institute Investigator award, an American Lung Association Career Investigator award, a Leukemia and Lymphoma Society Scholar award, and a Trust Fellowship of the M. D. Anderson Cancer Center.

2Address correspondence and reprint requests to Dr. Chen Dong, Department of Immunology, M. D. Anderson Cancer Center, 7455 Fannin Street, Unit 902, Houston, TX 77030. cdong@mdanderson.org or Dr. Daniel Graf, Institute of Immunology, Biomedical Sciences Research Center 'Alexander Fleming,' 34 Al. Fleming Street, 166 72 Vari, Hellas/Greece. graf@fleming.gr.

Disclosures

The authors have no financial conflict of interest.

The B and T lymphocyte attenuator (BTLA)[3] has recently joined the CD28 family of negative regulators (4,5), which is broadly expressed on hemopoietic cells including $CD4^+$ cells, $CD8^+$ cells, B cells, dendritic cells, macrophages, and NK cells (6,7). In the peripheral lymphoid tissue, mature T cells expressed low level of BTLA and TCR ligation increased BTLA expression. Two groups independently identified herpesvirus entry mediator (HVEM) as the unique ligand for BTLA (8,9). Similarly as for BTLA, HVEM was also broadly expressed on cells of the immune system such as T and B lymphocytes, NK cells, and dendritic cells, but it was also expressed on endothelial cells (10).

BTLA-deficient $CD4^+$ and $CD8^+$ T cells were previously shown to be hypersensitive to TCR stimulation (5,7). BTLA-deficient mice have persistent inflammation of the lung in a model of acute allergic airway inflammation (11). Also, targeting BTLA or HVEM prompted rapid rejection in a cardiac transplant study (12). These results indicate an inhibitory effect of BTLA in controlling T cell activation.

In the current study, we examined the function of BTLA in peripheral tolerance by using a novel strain of BTLA knockout (KO) mice and several in vivo tolerance-induction models. We show a crucial role for BTLA in controlling peripheral T cell tolerance.

Materials and Methods

Mice

C57BL/6J, OT-I, and OT-II transgenic mice were purchased from The Jackson Laboratory. Rat insulin promoter (RIP)-membrane-bound OVA (mOVA) mice were kindly provided by W. Heath of the Walter and Eliza Hall Institute of Medical Research (Parkville, Australia). BTLA KO mice on a C57BL/6 background were crossed with OT-I or OT-II mice to get $BTLA^{-/-}$ OT-I and $BTLA^{-/-}$ OT-II mice. All mice were housed in the specific pathogen-free animal facility at M. D. Anderson Cancer Center, and the animal experiments were performed with protocols approved by Institutional Animal Care and Use Committee. Eight- to 12-wk-old mice were used in the experiments.

Reagents for flow cytometry

CD4-Percy5.5, CD25-PE, CD44-allophycocyanin, CD62L-FITC, CD8-Percy5.5, Vα2-PE, IFN-γ-allophycocyanin, and IL-2-allophycocyanin Abs were from BD Biosciences. PD1-FITC and BTLA-PE Abs and isotype controls were from eBioscience. The OT-I tetramer was synthesized as described (13). Cells were analyzed on a FACSCalibur cytometer (BD Biosciences).

Induction and assessment of oral tolerance

Wild-type (WT) and BTLA KO mice daily received intragastrically 2 mg of chicken OVA protein (grade V; Sigma-Aldrich) dissolved in PBS for a total of five times. Control mice were given PBS alone. Seven days after the last treatment, all mice were immunized s.c. with 100 μg of OVA protein emulsified in CFA. One week later, spleens were obtained from the mice and splenocytes were restimulated with OVA protein to measure IL-2 production, T cell proliferation, and effector cytokine production.

[3]Abbreviations used in this paper: BTLA, B and T lymphocyte attenuator; HVEM, herpesvirus entry mediator; KO, knockout; mOVA, membrane-bound OVA; PLN, pancreatic lymph node; RIP, rat insulin promoter; WT, wild type.

Peptide-induced tolerance

WT and BTLA$^{-/-}$ OT-II mice were injected i.v. with 500 μg of OVA peptide on days 0 and 3. Control mice were given PBS alone. Ten days later, the mice were sacrificed and splenocytes were harvested. CD4 T cells were isolated by staining with anti-CD4 MACS beads and an autoMACS cell separator (Miltenyi Biotec). The CD4^{+} cells were subsequently stimulated in vitro with anti-CD3, and IL-2 proliferation and IFN-γ production were measured.

