Enhanced Immune Responses in Transgenic Mice Expressing a Truncated Form of the Lymphocyte Semaphorin CD100

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Enhanced Immune Responses in Transgenic Mice Expressing a Truncated Form of the Lymphocyte Semaphorin CD100

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CD100/Sema4D is a 150-kDa transmembrane protein that belongs to the semaphorin family. Binding of CD100 to CD72 enhances the immune response by turning off the negative signaling effects of CD72. To investigate the physiological functions of CD100 in vivo, we generated transgenic mice expressing a truncated form of CD100. A large amount of the soluble form of CD100 was detected in the sera of mice expressing a truncated form of CD100, although the amount of CD100 was only slightly elevated on the surface of B cells. In the mutant mice the development of conventional B and T cells appeared normal in terms of the surface marker phenotypes, while the number of CD5+ B-1 cells in the peritoneal cavity increased in comparison with wild-type mice. In vitro proliferation and Ig production of B cells in response to CD40 stimulation were considerably enhanced in mice expressing a truncated form of CD100. Additionally, in vivo both Ab responses against T cell-dependent Ags and generation of Ag-specific T cells were enhanced. Furthermore, introduction of the CD100-transgene could restore in vitro B cell responses as well as in vivo Ab production against T cell-dependent Ag in CD100-deficient mice. Collectively, these results not only indicate that CD100 has an important role in the immune system, but also that the soluble form of CD100 released from the cell surface can exert functions in vivo. The Journal of Immunology, 2001, 167: 4321–4328.

Although CD100 is a semaphorin member of the semaphorin family, which consists of a large number of phylogenetically conserved proteins, carrying a large Sema domain (∼500 aa residues) in their extracellular regions (1, 2). Many members of the semaphorin family are known to play important roles in axon guidance, acting as chemorepulsion factors during neuronal development (2–6). Among the semaphorin family, CD100 is the first semaphorin member, for which physiological expression has been identified in the immune system (7). CD100 mRNA has been detected in large quantities in lymphoid organs, including spleen, thymus, and lymph nodes (8, 9). Although the expression of CD100 is already abundant in resting T cells and is weak in resting B cells, it is significantly up-regulated in both T and B cells following activation of the respective cells (10–12).

Ab cross-linking of CD100 has been shown to provide proliferative signals to human peripheral blood T lymphocytes in the presence of submitogenic doses of CD3 or CD2 Abs (7, 10). In addition, tyrosine phosphatases, including CD45, have been shown to be associated with CD100 in either T or B cells (12, 13). Furthermore, a serine/threonine kinase activity has been reported to be associated with the cytoplasmic portion of CD100 (14, 15). These findings suggest that CD100 may function as a receptor.

The 150-kDa transmembrane protein CD100 belongs to the semaphorin family, which consists of a large number of phylogenetically conserved proteins, carrying a large Sema domain (∼500 aa residues) in their extracellular regions (1, 2). Many members of the semaphorin family are known to play important roles in axon guidance, acting as chemorepulsion factors during neuronal development (2–6). Among the semaphorin family, CD100 is the first semaphorin member, for which physiological expression has been identified in the immune system (7). CD100 mRNA has been detected in large quantities in lymphoid organs, including spleen, thymus, and lymph nodes (8, 9). Although the expression of CD100 is already abundant in resting T cells and is weak in resting B cells, it is significantly up-regulated in both T and B cells following activation of the respective cells (10–12).

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receptor (BCR)-mediated cell death, 2) promote B cell survival and proliferation, and 3) enhance the production and release of CD23 in B cells (22–26). Many of the effects due to CD72 mAbs, which appear to be very similar to those due to CD100 stimulation, imply that CD72 transmits positive signals for B cell activation (8, 11). On the other hand, recent findings suggest a potential role of CD72 as a negative regulator of B cell responses. Indeed, the cytoplasmic domain of CD72 has two immunoreceptor tyrosine-based inhibitory motifs. Adachi et al. (27) have shown that cross-linking of BCR enhances the phosphorylation of tyrosines on CD72 and association of CD72 with the protein tyrosine phosphatase Src homology 2 domain containing tyrosine phosphatase-1 (SHP-1), suggesting that CD72 might negatively regulate B cell activation. Consistent with this finding, B cells from CD72-deficient mice become hyperproliferative in response to various stimuli (28). These contradicting findings raise the question of how the positive outputs are generated from the binding of CD100 to CD72. Recently, we have demonstrated that CD100 binding induces the dephosphorylation of tyrosines on CD72 and the dissociation of SHP-1 from the latter. Therefore, CD100 appears to turn off the negative signaling effects of CD72, resulting in an enhancement of B cell activation (11). Indeed, CD100-deficient mice displayed hyporesponsiveness of B cells and impaired B-1 cell development, which are almost opposite the phenotypes of CD72-deficient mice (21, 28).

