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CD100 belongs to the semaphorin family, several members of which are known to act as repulsive axonal guidance factors during neuronal development. We have previously demonstrated that CD100 plays a crucial role in humoral immunity. In this study, we show that CD100 is also important for cellular immunity through the maturation of dendritic cells (DCs). CD100−/− mice fail to develop experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein peptide, because myelin oligodendrocyte glycoprotein-specific T cells are not generated in the absence of CD100. In vitro studies with T cells from OVA-specific TCR-transgenic mice demonstrate that Ag-specific T cells lacking CD100 fail to differentiate into cells producing either IL-4 or IFN-γ in the presence of APCs and OVA peptide. In addition, DCs from CD100−/− mice display poor allostimulatory capabilities and defects in costimulatory molecule expression and IL-12 production. The addition of exogenous soluble rCD100 restores normal functions in CD100−/− DCs and further enhances functions of normal DCs. Furthermore, treatment of Ag-pulsed DCs with both soluble CD100 and anti-CD40 before immunization significantly enhances their immunogenicity. This treatment elicits improved T cell priming in vivo, enhancing both primary and memory T cell responses. Collectively, these results demonstrate that CD100, which enhances the maturation of DCs, is essential in the activation and differentiation of Ag-specific T cells. The Journal of Immunology, 2002, 169: 1175–1181.
CD100 in the maturation of DCs, which is necessary for the stimulation of Ag-specific T cell responses.

Materials and Methods

Mice

To generate CD100−/− mice on either the C57BL/6 or BALB/c backgrounds, CD100−/− mice were backcrossed for more than eight generations with C57BL/6 or BALB/c mice. Mice expressing the Tg OVA (OVA323–339)-specific αβ TCR (OVA-TCR Tg) on a BALB/c background have been previously described (23, 24). OVA-TCR Tg mice on a C57BL/6 background were established by crossing the OVA-TCR Tg mice with CD100−/− BALB/c mice. Mice were maintained in a pathogen-free environment.

Induction of EAE

EAE was induced in 8- to 12-wk-old CD100+/+ or CD100−/− mice on a C57BL/6 background following s.c. injection of 100 μg mouse/rat myelin oligodendrocyte glycoprotein (MOG) peptide (MEGVWYRSPFSRVMHLYRNGK) and pertussis toxin (100 ng; List Biological Labs, Campbell, CA), as described previously (25). For T cell priming analysis for MOG or OVA, 8- to 12-wk-old CD100+/+ or CD100−/− mice on a C57BL/6 background were immunized with 100 μg MOG or OVA in CFA into the hind footpads. Seven days after the immunization, CD4+ T cells were purified from the draining lymph nodes by MACS (Miltenyi Biotec, Bergisch Gladbach, Germany), and 1 × 105 cells were cultured for 72 h with MOG or OVA in the presence of irradiated (3000 rad) splenocytes (1 × 106 cells) from C57BL6 mice. For proliferation assay, cells were pulsed with 2 μCi [3H]thymidine for the last 12 h. Levels of IL-4 and IFN-γ in the culture supernatants were measured using ELISA kit (R&D Systems, Minneapolis, MN).

Stimulation of naive TCR-Tg T cells

CD62L−/−CD4+ naive T cells from OVA-TCR Tg mice were purified by positive selection using a FACS Vantage flow cytometer (BD Biosciences, Mountain View, CA). The purity of the cells was >99%. In the proliferation assays, 2 × 104 isolated naive T cells were cultured for 72 h with varying concentrations of OVA peptide in the presence of irradiated (3000 rad) splenocytes (2 × 105 cells) derived from either CD100+/+ or CD100−/− mice on a BALB/c background in 96-well microtiter plates. Cells were pulsed with 2 μCi [3H]thymidine for the last 12 h. In the cytokine assays, 1 × 105 naive T cells were cultured for 1 wk with varying concentrations of OVA peptide in the presence of 2 × 106 irradiated splenocytes derived from CD100+/+ or CD100−/− mice in 24-well plates. CD4+ T cells were prepared using MACS, and 1 × 105 cells were restimulated for 48 h with 5 μg/ml anti-CD3 (2C11; BD Pharmingen, San Diego, CA) coated on the plates in the presence of 5 μg/ml anti-CD28 mAb (37.51; BD Pharmingen) in round-bottom 96-well plates, as previously described (24). The levels of IL-4 and IFN-γ were measured by ELISA.