Diabetes induction and measurement

CD8 cells from WT and BTLA$^{-/-}$ OT-I mice were purified using anti-CD8 Miltenyi beads and an autoMACS cell separator (Miltenyi Biotec). Five million cells were i.v. injected into RIP-mOVA recipient mice. Mice were monitored daily for urine glucose levels (Diastix; Bayer Pharmaceuticals), and high reads were confirmed by blood glucose measurements (Ascencia Elite; Bayer Pharmaceuticals). Diabetes was scored after three consecutive reads higher than 13.5 mM/L.

Proliferation and cytokine analysis

Purified OT-I cells were adjusted to 20 million cells/ml and labeled in RPMI 1640 containing 1% FBS and 10 μM CFSE for 15 min at 37°C. Cells were then washed twice with RPMI 1640 and 10% FBS and one time with PBS before injection into RIP-mOVA mice. For intracellular cytokines, cell suspensions were culture for 5 h with 5 μg/ml SIINFEKL peptide and 1 μl/ml GolgiPlug, and further intracellular staining was performed with the Cytofix/Cytoperm kit (BD Biosciences).

Results and Discussion

BTLA is highly expressed on tolerant T cells

We previously reported that T cells activated in the absence of CD28 and ICOS costimulation become tolerant (14). In our preliminary microarray analysis, we found that these tolerant T cells have significant up-regulated expression of BTLA compared with effector and naive T cells (data not shown). We examined BTLA levels by real-time PCR, and confirmed that tolerant T cells expressed the highest BTLA mRNA in comparison with naive and effector cells (Fig. 1*A*). Furthermore, the surface expression of BTLA proteins was also up-regulated in tolerant cells by flow cytometry (Fig. 1*B*). Because BTLA is highly up-regulated on tolerant T cells, we speculated that the BTLA signal might participate in T cell tolerance induction.

Generation and characterization of BTLA KO mice

To be able to examine the function of BTLA in vivo, we generated a BTLA KO mouse by removing part of the promoter as well as the complete exon 1, which had the signal peptide necessary for transmembrane integration. By Northern Blot analysis, we confirmed the complete absence of BTLA mRNA expression in our BTLA-deficient mice (data not shown). The BTLA-deficient mice had no obvious defects. We observed increased memory CD8^{+} cells in the KO mice (data not shown) as reported previously (15). There was no difference in the percentages of regulatory T cells in the thymus and spleen, and both WT and KO regulatory T cells suppressed naive T cell activation in vitro to the same level (data not shown). In keeping with previous findings (5,7), BTLA-deficient T cells showed a heightened response to anti-CD3 stimulation. However, both WT and KO naive T cells could be suppressed similarly by regulatory T cells (data not shown). These data argued that the hypersensitivity of T cells was not due to the dysfunction of regulatory T cells in KO mice.

Resistance to oral tolerance by BTLA KO mice

Oral tolerance is a form of peripheral tolerance in which Ag-specific T cell tolerance is induced against oral Ags (16). We thus examined the function of oral tolerance induction in both WT and KO mice. We found that spleen cells from OVA-fed WT mice exhibited reduced production of IL-2, reduced proliferation, and reduced secretion of IFN-γ upon restimulation as compared with the PBS-fed group (Fig. 2*A*), indicating that profound T cell tolerance had been induced on the OVA protein. In contrast, T cells from KO mice produced significant more amounts of IL-2 and IFN-γ and proliferated more robustly than those from WT mice (Fig. 2*A*). More importantly, T cells from OVA- and PBS-fed KO mice produced similar amounts of IL-2 and IFN-γ and proliferated to the same extent upon Ag stimulation. These data demonstrated that BTLA was essential for the induction and/or maintenance of oral tolerance and that deficiency in BTLA impairs tolerance induction through the oral tract.

BTLA deficiency is required for peptide-induced tolerance in CD4$^+$ T cells

To examine the relevance of BTLA in CD4$^+$ T cell tolerance, we used a peptide-induced tolerance model with OT-II TCR transgenic mice (17). We found that OVA-treated WT OT-II cells failed to proliferate and produced little IL-2 and IFN-γ (Fig. 2*B*). In contrast, OVA-treated BTLA$^{-/-}$ OT-II cells displayed considerable IL-2 production and cell proliferation after anti-CD3 restimulation (Fig. 2*B*). Despite this significant difference, BTLA$^{-/-}$ OT-II cells from OVA peptide-treated mice still exhibited greatly reduced proliferation and IL-2 expression, although they produced greater amounts of IFN-γ than those from PBS-injected mice, indicating that a molecule or molecules other than BTLA are also required for the induction of Ag-specific CD4$^+$ T cell tolerance in this model. Moreover, after similar OVA peptide treatment, BTLA$^{-/-}$ OT-II cells transferred into Ly5.1 congenic mice exhibited enhanced IL-2 production compared with WT OT-II cells in the same type of recipients (data not shown), indicating an important role for BTLA in T cells in tolerance induction.