In the present study, to elucidate in vivo functions of CD100 as a ligand and to clarify a role of the cytoplasmic tail in the expression and functions of CD100, we generated transgenic mice expressing a truncated form of CD100 (CD100-Tg). The results indicate that CD100 is critically involved in the regulation of B-1 cell development and immune responses and suggest the importance of sCD100 in regulating immune reactions.

Materials and Methods
Construction of the transgene and production of transgenic mice
A truncated form of CD100 cDNA was prepared by PCR from a full-length mouse CD100 cDNA derived from CD40-stimulated WEHI-231 cells (11). A pair of oligonucleotide primers made of a sense sequence including a Sall site (5'-GCTTCCCAAGCATGACTGAGGACCTTCTTCTTCTC-3') and an antisense sequence including a XhoI site and a Flag sequence (DYKDDDDK) (5'-CACTAGGACTCCAGGATCTGAGTTTCTGTCGTTGAGGAGGTAAGGAGG-3') and an antisense sequence were used. The resulting mutant CD100 cDNA contained the complete extracellular and transmembrane regions, but not the intracellular region. The Sall-XhoI cDNA fragment was inserted into the unique XhoI site of the rabbit β-globin gene in the pBluescript II KS+ vector, which carries the enhancer and promoter of the Ig heavy chain VH4C8, and 3'-untranslated rabbit β-globin gene. The vector was linearized by the Sall and NotI restriction enzymes and microinjected into the pronuclei of fertilized C57BL/6 mouse eggs. Offspring from the microinjected eggs were selected by PCR and Southern blot analysis.

The following oligonucleotide primers were used to identify endogenous CD100 expression or transgenic CD100 expression. Primer 1 (5'-TGG TAGACAGGACCCAG-3') was made of a sense sequence, and primer 2 (5'-TCGTCGTCCTGTCAGTCCCATC-3') was made of an antisense sequence complementary to cDNA of CD100. Primer 3 (5'-TCGTCGTCCTGTCAGTCCATC-3') was made of an antisense sequence complementary to a Flag sequence. Primer 1 was used in conjugation with primer 2 to identify the endogenous CD100 expression and was used in conjugation with primer 3 to identify the transgene. Two lines (lines 6 and 22) were established. The copy number of the transgenes was determined by Southern blot analysis of tail DNA. To introduce the transgene into a CD100-null background, the transgenic mice were backcrossed with CD100-deficient mice (21). All these mice were maintained in a pathogen-free environment.

Real-time monitoring quantitative PCR (QPCR)
The relative abundance of CD100 and GAPDH mRNAs was assessed by real-time monitoring QPCR. Briefly, B220+ B cells or Thy1+ T cells were purified from the spleen by MACS (Miltenyi Biotech, Bergisch Gladbach, Germany). The purity of the cells was >95%. Total RNA was isolated from B220+ B cells or Thy1+ T cells using Isoegen (Nippon Gene, Toyama, Japan). Total RNA was treated with DNase I to remove any contaminating genomic DNA before RT. Then, cDNA were synthesized using SuperScript II reverse transcriptase (Life Technologies). Primer 4′5′F (5′-AGCTTTCCGAGGACCGGATGTG-3′) was used in conjugation with primer 719-R (5′-CACATTGGCCGTATCTCGAC-3′) to amplify a 76-bp fragment in the transmembrane region of CD100. A TaqMan probe, 5′-Fam (6-carboxy fluorescein)-CCCCTTGGCTACGAGCTTACGT-3′ TAMRA (6-carboxy-tetramethylrhodamine), was included during QPCR. PCR primers and TaqMan probe for GAPDH were obtained from PerkinElmer (Norwalk, CT). QPCR amplification was conducted in triplicate in 50-μl reaction volumes consisting of TaqMan Universal PCR Master Mix, 900 nM of each primer, and 250 nM TaqMan probe. CDNA (500 ng) was amplified according to the thermal profile of 50°C for 2 min and 95°C for 10 min (95°C for 15 s, 60°C for 1 min) for 40 cycles and incubated at 25°C using an ABI 7700 (Applied Biosystems, Foster City, CA). Quantification was performed using software from ABI 7700 sequence detection systems.