DC-induced MLR

DCs were generated from the bone marrow progenitors of CD100+/+ or CD100−/− C57BL/6 mice (6–12 wk old) using GM-CSF, as previously described (26, 27). In most experiments, we used bone marrow-derived DCs 6 days after in vitro culture with GM-CSF because the expression of DCs 6 days after in vitro culture with GM-CSF has been previously described (23, 24). OVA-TCR Tg mice on a C57BL/6 background were backcrossed for more than eight generations with C57BL/6 or BALB/c mice. Mice expressing the Tg OVA (OVA323–339)-specific αβ TCR (OVA-TCR Tg) on a BALB/c background have been previously described (23, 24). OVA-TCR Tg mice on a CD100−/− background were established by crossing the OVA-TCR Tg mice with CD100−/− BALB/c mice. Mice were maintained in a pathogen-free environment.

IL-12 production assay

IL-12 was quantitated after culturing DCs (1 × 106 cells/ml/24 well) for 72 h in the presence or absence of either anti-CD40 (3/23, 10 μg/ml), sCD100 (20 μg/ml), or anti-CD72 (IOT 72.2) (10 μg/ml). The IL-12 p40 was detected using a mouse IL-12 ELISA kit (R&D Systems).

Assays for in vivo induction of primary and memory T cells

Immature DCs (26, 27), prepared from normal C57BL/6 mice, were pulsed for 6 h with 10 μg/ml OVA (Sigma-Aldrich, St. Louis, MO). Cells were then dislodged and incubated for 12 h in the presence or absence of CD100 (20 μg/ml) (15), control human IgG1 (10 μg/ml), and anti-CD40 (HM-40-3, 0.5 μg/ml). Following three PBS washes, DCs were resuspended in PBS for injection into the footpads of C57BL/6 mice (5 × 10⁵ cells per footpad). Seven days after immunization with Ag-pulsed DCs, CD4+ T cells were prepared from the draining lymph nodes of the treated mice by MACs positive selection. A total of 1 × 10⁸ T cells was stimulated for 72 h with various concentrations of OVA in the presence of irradiated (3000 rad) splenocytes (2 × 10⁶ cells/ml) derived from syngeneic mice in 96-well microtiter plates. Cells were pulsed with 2 μCi [3H]thymidine for the last 12 h. For the measurement of delayed-type hypersensitivity (DTH), mice were challenged 9 wk after left hind footpad immunization by the injection of 10 μg OVA in PBS into the right footpad and PBS alone into the left footpad. After 24 h, we recorded the increase in thickness of right vs left footpad, as described previously (27).

Results

CD100−/− mice fail to develop EAE

To determine the involvement of CD100 in a pathological immune response, we used the MOG peptide-induced EAE model. As shown in Fig. 1, s.c. immunization of CD100−/− mice with the MOG peptide, together with pertussis toxin, induced severe encephalomyelitis associated with rapidly ascending paralysis appearing at approximately day 10–14, as described previously (25). In contrast, the development of EAE was significantly reduced in CD100−/− mice, and moreover, the affected CD100−/− mice experienced a very mild disease course. To determine the mechanisms responsible for the defective induction of EAE in CD100−/− mice, CD4+ T cells were prepared from the draining lymph nodes of immunized mice. Following restimulation with the MOG peptide in vitro, Ag-specific T cell responses, particularly generation of cytokine-producing effector cells, were severely impaired in CD100−/− mice (Fig. 2A). In addition, this impairment could be reproduced after immunization with other protein Ags, including OVA (Fig. 2B) and keyhole limpet hemocyanin (16). These results indicate that a defect in generation of Ag-specific effector T cells is at least one of the reasons for why CD100−/− mice developed very mild EAE upon immunization with MOG peptide, although CD100 may be also involved in an effector phase. In the following
Seven days after immunization with MOG (100 μg/mouse) and then stimulated for 72 h with 20 μg MOG or OVA in the presence of irradiated (3000 rad) splenocytes from syngeneic mice. Proliferation was assessed during the final 12 h of culture by pulsing with 2 μCi [3H]thymidine. IL-4 and IFN-γ production in the culture supernatants was measured by ELISA. The results shown are representative of three independent experiments.

We next analyzed the role of CD100 in the differentiation of T cells in CD100−/− mice. As shown in Fig. 3A, there were no significant differences in either CD8α-positive or CD8α-negative splenic DCs between CD100+/+ and CD100−/− mice, suggesting that impaired T cell priming in CD100−/− mice is due to the defective T cell-APC interactions rather than the reduced number of DCs in CD100−/− mice.