BTLA protects against CD8$^+$ T cell-mediated autoimmunity

To study the role of BTLA in peripheral tolerance in CD8$^+$ T cells, we used the RIP-mOVA diabetic model (18). BTLA KO were bred with OT-I TCR transgenic mice, and the resulting BTLA$^{-/-}$ OT-I mice contained similar naive and memory populations in their CD8 cells (data not shown). Consistent with our previous report (19), control mice receiving WT OT-I cells did not develop diabetes for at least 20 days (Fig. 3*A*). However, up to 60% of the mice that received CD8$^+$ T cells from BTLA-deficient mice developed diabetes as early as 7 days after transfer (Fig. 3*A*). Pancreas from mice transferred with BTLA$^{-/-}$ OT-I cells showed aggressive insulitis with infiltration in the islets, whereas control mice showed only peri-insulitis or no infiltration (Fig. 3, *B* and *C*). Thus, BTLA is required for controlling the autoimmunity mediated by islet-specific CD8$^+$ T cells.

It has been shown that OT-I cells transferred into RIP-mOVA mice undergo deletion after a brief proliferation (20). We thus analyzed the fate of donor cells from various peripheral lymph nodes and spleens of recipient RIP-mOVA mice by staining with a MHC-I tetramer. Two weeks after the transfer, both groups of OT-I cells recovered from recipient mice expressed high levels of CD44, indicating that they were all Ag-experienced (data not shown). However, mice transferred with BTLA$^{-/-}$ OT-I cells contained two times more OT-I cells in pancreatic lymph nodes (PLN) compared with the control mice (Fig. 3*D*). Thus, BTLA signaling appears to mediate immune tolerance by restricting the expansion or survival of autoreactive CD8$^+$ T cells. Furthermore, the BTLA$^{-/-}$ OT-I T cells recovered from recipient mice produced more IFN-γ than those from control mice (data not shown), indicating that these cells were not tolerized. Interestingly, BTLA$^{-/-}$ OT-I cells recovered from PLN of RIP-mOVA mice expressed higher levels of PD-1 on their surface than those from control mice (data not shown). It has been shown that both BTLA and PD1 were necessary for controlling the duration of

allergic airway inflammation and MHC-mismatched allograft rejection (11,12); thus, it is reasonable to propose that BTLA and PD1 cooperate to control the autoimmunity in the pancreatic tissue. This idea is supported by our previous finding that PD-1 and PD-L1 interaction mediates OT-I cell tolerance induction in RIP-mOVA mice (19).

The above results indicate a critical role for BTLA in the control of autoimmune diabetes mediated by $CD8^+$ T cells. However, it is unclear whether BTLA regulates $CD8^+$ T cell tolerance during the initial priming or beyond. We thus tested the early phase when OT-I cells first encountered Ag-presenting dendritic cells in PLN. $CD8^+$ T cells from WT or BTLA KO OT-I mice were labeled with CFSE and subsequently injected i.v. into RIP-mOVA mice. We then evaluated the cell division of CFSE-labeled OT-I cells in various peripheral lymph nodes and spleen 55 h after their transfer into RIP-mOVA mice. At this stage, divisions of OT-I cells occurred primarily in PLNs; $BTLA^{-/-}$ OT-I cells proliferated much more than WT OT-I cells (Fig. 4*A*). In addition, the numbers of IFN-γ- and IL-2-producing cells were doubled in the absence of BTLA (Fig. 4, *B* and *C*). Interestingly, cytokine-expressing cells were among those exhibiting the highest numbers of cell division, which suggested a role for BTLA in controlling T cell activation programs. In summary, these results indicate that BTLA signaling controls the activation and proliferation of OT-I cells during the priming phase. Subsequently, these effector cells activated in the absence of BTLA exhibited an advantage in expansion and/or survival. Together, BTLA plays an important role in the peripheral tolerance of $CD8^+$ T cells.