Flow cytometry and Abs
Single-cell suspensions were prepared from mouse spleen (8–12 wk old). Bone marrow cells were obtained from one tibia and one femur. Peritoneal cavity cells were collected by flushing the peritoneal cavity with PBS containing 2% FCS and 10 U/ml heparin. One million cells of the various tissues were used for the staining with the following antibodies: anti-CD3 (145-2C11), anti-CD4 (GK1.5), and anti-CD100 (BMA-12) conjugated with FITC, PE, or biotin. Streptavidin-FITC, -PE, and -allophycocyanin were used as second-step reagents for biotinylated Abs. These Abs and reagents, except for anti-CD4 and anti-CD100, were purchased from BD PharMingen (San Diego, CA). Goat anti-IgD was purchased from Nordic Immunological Laboratories (Tilburg, The Netherlands), and anti-CD100 (BMA-12) was established at our laboratory (11). Data analysis was performed using FlowJo software (Treestar, San Carlos, CA).

Serum sCD100 assays
The levels of serum sCD100 were determined by sandwich ELISA as previously described (17). Ninety-six-well microplates (Maxisorp Nunc-Immuno plate; Nunc, Roskilde, Denmark) were coated with BMA-12 (100 μl of 5 μg/ml in 0.1 mM NaHCO3, pH 9.0) at 4°C overnight. The wells were incubated in a blocking solution (200 μl of 50 mM Tris-HCl (pH 8.1), 1 mM MgCl2, 0.15 M NaCl, 1% BSA, and 0.05% Tween 20) for 1 h at room temperature. Samples and controls (recombinant sCD100 protein consisting of the extracellular region of the mouse CD100 fused with a Flag peptide) (11) diluted with the blocking solution were added to the wells (50 μl/well) and were incubated to determine the concentration of sCD100. Samples and controls diluted with the blocking solution were added to the wells (50 μl/well) and incubated for 2 h at room temperature. The wells were washed three times with PBS containing 0.05% Tween 20 and incubated with biotinylated BMA-8 (2 μg/ml) for 2 h at room temperature (11). The incubation continued with alkaline phosphatase-conjugated streptavidin and was subsequently developed by phosphatase substrate (Sigma-Troestan-104; Sigma, St. Louis, MO). Absorbance values were obtained at 405 nm (correction wavelength set at 620 nm).

Assays for proliferation and Ig production
Nonadherent splen B cells were isolated with a combination of anti-Thy-1.2 (FTDS, Serotec, Oxford, U.K.) and rabbit complement (Wako Pure Chemical Industries, Osaka, Japan) to remove T cells. The remaining B cells were fractionated with a Percoll gradient of 50, 60, 66, and 70%, and cells at the interface between 66 and 70% were collected. The purity of the resting B cell preparations was >90%. Purified B cells (1 × 107 cells/well) were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% FCS (Life Technologies), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 2-ME (5 × 10-5 M) in the presence or the absence of IL-4 (Genzyme, Cambridge, MA), CD40 mAb (HM40-3; BD PharMingen), or 0.2 μg/ml LPS (Sigma) in 96-well microtiter plates. For B cell proliferation assays, cultures were pulsed with 2 μCi of [3H]thymidine for the last 16 h of culture period, and the incorporation of [3H]thymidine was measured. For Ig production assays, culture supernatants were harvested with 10 U/ml IL-4 and 0.5 μg/ml CD40 mAb were harvested from 7-day cultures, and the levels of Ig were measured by ELISA (29).