To elucidate how CD100 is involved in Ag-specific T cell activation, we bred OVA-TCR Tg mice with CD100−/− BALB/c mice to generate OVA-TCR Tg CD100−/− mice. CD62Ibright CD4+ naive T cells derived from either OVA-TCR Tg CD100−/− or OVA-TCR Tg CD100+/+ mice were stimulated with varying concentrations of an OVA-derived peptide and with CD100+ or CD100− BALB/c APCs. The absence of CD100 on either the T cells or APCs affects the proliferative responses of OVA-TCR Tg T cells to an OVA-derived peptide to some extent (Fig. 4A).

We next examined the role of CD100 in the differentiation of T cells into IFN-γ- or IL-4-producing cells. Following a 7-day culture with an OVA-derived peptide, CD4+ T cells were restimulated with anti-CD3 and anti-CD28. We then measured the concentrations of IFN-γ and IL-4 in the culture supernatants. CD100+/+ or CD100−/− T cells cultured with either CD100+/+ or CD100−/− APCs produced large amounts of IFN-γ and IL-4. In contrast, CD100−/− OVA-TCR Tg T cells cultured with either CD100+/+ or CD100−/− APCs exhibited significantly reduced...
production of these cytokines, indicating that surface expression of CD100 on T cells is important for effective T cell differentiation into functional effector cells (Fig. 4, B and C).

Involvement of CD100 in DC allostimulating

We next examined whether CD100 contributes to the interactions between T cells and DCs using a MLR. DCs generated from bone marrow progenitors of CD100+/+ or CD100−/− C57BL/6 mice by stimulating them with GM-CSF were used to stimulate CD4+ T cells isolated from CD100+/+ or CD100−/− BALB/c mice. As shown in Fig. 5A, the absence of CD100 on either the T cells or DCs substantially affects T cell proliferation. The response was markedly reduced when both T cells and DCs lacked CD100. This is consistent with a CD100 requirement for proliferative responses of OVA-TCR Tg T cells (Fig. 4A). We next tested the effects of sCD100 in a MLR using CD100+/+ CD4+ T cells as responders and CD100+/+ or CD100−/− DCs as stimulators. As shown in Fig. 5B, sCD100 significantly enhanced the MLR induced by both CD100+/+ and CD100−/− DCs. These results suggest that an optimal MLR requires the expression of CD100 on T cells and on DCs. Indeed, activation by anti-CD40 enhances the expression of CD100 on DCs (Fig. 3B), suggesting that DC-derived CD100 has some contribution in an autocrine manner.

The expression of CD72 is observed on a small fraction of activated T cells (28). However, we previously showed that CD100 does not have any effects on either resting or mitogen-stimulated T cells (16). To determine whether CD100 acts on DCs to enhance their activation and maturation, but does not stimulate T cells directly in this experimental system, DCs were stimulated with anti-CD40 and sCD100 to induce maturation, fixed with paraformaldehyde, and then used to stimulate allogeneic T cells in a MLR. As shown in Fig. 5C, both CD100+/+ and CD100−/− fixed mature DCs could induce similar levels of T cell proliferation in CD100+/+ and CD100−/− T cells when DCs were fully activated and then fixed. In particular, it is noteworthy that there is no difference between responses of CD100−/− T cells and CD100+/+ T cells. Furthermore, sCD100 did not enhance the MLR using CD100−/− T cells and fixed mature CD100−/− DCs (Fig. 5D), showing that sCD100 does not act on T cells. These results demonstrate that CD100 acts directly on DCs, but not on T cells.

Impaired expression of costimulatory molecules and IL-12 production in CD100−/− DCs