It is interesting to note that in all of these three tolerance models, BTLA deficiency exerts its influence differently to a certain extent, indicating that BTLA is not simply a negative regulator (dampening signaling in general) but rather affects the expression of cytokines differentially and thus might fine tune immune responses. Indeed, the only ligand for BTLA is HVEM (8, 9). However, HVEM can bind to BTLA, LIGHT/LTβ (4), and CD160 (21), which adds another layer of complexity to the regulation of BTLA function.

The CD28 family negative costimulatory molecules BTLA, CTLA-4, and PD-1 thus all play roles in tolerance and autoimmunity control. These molecules may play nonredundant roles at various stags of T cell activation. For example, CTLA4 regulates naive T cell activation and PD1 becomes expressed after T cell activation (1). BTLA, however, is expressed on both naive and activated T cells, also highly on tolerant cells, and potentially regulates all phases of T cell activation. In contrast, BTLA may cooperate with CTLA-4 and PD-1 to control T cell tolerance and autoimmunity. Although at this stage, it is unclear about their specificity or redundancy, these inhibitory pathways may be targeted for controlling chronic infection or boosting tumor immunity.

Acknowledgments

We thank the entire Dong laboratory for help and discussions and Pamela Grant for help with histology.

References

1. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515–548. [PubMed: 15771580]
2. Martin-Orozco N, Dong C. Inhibitory costimulation and anti-tumor immunity. Semin Cancer Biol 2007;17:288–298. [PubMed: 17683946]
3. Chambers CA, Allison JP. Costimulatory regulation of T cell function. Curr Opin Cell Biol 1999;11:203–210. [PubMed: 10209159]
4. Murphy KM, Nelson CA, Sedy JR. Balancing co-stimulation and inhibition with BTLA and HVEM. Nat Rev Immunol 2006;6:671–681. [PubMed: 16932752]

5. Watanabe N, Gavrieli M, Sedy JR, Yang J, Fallarino F, Loftin SK, Hurchla MA, Zimmerman N, Sim J, Zang X, et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. Nat Immunol 2003;4:670–679. [PubMed: 12796776]
6. Hurchla MA, Sedy JR, Gavrieli M, Drake CG, Murphy TL, Murphy KM. B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic $CD4^+$ T cells. J Immunol 2005;174:3377–3385. [PubMed: 15749870]
7. Han P, Goularte OD, Rufner K, Wilkinson B, Kaye J. An inhibitory Ig superfamily protein expressed by lymphocytes and APCs is also an early marker of thymocyte positive selection. J Immunol 2004;172:5931–5939. [PubMed: 15128774]
8. Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hildner K, Scheu S, Pfeffer K, Ware CF, Murphy TL, Murphy KM. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. Nat Immunol 2005;6:90–98. [PubMed: 15568026]
9. Gonzalez LC, Loyet KM, Calemine-Fenaux J, Chauhan V, Wranik B, Ouyang W, Eaton DL. A coreceptor interaction between the CD28 and TNF receptor family members B and T lymphocyte attenuator and herpesvirus entry mediator. Proc Natl Acad Sci USA 2005;102:1116–1121. [PubMed: 15647361]
10. Morel Y, Truneh A, Sweet RW, Olive D, Costello RT. The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. J Immunol 2001;167:2479–2486. [PubMed: 11509586]
11. Deppong C, Juehne TI, Hurchla M, Friend LD, Shah DD, Rose CM, Bricker TL, Shornick LP, Crouch EC, Murphy TL, et al. Cutting edge: B and T lymphocyte attenuator and programmed death receptor-1 inhibitory receptors are required for termination of acute allergic airway inflammation. J Immunol 2006;176:3909–3913. [PubMed: 16547224]
12. Tao R, Wang L, Han R, Wang T, Ye Q, Honjo T, Murphy TL, Murphy KM, Hancock WW. Differential effects of B and T lymphocyte attenuator and programmed death-1 on acceptance of partially versus fully MHC-mismatched cardiac allografts. J Immunol 2005;175:5774–5782. [PubMed: 16237069]
13. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. Phenotypic analysis of antigen-specific T lymphocytes. Science 1996;274:94–96. [PubMed: 8810254]
14. Nurieva R, Thomas S, Nguyen T, Martin-Orozco N, Wang Y, Kaja MK, Yu XZ, Dong C. T-cell tolerance or function is determined by combinatorial costimulatory signals. EMBO J 2006;25:2623–2633. [PubMed: 16724117]
15. Krieg C, Boyman O, Fu YX, Kaye J. B and T lymphocyte attenuator regulates $CD8^+$ T cell-intrinsic homeostasis and memory cell generation. Nat Immunol 2007;8:162–171. [PubMed: 17206146]
16. Faria AM, Weiner HL. Oral tolerance. Immunol Rev 2005;206:232–259. [PubMed: 16048553]
17. Jeon MS, Atfield A, Venuprasad K, Krawczyk C, Sarao R, Elly C, Yang C, Arya S, Bachmaier K, Su L, et al. Essential role of the E3 ubiquitin ligase Cbl-b in T cell anergy induction. Immunity 2004;21:167–177. [PubMed: 15308098]
18. Kurts C, Carbone FR, Barnden M, Blanas E, Allison J, Heath WR, Miller JF. $CD4^+$ T cell help impairs $CD8^+$ T cell deletion induced by cross-presentation of self-antigens and favors autoimmunity. J Exp Med 1997;186:2057–2062. [PubMed: 9396776]
19. Martin-Orozco N, Wang YH, Yagita H, Dong C. Cutting edge: programmed death (PD) ligand-1/PD-1 interaction is required for $CD8^+$T cell tolerance to tissue antigens. J Immunol 2006;177:8291–8295. [PubMed: 17142723]
20. Kurts C, Kosaka H, Carbone FR, Miller JF, Heath WR. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive $CD8^+$ T cells. J Exp Med 1997;186:239–245. [PubMed: 9221753]
21. Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human $CD4^+$ T cells through interaction with herpesvirus entry mediator. Nat Immunol 2008;9:176–185. [PubMed: 18193050]