Immunization and serum Ab assays
To induce Ab responses to TD Ag, 8- to 12-wk-old mice were immunized i.p. with 100 μg of 4-hydroxy-3-nitrophenoxyacetyl-chicken γ-globulin conjugates (NP-βCGG) as an alum-precipitated complex on day 0, boosted on...
The transmembrane region (TM; □), the coding sequence of the truncated mouse CD100 cDNA including the extracellular region (EC; □), the transmembrane region (TM; □), and the rabbit β-globin 3′-untranslated region (□). B, Real-time monitoring QPCR analysis for expression of the transgene. Total RNA was isolated from splenic T and B cells of wild-type (□) or CD100-Tg (■) mice and then treated with DNase I to exclude genomic DNA. One pair of primers with an internal probe was used to quantify the levels of CD100 expression as described in Materials and Methods. CD100 relative expression levels to GAPDH were displayed as CD100/GAPDH ratio. C, Expression levels of CD100 on B and T cells of CD100-Tg mice. Splenocytes from wild-type (dotted line) or CD100-Tg mice (thick line) were stained with biotinylated anti-mouse CD100 mAb (BMA-12) or biotinylated isotype-matched controls (thin line) plus PE-conjugated streptavidin and FITC-conjugated anti-B220 mAb/FITC-conjugated anti-Thy-1. B220- or Thy-1-positive cells were gated for the analysis of CD100 expression by flow cytometry. D, Elevated levels of sCD100 in sera of CD100-Tg mice. Sera from CD100-Tg mice at 8–12 wk of age were collected, and the concentrations of serum sCD100 were measured by ELISA as described in Materials and Methods. The horizontal lines represent the mean value for each group.

Development of lymphocytes in CD100-Tg mice

We first performed flow cytometric analyses on cells from the bone marrow, spleen, and peritoneal cavity of CD100-Tg mice to

Results

Generation of transgenic mice expressing CD100

The expression of CD100 is relatively weak in resting B cells, but is up-regulated upon activation (10–12). To investigate the effect of increased CD100 expression on immune responses, we generated transgenic mice expressing increased levels of CD100. As shown in Fig. 1A, the transgene contains the cDNA sequence encoding the extracellular and transmembrane regions of CD100, but not the cytoplasmic region, next to the β enhancer and the promoter of Ig heavy chain gene. Offspring from eggs in which the fragment was microinjected were selected by PCR. Among several lines of mice carrying the transgene, two lines (lines 6 and 22) with higher expression of the transgene were selected by RT-PCR. Since these two lines showed essentially the same phenotypes, line 6 was subjected to further detailed analysis as described below.

From Southern blot analysis, ~20 copies of the transgenes were found to be present in this transgenic line. The real-time monitoring QPCR revealed that B cells and T cells from CD100-Tg mice expressed ~8.8- and 2.7-fold more CD100 mRNA than those from wild-type mice, respectively (Fig. 1B). However, only a slight increase in CD100 expression was observed on the cell surface of CD100-Tg B cells (Fig. 1C). We could not detect any increase in CD100 expression on the cell surface of T cells. Interestingly, a large amount of sCD100 was detected in sera of CD100-Tg mice, but not of wild-type littermates (Fig. 1D). We have previously shown that the extracellular region of CD100 on the surface of lymphocytes was cleaved and released in the form of sCD100 upon activation (17). It thus appears that a truncated form of CD100 is unstable on the surface of lymphocytes and is easily cleaved and released from the surface of lymphocytes without stimulation, resulting in the accumulation of sCD100 in sera of CD100-Tg mice.

FIGURE 1. Generation of CD100-Tg mice. A, Schematic structures of the transgenic constructs. Functional elements include the enhancer μ (■), the promoter of the Ig heavy chain VH4C8 (□), the coding sequence of the truncated mouse CD100 cDNA including the extracellular region (EC; □), the transmembrane region (TM; □), and the rabbit β-globin 3′-untranslated region (□). B, Real-time monitoring QPCR analysis for expression of the transgene. Total RNA was isolated from splenic T and B cells of wild-type (□) or CD100-Tg (■) mice and then treated with DNase I to exclude genomic DNA. One pair of primers with an internal probe was used to quantify the levels of CD100 expression as described in Materials and Methods. CD100 relative expression levels to GAPDH were displayed as CD100/GAPDH ratio. C, Expression levels of CD100 on B and T cells of CD100-Tg mice. Splenocytes from wild-type (dotted line) or CD100-Tg mice (thick line) were stained with biotinylated anti-mouse CD100 mAb (BMA-12) or biotinylated isotype-matched controls (thin line) plus PE-conjugated streptavidin and FITC-conjugated anti-B220 mAb/FITC-conjugated anti-Thy-1. B220- or Thy-1-positive cells were gated for the analysis of CD100 expression by flow cytometry. D, Elevated levels of sCD100 in sera of CD100-Tg mice. Sera from CD100-Tg mice at 8–12 wk of age were collected, and the concentrations of serum sCD100 were measured by ELISA as described in Materials and Methods. The horizontal lines represent the mean value for each group.