The ligation of CD40 on DCs and macrophages by CD40 ligand on the surface of activated T cells induces expression of various costimulatory molecules and cytokines such as CD80, CD86, and IL-12 (29, 30). We, therefore, examined the role of CD100 in the CD40-induced expression of CD40, CD80, and I-A α molecules on the cell surface of DCs. Although a 14-h stimulation with anti-CD40 up-regulated the expression of CD40, CD80, and I-A α on the surface of CD100+/+ DCs, the expression of these molecules was impaired in CD100−/− DCs (Fig. 6A). The effects of anti-CD40 treatment were restored in CD100−/− DCs by addition of sCD100, a treatment that enhanced these changes in CD100+/+ DCs.
FIGURE 6. sCD100 enhances the maturation of DCs. A, Bone marrow-derived DCs generated from CD100+/+ or CD100−/− mice were cultured for 14 h with or without the combination of anti-CD40 (0.5 μg/ml) and sCD100 (20 μg/ml). Cells were then stained with PE anti-B220; FITC anti-CD11c; and biotin anti-CD40, biotin anti-CD80, or biotin anti-I-A plus streptavidin APC. CD11c-positive and B220-negative cells were analyzed for the expression of CD40, CD80, and anti-I-A. B, CD100+/+ (○) or CD100−/− (●) DCs were cultured for 72 h with varying concentrations of sCD100 in the presence of anti-CD40 (10 μg/ml). IL-12 in the culture supernatants was measured by ELISA. C, CD100+/+ (open bars) or CD100−/− (shaded bars) DCs were cultured with anti-CD40 (10 μg/ml), sCD100 (10 μg/ml), or anti-CD72 (10 μg/ml) for 72 h. IL-12 in the culture supernatants was measured by ELISA. *, Value of p < 0.01 was analyzed by unpaired t test.

DC-derived IL-12 is necessary for the induction of IFN-γ-producing Th1 effector cells (31). CD40-induced IL-12 production was severely impaired in CD100−/− DCs. The addition of sCD100 significantly enhanced CD40-induced IL-12 production in both CD100+/+ and CD100−/− DCs (Fig. 6B). These results suggest a critical role of CD100 in the expression of immunoregulatory molecules during DC maturation. Furthermore, agonistic CD72 mAb could mimic the effects of CD100 on DCs, and not only rescued the defects of CD100−/− DCs, but also enhanced IL-12 production of both CD100+/+ and CD100−/− DCs (Fig. 6C), suggesting that the effects of CD100 on DCs are also mediated through CD72.

Enhancement of Ag-specific primary and memory T cell responses by sCD100-treated Ag-pulsed DCs

We then analyzed the effect of CD100 on the ability of DC to stimulate Ag-specific T cells in vivo. Bone marrow-derived DCs from CD100+/+ mice on a C57BL/6 background were pulsed with OVA protein for 6 h. After priming with OVA, DCs were treated with sCD100, anti-CD40, or sCD100 plus anti-CD40. DCs were injected s.c. into the hind footpads of syngeneic mice. We assessed the immunogenicity of the injected DCs by in vitro restimulation of Ag-specific T cells. DCs treated with sCD100 plus anti-CD40 induced strong OVA-specific T cell responses, while DCs treated with anti-CD40 alone induced relatively weak responses (Fig. 7A). No response was observed in mice immunized with either untreated DCs or DCs treated with sCD100 alone. These results indicate that CD100 synergistically enhances the ability of CD40-stimulated DCs to prime Ag-specific T cells.

We next tested an effect of sCD100 treatment on the ability of DCs to establish T cell memory. Following immunization with OVA-pulsed DCs stimulated with sCD100 plus anti-CD40, DTH responses were evident 9 wk later (Fig. 7B). We did not observe a significant DTH response in the mice injected with DCs that were pulsed with OVA and stimulated with anti-CD40 alone. These results suggest that sCD100 treatment enhances the capacity of Ag-pulsed DCs to induce both primary T cell responses and Ag-specific T cell memory in vivo.

FIGURE 7. sCD100 enhances the ability of DCs to induce Ag-specific T cell priming and memory. Ag-pulsed DCs were treated for 12 h with control human IgG (●), sCD100 (■), anti-CD40 (○), or sCD100 plus anti-CD40 (□), and then 5 × 10⁵ DCs were injected in the hind footpad. In the T cell priming assays (A), CD4− T cells isolated from the popliteal lymph node 7 days after immunization were stimulated for 72 h with varying concentrations of OVA in the presence of irradiated (3000 rad) splenocytes. [3H]Thymidine was added for the last 12 h. In the assays for T cell memory (B), mice were challenged 9 wk after immunization by the injection of 10 μg OVA in the right footpad or with PBS alone in the left footpad. Footpad swelling was assessed 24 h later. Each bar represents the increase in thickness of right vs left footpad (mean ± SD, 10 mice per group). **, Value of p < 0.01 compared with the group of mice injected with OVA-pulsed DCs treated with sCD100 or anti-CD40 alone.
Discussion
In this study, we show that CD100 is required for the induction of cellular immune responses, including pathological immune responses. Although our previous studies using CD100−/− mice have shown that CD100 plays a crucial role in not only humoral immune responses, but also cellular immunity (16), precise mechanisms as to how CD100 is involved in Ag-specific T cell activation in vivo were not clear. We provide in this study direct evidence that CD100 plays a critical role in T cell-APC interactions by enhancing CD40-induced DC activation. Both CD100 and CD40 ligand may be concomitantly expressed on the surface of activated T cells, which can bind to CD72 and CD40, respectively, on the surface of B cells and professional APCs. In B cells, CD100 stimulation synergistically enhances CD40-mediated responses by causing SHP-1, a negative regulator of B cell functions, to dissociate from CD72. In CD100−/− B cells, SHP-1 is constitutively associated with CD72, resulting in impaired B cell responses in CD100−/− mice (16). Because several src kinases including lyn, which can be substrates for SHP-1, are shown to be involved in CD40-mediated signals in DCs (31), it is possible that the CD100-mediated enhancement of DC activation might function through similar signaling pathways. As shown in Fig. 3C, CD72 is expressed on the surface of splenic DCs, which is consistent with the previous findings (22). Furthermore, agonistic CD72 mAb could mimic the effects of CD100 on anti-CD40-stimulated DCs (Fig. 6C), suggesting that the similar mechanism may be involved in CD100-induced enhancement of DC activation. However, it still remains to be determined whether CD72 is the only receptor for CD100 on DCs or whether APCs express another receptor for CD100. Further studies will be required to clarify the involvement of CD72 in CD40-mediated signals in DCs.