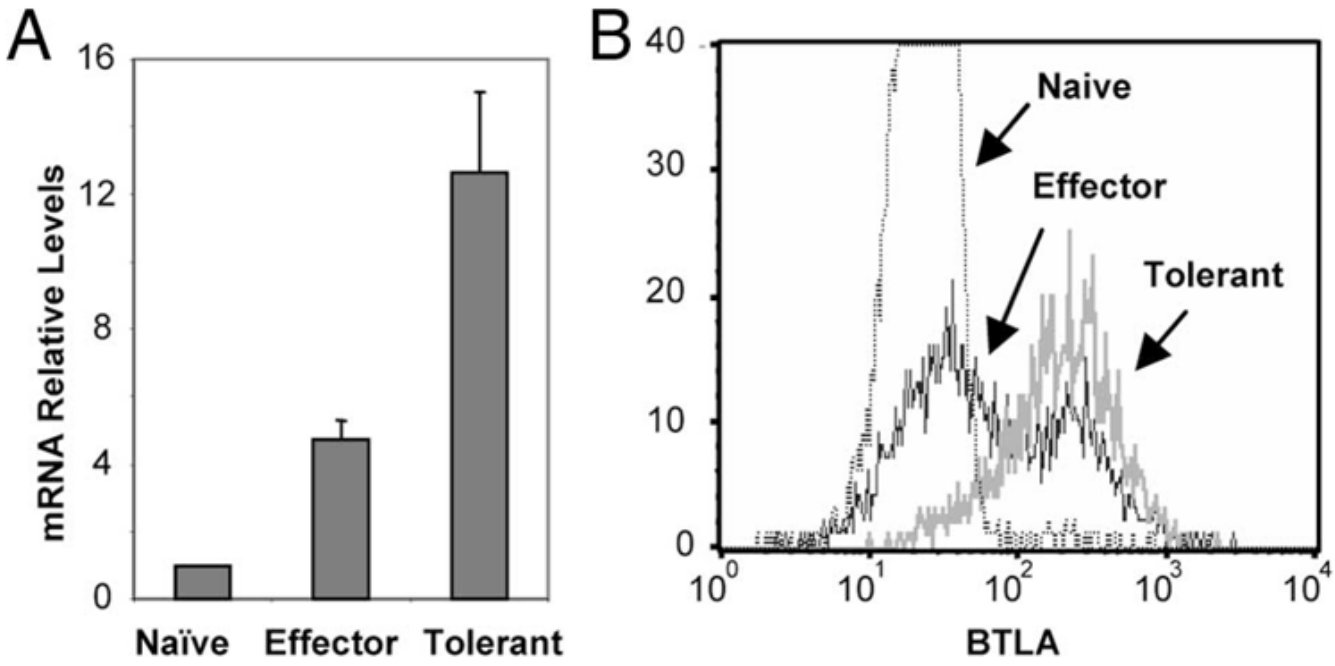

FIGURE 1.