T cell priming

Mice were immunized by injecting 1, 10, or 100 µg of keyhole limpet hemocyanin (KLH) in CFA into the hind footpads. Nine days after priming, CD4+ T cells were purified from the draining lymph nodes using MACS, and 1 × 106 cells were stimulated for 72 h with various concentrations of KLH in the presence of irradiated (3000 rad) splenocytes (5 × 105) of wild-type littermates in 96-well microtiter plates. For proliferation assays, cells were pulsed with 2 Ci of [3H]thymidine for the last 12 h.

Levels of IL-4 and IFN-γ in the culture supernatants were measured using ELISA kits (R&D Systems, Minneapolis, MN).

FIGURE 2. Flow cytometric analysis of lymphocytes from CD100-Tg and wild-type mice. Single-cell suspensions from bone marrow, spleen, and peritoneal cavity were isolated from CD100-Tg and wild-type littermates and stained with the following combinations. A, FITC-conjugated anti-CD43 and PE-conjugated anti-B220; B, bone marrow cells were stained with FITC-conjugated anti-IgD, PE-conjugated anti-B220, and biotinylated anti-IgM plus allopurinocyanin-conjugated streptavidin, then B220-positive cells were gated for further analysis of surface expression of IgM and IgD. C, FITC-conjugated anti-IgD and biotinylated anti-IgM plus PE-conjugated streptavidin. D, FITC-conjugated anti-CD8 and PE-conjugated anti-CD4. E, FITC-conjugated anti-B220 and PE-conjugated anti-Thy-1.2. F, FITC-conjugated anti-B220 and PE-conjugated anti-CD5. The results shown are representative of five independent experiments. Gates are indicated by boxes, and the percentage of gated cells over total cells is indicated within each plot.
examine the development of lymphocytes. The mutant mice had relatively increased numbers of CD5<sup>B220</sup> B-1 cells (wild type, 13.7 ± 2.0%; CD100-Tg, 17.2 ± 1.0%; p < 0.05; Fig. 2F). This is in good agreement with our previous observations in CD100-deficient mice, where the number of B-1 cells was reduced (21), suggesting a role of CD100 in the development of B-1 cells. We also analyzed the subpopulations of B-1, B-1a (CD5<sup>B220</sup>B<sub>Mac-1</sub>) and B-1b (CD5<sup>B220</sup>B<sub>Mac-1</sub>) cells by three-color flow cytometric analysis. B-1a cells were found to increase in CD100-Tg mice (wild type, 9.4 ± 2.9%; CD100-Tg, 14.7 ± 1.9%; p < 0.05). However, we could not detect significant changes in B-1b cells in CD100-Tg mice (wild type, 4.2 ± 1.7%; CD100-Tg, 2.5 ± 0.8%). Except for these phenotypic changes, we did not observe any other differences in the surface phenotypes (Fig. 2, A–E), numbers, and ratios of T and B cells between CD100-Tg mice and wild-type mice (data not shown). T cells and conventional B cells appeared to develop normally in the transgenic mice.

In vitro responses of lymphocytes in CD100-Tg B cells

CD100 stimulation synergistically enhances CD40-induced B cell proliferation and Ig production (8, 11). CD100-deficient B cells show hyporesponsiveness to various stimuli (21). To examine the influence of increased CD100 expression on B cell responses, small resting spleen B cells were purified from CD100-Tg or wild-type mice and stimulated with various stimuli. As shown in Fig. 3A, B cells from CD100-Tg mice showed enhanced proliferative responses to CD40 mAb compared with those from wild-type mice, although the differences were obscured when cells were stimulated with a high dose of IL-4. CD100-Tg B cells also showed enhanced responses to LPS stimulation (Fig. 3B). In addition, in vitro IgG1 production of B cells induced by the combination of CD40 mAb and IL-4 was elevated in CD100-Tg mice (Fig. 3C). These results are inconsistent with our previous observations that either sCD100- or CD100-expressing transfectants enhance in vitro B cell responses (11, 17).