Our present study demonstrates that CD100 expressed on either T cells or DCs can contribute to the activation and maturation of DCs. Although the development of DCs is not affected in CD100−/− mice, anti-CD40-induced maturation of CD100−/− DCs was defective in the context of expression of costimulatory molecules and production of IL-12. It thus appears that CD100 derived from DCs can also play a role in the maturation of DCs in an autocrine manner. However, addition of sCD100 significantly enhanced CD40-induced IL-12 production and immunogenicity of normal DCs, suggesting that exogenous CD100 probably derived from T cells may be required for full activation of DCs. Indeed, the expression of CD100 on the surface of T cells is much more abundant than that on DCs (Fig. 3B). In our OVA-TCR Tg system, the expression of CD100 on T cells, but not on APCs, was particularly important for the differentiation of naive TCR-Tg T cells into cytokine-producing effector cells (Fig. 4, B and C). Hence, T cells may be a major source of CD100 in physiological T cell-dependent immune responses through T cell-APC interactions.

DCs play a role in linking the innate and adaptive immune responses, in which the innate immune system has been shown to be critically important in the activation of the adaptive immune system (32). In the innate immune system, microbial components stimulate DCs through Toll-like receptors (TLRs) (33). CD100−/− B cells are hyporesponsive to LPS, as shown in previous studies (16). LPS-induced IL-12 production was also affected in CD100−/− DCs (A. Kumanogoh, unpublished data), suggesting that CD100 may affect signals of TLR4 in an autocrine manner. Therefore, CD100 may play a role in shaping both the innate and adaptive immune responses by regulating the activation and maturation of DCs. It is of value to determine the involvement of CD100-CD72 interactions in TLR signals in DCs.

We have also shown that sCD100 can significantly enhance the ability of DCs to induce T cell priming and T cell memory. DCs have been used as adjuvant to enforce immunity against infection and tumors. Taken together with the fact that CD100−/− mice are resistant to EAE, our findings suggest that CD100 is a potential target not only for immunointervention of autoimmune diseases, but also for reinforcement of DC-based vaccination.

Besides CD100, several other semaphorins also have been shown to modify functions of immune cells, particularly monocytes. For instance, viral semaphorins, A39R encoded by vaccinia virus and AVHsema encoded by alcelaphine herpesvirus, have been shown to bind to its cellular receptor, virus-encoded semaphorin protein receptor/CD232/plexin-C1, and to induce robust responses in human monocytes (34, 35). In addition, a glycosylphosphatidylinositol-anchored semaphorin, CD108/Sema7A/Sema-K1, which may be a mammalian counterpart of AVHsema, has been demonstrated recently to bind to CD232/virus-encoded semaphorin protein receptor/plexin-C1 (19). Furthermore, Sema3A/H-SemIII, the representative semaphorin member identified as an axonal guidance factor, has been reported to inhibit monocyte migration (36). Although the physiological and pathological significance of these semaphorins has not yet been determined, it is possible that they may play important roles in cellular immune responses, as we have shown in this study for CD100. Future studies would not only clarify the functional significance of the semaphorin family in the immune system, but also open up a novel paradigm of the immunoregulatory semaphorin network.

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References