Characterization of a novel strain of BTLA KO mice. *A* and *B*, Naive $CD4^+$ T cells were sorted by flow cytometry (gated on $CD4^+CD25^-$ $CD44^{low}CD62L^{high}$) and stimulated with anti-CD3 and splenic APC from WT or mice lacking B7.1, B7.2, and B7h to generate effector and tolerant T cells. After 4 days, cells were washed and restimulated with anti-CD3 for 5 h for mRNA (*A*) or 24 h for cell surface expression (*B*) measurement. *A*, Real-time PCR analysis was conducted and the expression levels in naive T cells were set at 1. *B*, BTLA protein levels on the cell surface of naive (dotted line), effector (dark line), and tolerant (gray line) T cells were examined by FACS staining and analyzed on gated live $CD4^+$ cells.

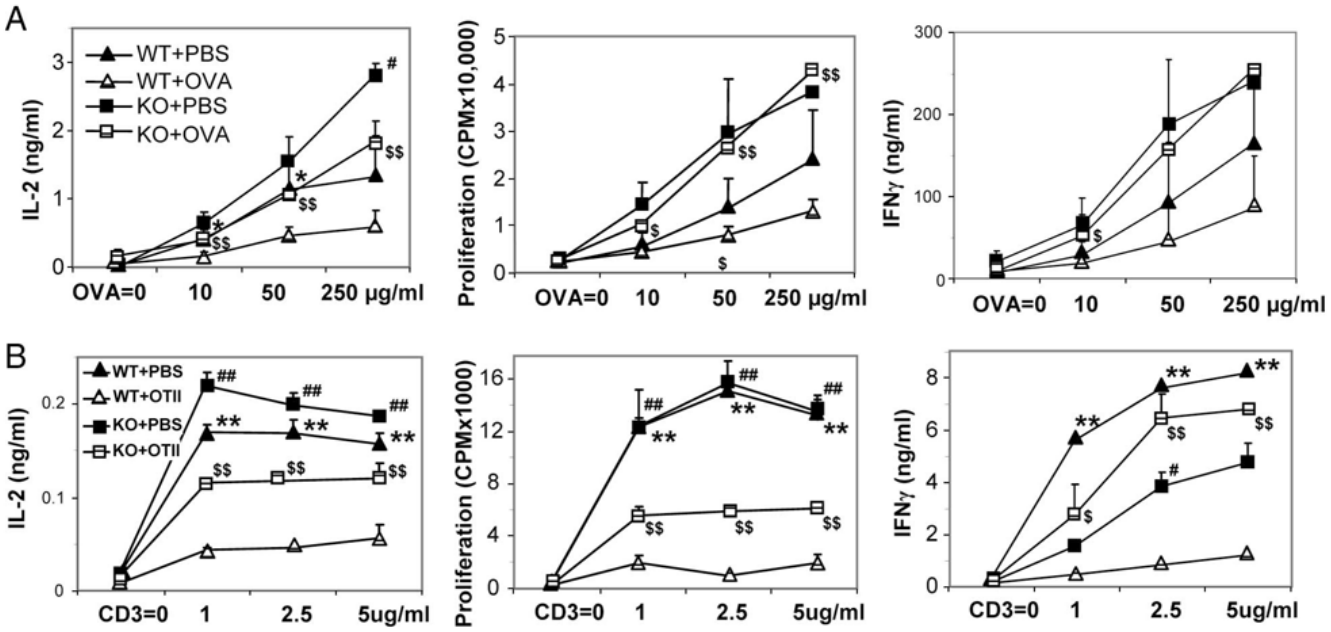

FIGURE 2.

BTLA regulates oral tolerance and peptide-induced tolerance. *A*, WT and BTLA KO mice were fed five times with OVA protein or PBS and subsequently immunized with OVA protein in CFA. Seven days later, splenocytes were stimulated with OVA protein. Data are representative of two individual experiments. Each experimental group consisted of five mice. *B*, WT OT-II and $BTLA^{-/-}$ OT-II mice were injected i.v. with OVA peptide or PBS twice. On day 10, $CD4^+$ T cells were isolated and stimulated in vitro with CD3. IL-2 were measured at 24 h, IFN-γ and proliferation were measured at 72 h with [^{3}H]thymidine added at the last 7 h of culture. The data shown are representative of two individual experiments. *p* values were calculated with Student's *t* test. *, $p < 0.05$ and **, $p < 0.01$ for WT-PBS vs WT-OVA (or WT-OT-II); #, $p < 0.05$ and ##, $p < 0.01$ for KO-PBS vs KO-OVA (or KO-OT-II); $, $p < 0.05$ and $$, $p < 0.01$ for WT-OVA (or WT-OT-II) vs KO-OVA (or KO-OT-II).