Ab responses of CD100-Tg mice

To explore the influence of the excessive CD100 on in vivo Ab responses, CD100-Tg mice were immunized with a TD Ag, NP-CGG. Although the expression of the transgene did not have so much effect on the primary Ab responses, Ab titers were still sustained at the later phase of primary responses (day 28). The effects

![Image of graphs showing in vitro responses of lymphocytes in CD100-Tg B cells](http://www.jimmunol.org/)

**FIGURE 3.** In vitro B cell responses of CD100-Tg mice. A, Enhanced proliferative B cell responses in CD100-Tg mice. Small resting B cells were purified from wild-type (○) or CD100-Tg (●) mice and cultured with or without CD40 mAb (0, 0.1, 0.4, and 1 μg/ml) and IL-4 (0, 2, and 20 U/ml) for 24 or 72 h. Cells were pulsed with 2 μCi of [3H]thymidine for the last 16 h. B, Enhanced proliferative B cell responses to LPS in CD100-Tg mice. Small resting B cells were purified from wild-type (○) or CD100-Tg (●) mice and cultured with or without LPS (0.2 μg/ml) as indicated for 24 or 72 h. Cells were pulsed with 2 μCi of [3H]thymidine for the last 16 h. C, Enhanced in vitro Ig production induced by CD40 mAb plus IL-4 in CD100-Tg mice. Small resting B cells were purified from CD100-Tg (●) or wild-type littermates (○) were cultured with or without CD40 mAb (0.5 μg/ml) and IL-4 (10 U/ml) for 7 days. Ig production was measured by ELISA as described in Materials and Methods. *, p < 0.05; **, p < 0.01; ***, p < 0.005. Each value was analyzed by unpaired t test.
of the transgene were much more obvious in the secondary immune responses (Fig. 4). Particularly, Ab responses persisted for a long period in CD100-Tg mice. We next examined the Ab responses against TI Ags: TNP-LPS as a type I TI-Ag (TI-1) and NP-Ficoll as a type II TI-Ag (TI-2). We could not observe clear differences in comparable Ab responses against TNP-LPS between CD100-Tg and wild-type mice (Fig. 5A), although Ab titers fluctuated among individual mice. On the other hand, CD100-Tg mice mounted relatively reduced responses against NP-Ficoll, particularly in the IgG1 Ab response (Fig. 5B), suggesting that CD100 may modulate BCR-mediated signaling.

In vivo T cell priming in CD100-Tg mice

We examined the generation of Ag-specific T cells in CD100-Tg mice because CD100-deficient mice exhibited severe impairments of T cell priming (21). We immunized wild-type or CD100-Tg mice with a low dose (1 µg/mouse; Fig. 6A), an intermediate dose (10 µg/mouse; Fig. 6B), or a high dose (100 µg/mouse; Fig. 6C) of KLH in CFA s.c. in the hind foot pad. Nine days after immunization, CD4+ T cells were prepared from the draining lymph nodes and stimulated with various concentrations of KLH in the presence of APC from normal mice. Enhancement of Ag-induced proliferation and cytokine production of CD4+ T cells was observed in CD100-Tg mice when immunized with a low dose of KLH. Enhanced IFN-γ production was still observed in CD100-Tg mice immunized with an intermediate dose of KLH, although there was no difference when mice were immunized with a high dose of Ags. Taken together, these findings suggest that excessive amounts of sCD100 result in enhancement of T cell priming in vivo when the mice are immunized with suboptimal doses of Ags.

CD100-Tg restored CD100 deficiency

To test whether the transgenic expression of CD100 can restore the impaired in vitro and in vivo B cell responses in CD100-deficient mice, CD100-Tg mice were backcrossed with CD100-deficient mice. As expected, the transgenic expression of CD100 could restore in vitro responses of CD100-deficient B cells to various stimuli (Fig. 7A). When CD100-Tg mice in the CD100-deficient background were immunized with a TD Ag, NP-CGG, they produced Ag-specific Abs comparable to those of wild-type mice (Fig. 7B). This result confirms the involvement of CD100 in immune responses and suggests the importance of elevated amounts of sCD100 in immune reactions.

Discussion

Expression of truncated and soluble forms of CD100 enhances immune responses

Previous studies suggest that CD100 may exert its functions as both a ligand and a receptor (7, 8, 10, 11, 14–17). To study the functions of CD100 as a ligand and the role of its cytoplasmic tail in functions and expression of CD100, we generated transgenic mice expressing the truncated CD100 lacking its cytoplasmic region. A large amount of sCD100 was observed in sera of CD100-Tg mice, although the expression of transgenic CD100 on B cells was only slightly elevated. These findings suggest that a truncated form of CD100 may be rapidly cleaved and shed from the cell surface. A serine kinase activity has previously been shown to be associated with the cytoplasmic tail of CD100 (14). In addition, it has recently been reported that the release of sCD100 is significantly enhanced by a serine kinase inhibitor (15). Taken together, all these findings suggest that the cytoplasmic tail of CD100 plays a role in the regulation of proteolytic cleavage of its extracellular domain.
FIGURE 6. Enhanced generation of Ag-specific T cells in CD100-Tg mice. In vitro responses of CD4+ T cells from draining lymph nodes. Wild-type (○) and CD100-Tg (●) mice were immunized with a low dose (1 μg/mouse; A), an intermediate dose (10 μg/mouse; B) or a high dose (100 μg/mouse; C) of KLH in CFA in the hind foot pad. Nine days after priming, CD4+ T cells were prepared from the draining lymph nodes and stimulated with various concentrations of KLH in the presence of irradiated (3000 rad) APCs from wild-type mice for 3 days. Cells were pulsed with 3-day culture supernatants were measured by ELISA.

CD100-Tg mice displayed an increased number of B-1 cells, enhanced in vitro B cell responses, elevated levels of Ab production against TD Ags, and accelerated in vivo T cell priming, which are phenotypes almost opposite to those of CD100-deficient mice (21). Furthermore, introduction of the transgene could restore in vitro and in vivo B cell responses of CD100-deficient mice. Such effects of transgene expression can be largely attributed to increased sCD100 rather than to the truncated membrane-bound CD100 on B cells because of only a small increase in CD100 on the surface of B cells. We have recently shown that there is a good correlation between the levels of sCD100 and the titers of specific Abs against TD Ag in sera of immunized mice or the titers of autoantibodies in sera of MRL/lpr mice (17). Furthermore, in vivo treatment with recombinant sCD100 protein has been shown to enhance humoral immune responses of normal mice and to restore Ag-specific T cell priming of CD100-deficient mice (11, 21). Thus, our present and previous observations strongly indicate that sCD100 plays a critical role in the regulation of physiological and pathological immune responses.

Although the semaphorin family consists of secreted-type and transmembrane-type proteins, their representative biological functions, such as repulsive axon guidance, have been reported on secreted-type members, acting as soluble factors (2). In contrast, the biological functions of transmembrane-type members have not been fully elucidated. CD100 has been reported to exist not only as a membrane-bound form, but also as a soluble form (10, 11, 15–17). We have directly demonstrated that sCD100 generated from the cell surface of lymphocytes by proteolytic cleavage had potent costimulatory activity as well as recombinant sCD100 protein (17). Many receptors, including IL-6R and CD62 ligand, are known to be cleaved by proteases such as metalloproteases and released as soluble forms (31–33). Although it is not clear whether the transmembrane-type CD100 needs to be converted into a soluble form to exert its functions, sCD100 seems to work as the active form in immune responses. From this point of view, the transmembrane-type of CD100 may exist as a reservoir for sCD100 to allow for a broader spectrum of immune reactions. If this is the case, proteolytic cleavage of CD100 would be one of the critical steps in regulating the function of CD100 in immune response (10). The phenotypes of CD100-Tg mice can be mostly explained by biological activities of sCD100 as a ligand. However, human CD100 has been shown to mediate signals as a receptor (7, 10, 14), suggesting that some phenotypes may be due to a dominant negative effect of sCD100 on signals via CD100. At present we cannot rule out this possibility. Further studies will be necessary to elucidate the functions of mouse CD100 as a receptor.