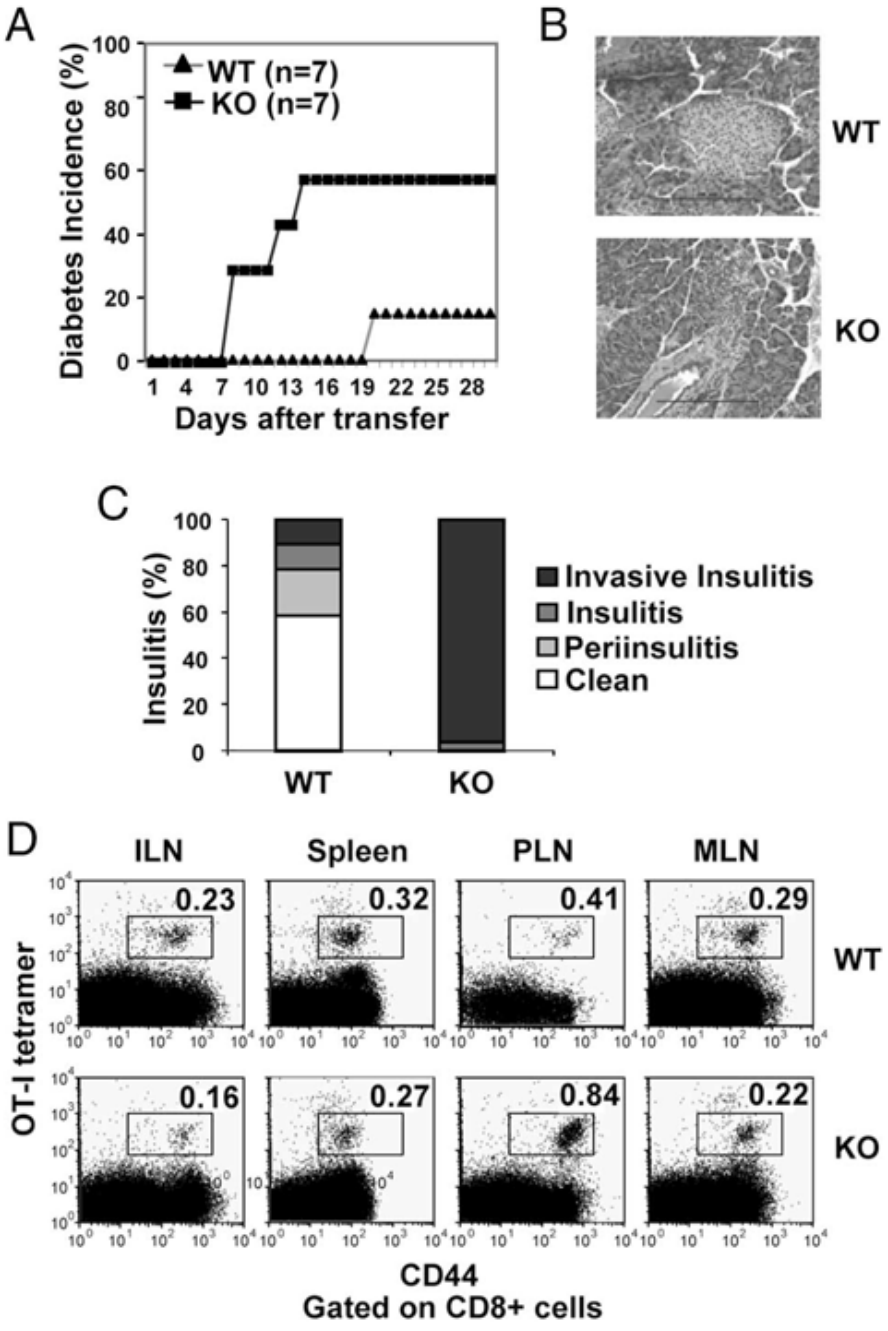

FIGURE 3.

BTLA deficiency results in CD8 T cell-mediated autoimmune diabetes. *A*, Purified WT and BTLA$^{-/-}$ OT-I (KO) cells were transferred into RIP-mOVA mice and the disease process was monitored by diabetes incidence. The results are compiled from two independent experiments with three or four mice per group. *B* and *C*, H&E-stained pancreas sections of RIP-mOVA recipient mice (B) and quantification of infiltrated islets from pancreas of RIP-mOVA recipient mice (*C*). *D*, Two weeks after the transfer, PLN, inguinal LN (ILN), mesenteric LN (MLN), and spleens were analyzed for the presence of OT-I cells with SIINFEKEL tetramers. Dot plots represent gated CD8 cells. Data are representative of two individual experiments.