Role of CD100 in B cell development

The CD5+ B-1 cell population in the adult mouse is thought to be a remnant of a distinct fetal B cell differentiation pathway that is maintained without the help of T cells (34, 35). The number of CD5+ B-1 cells was significantly reduced in the peritoneal cavity
of CD100-deficient mice (21). In contrast, the number of B-1 cells is reported to be elevated in CD72-deficient mice (28). In CD100-Tg mice the number of B-1 cells was elevated as well. Other mutant mice carrying defects in SHP-1 signals, such as SHP-1- and CD22-deficient mice, also show an expansion of B-1 cells (36–38). Collectively, these observations indicate that SHP-1 participates in the negative regulation of development or maintenance of B-1 cells. Our findings, therefore, suggest that CD100 may contribute to autonomous expansion of B-1 cells by inactivating SHP-1 signals through CD72.

The transgenic expression of the truncated form of CD100 did not show any effect on the development of conventional B cells at present. This is in good agreement with our previous observation that conventional B cells develop normally in CD100-deficient mice (21). It thus appears that CD100 is not involved in the regulation of conventional B cell development. However, CD72-deficient mice have been reported to display a subtle defect in early B cell development in the bone marrow (28). Although we currently do not have any clue to explain this discrepancy, it may be possible that an unknown ligand other than CD100 plays a role in regulating CD72 signals in bone marrow.

Role of CD100 in B cell responses

The fact that in vitro B cell responses and in vivo Ab responses against TD Ags were enhanced in CD100-Tg mice substantiates an important role of CD100 in B cell functions, which has been proposed from our previous observations of recombinant sCD100 or CD100-deficient mice (11, 21). Furthermore, the phenotypes of CD100-Tg mice are similar to those of CD72-deficient mice (28). Thus, the present findings confirm our assumption that CD100 turns off negative signals of CD72 (11). However, Ab responses against TI-2 Ags in CD100-Tg mice were weaker than those in wild-type control mice. Conversely, we previously showed that TI-2 Ab responses of certain isotypes, such as IgG1, were enhanced in CD100-deficient mice (21). TI-2 Ags, which have highly repetitive structures and can efficiently cross-link surface Igs, are known to have biphasic effects on B cells: heavy cross-linking of BCR renders B cell unresponsive, while moderate cross-linking of BCR stimulates B cells (35). Therefore, it is possible that CD100 may attenuate signals from heavily cross-linking of BCR and rescue B cells from apoptosis or anergy, resulting in the modulation of Ab responses against TI-2 Ag. Although it is not statistically as significant, some CD100-Tg mice showed greater IgG3 responses to TI-1 Ag. It may reflect enhancing effects of CD100 on LPS-induced B cell responses.

Role of CD100 in activation of Ag-specific T cells

Ag-specific T cell responses in CD100-deficient mice have been previously shown to be impaired, which was shown to be rescued by in vivo administration of recombinant sCD100 protein (21). In the present study, we demonstrated that CD100-Tg mice showed enhanced Ag-specific T cell responses. Although Ab cross-linking of CD100 has been shown to provide a proliferative signal to human T cells in the presence of submicromolar doses of anti-CD3 or anti-CD2 Abs (7, 10), our results instead suggest that CD100 plays the role of a ligand in T cell activation as well as in B cell activation. Then, the question is whether CD100 acts directly on T cells or on APCs. We have previously shown that defects in CD100 do not show any effect on anti-CD3- or mitogen-stimulated T cells, suggesting an indirect involvement of CD100 in T cell activation (21). CD72, the lymphocyte receptor for CD100, is known to be expressed on some fractions of APCs (26). We also observed that CD40 stimulation significantly induced CD72 expression on APCs, including dendritic cells and macrophages (A. Kumanogoh, C. Watanabe, and H. Kikutani, unpublished observations). In addition, sCD100 significantly enhanced the costimulatory activity of dendritic cells (A. Kumanogoh, C. Watanabe, and H. Kikutani, unpublished observations), suggesting the involvement of CD100 in the priming of Ag-specific T cells through modulating the functions of APCs.

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References