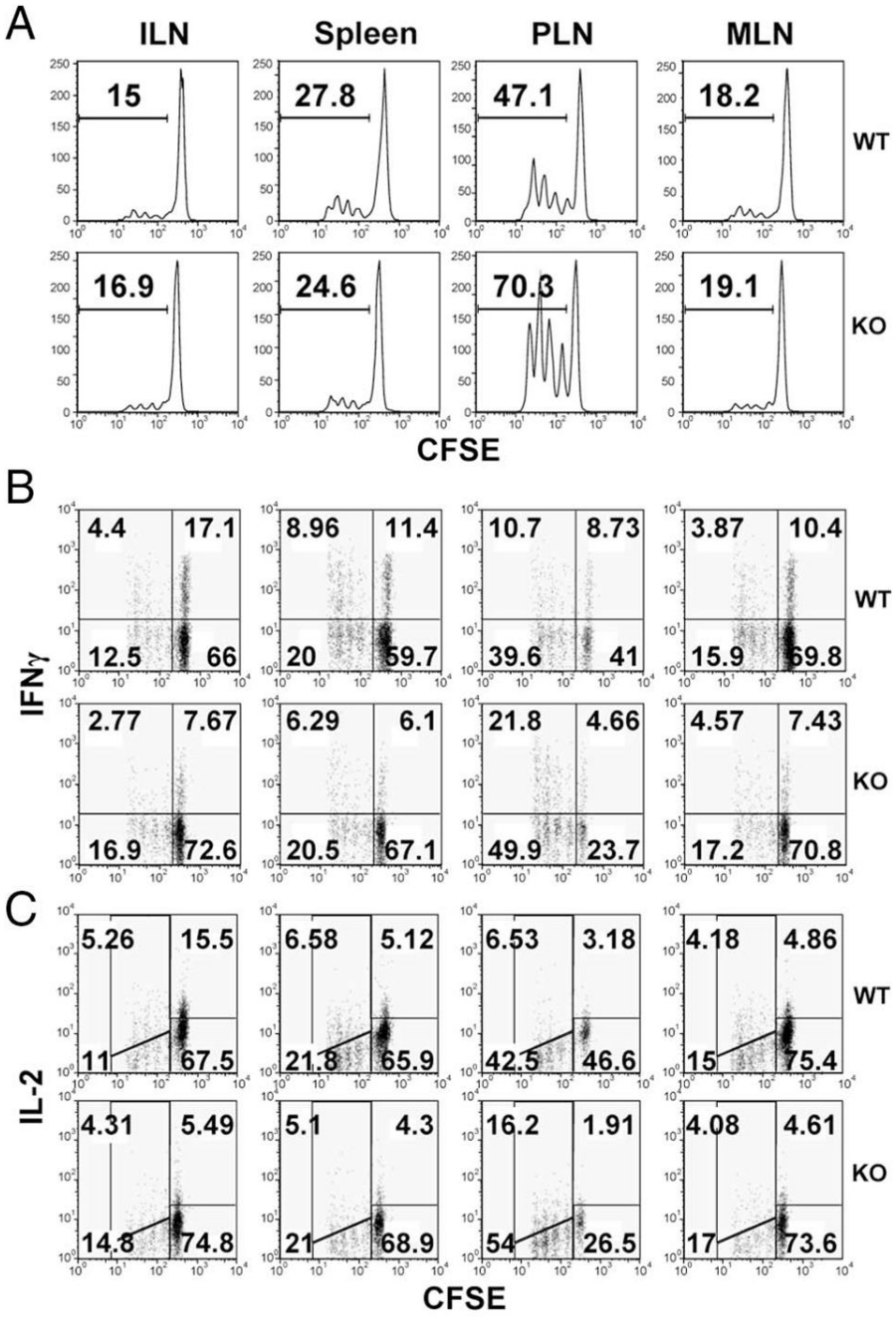

FIGURE 4.

BTLA controls effector differentiation of autoreactive CD8 T cells. Purified WT and BTLA$^{-/-}$OT-I cells were labeled with CFSE and transferred into RIP-mOVA mice, and 50 h after the transfer the proliferation (*A*), IFN-γ (*B*), and IL-2 (*C*) of OT-I cells were assessed. The numbers in the dot plots represent the percentage of dividing cells. Data are representative of two individual experiments.