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*J Immunol* 2006; 177:3406-3412; doi: 10.4049/jimmunol.177.5.3406

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CD100 Enhances Dendritic Cell and CD4⁺ Cell Activation Leading to Pathogenetic Humoral Responses and Immune Complex Glomerulonephritis¹

Ming Li,* Kim M. O’Sullivan,* Lynelle K. Jones,* Timothy Semple,* Atsushi Kumanogoh, † Hitoshi Kikutani, † Stephen R. Holdsworth,* and A. Richard Kitching2*

CD100, a member of the semaphorin family, is a costimulatory molecule in adaptive immune responses by switching off CD72’s negative signals. However, CD100’s potential pathogenetic effects in damaging immune responses remain largely unexplored. We tested the hypothesis that CD100 plays a pathogenetic role in experimental immune complex glomerulonephritis. Daily injection of horse apoferritin for 14 days induced immune complex formation, mesangial proliferative glomerulonephritis and proteinuria in CD100-intact (CD100+/+) BALB/c mice. CD100-deficient (CD100−/−) mice were protected from histological and functional glomerular injury. They exhibited reduced deposition of Igs and C3 in glomeruli, reduced MCP-1 and MIP-2 intrarenal mRNA expression, and diminished glomerular macrophage accumulation. Attenuated glomerular injury was associated with decreased Ag-specific Ig production, reduced CD4⁺ expression, and diminished glomerular macrophage accumulation. They exhibited reduced deposition of Igs and C3 in glomeruli, reduced MCP-1 and MIP-2 intrarenal mRNA expression, and diminished glomerular macrophage accumulation. Attenuated glomerular injury was associated with decreased Ag-specific Ig production, reduced CD4⁺ cell activation and cytokine production. Following Ag injection, CD4⁺ cell CD100 expression was enhanced and dendritic cell CD86 expression was up-regulated. However, in CD100−/− mice, dendritic cell CD86 (but not CD80) up-regulation was significantly attenuated. Following i.p. immunization, CD86, but not CD80, promotes early Ag-specific TCR-transgenic DO11.10 CD4⁺ cell proliferation and IFN-γ production, suggesting that CD100 expression enables full expression of CD86 and consequent CD4⁺ cell activation. Transfer of CD100−/− DO11.10 cells into CD100−/−/H11546 mice resulted in decreased proliferation demonstrating that CD100 from other sources in addition to CD100 from Ag-specific CD4⁺ cells plays a role in initial T cell proliferation. Although T cell-B cell interactions also may be relevant, these studies demonstrate that CD100 enhances pathogenetic humoral immune responses and promotes the activation of APCs by up-regulating CD86 expression. The Journal of Immunology, 2006, 177: 3406–3412.

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Received for publication May 24, 2005. Accepted for publication May 25, 2006.

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This work was supported by grants from the National Health and Medical Research Council of Australia.

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0022-1767/06/$02.00

The Journal of Immunology
Materials and Methods

**Mice and induction of immune complex GN**

CD100+/− BALB/c mice (7) and DO11.10 mice (19) (The Jackson Laboratory) were bred at Monash Medical Centre (Clayton, Victoria, Australia). Male BALB/c mice (8–12 wk of age) were obtained from Monash University Centre Animal Services. All mice were maintained in specific pathogen-free conditions. Studies were approved by the Monash University (Monash Medical Centre Committee B) Animal Ethics Committee. Four milligrams of horse spleen apoferritin (Sigma-Aldrich) in 78 μl of NaCl were injected i.p. daily into CD100+/− and CD100−/− BALB/c recipients for 14 days. Experiments ended on day 15. To obtain baseline values, age- and sex-matched nonimmunized BALB/c CD100+/− and in some experiments CD100−/− mice were used. Histological examination was performed on coded slides, results are expressed as mean ± SEM, and the significance of differences between groups was determined by unpaired t-test or one-way ANOVA according to the data to be analyzed. The GN experiments was performed twice with eight mice in each group (CD100+/− and CD100−/− mice) in the first experiment and seven mice in each group in the second. Results are presented from one of two consistent experiments.

**Assessment of renal injury, leukocyte infiltration, and chemokine mRNA**

Tissue sections (3 μm) from paraffin-embedded kidney tissue were stained with periodic acid-Schiff’s reagent (PAS) and deposition of PAS material assessed (minimum, 50 glomeruli/mouse) using a 0–3* scale: 0, no accumulation of PAS material; 1, mild; 2, moderate; and 3, more severe accumulation of PAS material. Total glomerular cell nuclei were counted (minimum, 20 glomeruli/mouse; expressed as cells per glomerular cross section (c/gcs)). Urinary protein excretion was determined by a modified Bradford method on urine collected over the final 24 h of experiments. Serum creatinine concentrations at the completion of experiments were measured by an enzymatic creatinase assay. Macrophages and neutrophils were demonstrated in glomeruli by three-layer immunoperoxidase staining of peroxidase-lsine paraformaldehyde-fixed frozen 6-μm kidney sections (20). The primary mAbs were FA11, anti-mouse CD68 (21) for macrophages, and RB6-8C5, anti-Gr-1 (DNAX Research Institute) for neutrophils. Isotype control IgG was used as a negative control. Total kidney RNA preparation and analysis were described (22) as previously using the Quant System (BD Pharmingen; template set MCK-5c) as previously described (22), normalized to the housekeeping gene L32, and results are expressed as arbitrary units.

**Detection of Ig and complement in glomeruli and serum Ag-specific Abs**

For detection of mouse Ig, IgG1 and IgG2a frozen sections (6 μm) were stained using FITC-sheep anti-mouse Ig (Silenus; dilution, 1/100), FITC-rat anti-mouse IgG1 (BD Pharmingen; dilution, 1/200) and FITC-rat anti-monotypic IgG2a (BD Pharmingen; dilution, 1/100). C3 was detected using FITC-conjugated goat anti-mouse C3 (Cappel; dilution, 1/100). Fluorescence intensity was assessed semiquantitatively (0–3*; minimum, 20 glomeruli). Titers of mouse anti-horse apoferritin were measured by ELISA on the last 18 h, and the incorporation of [3H]Thy were detected with a liquid scintillation beta counter (Wallac 1400; Cambridge Scientific). Results are expressed as follows: stimulation index = stimulated group cpm/unstimulated group cpm.

**Measurement of cytokine production by Ag-stimulated lymphocytes**

Splenocytes were prepared as above. After three washes with HKF2.5, cells were incubated in 24-well plates (4 × 105 cells/ml in 10% FCS RPMI 1640, supplemented with 10% FCS RPMI 1640, supplied with L-glutamine, 2-ME) in the presence or absence of horse apoferritin (72 h). Cells were pulsed with 0.5 μCi/well thymidine ([3H]Thy) for the last 18 h, and the incorporation of the [3H]Thy was detected with a liquid scintillation beta counter (Wallac 1400; Cambridge Scientific). Results are expressed as follows: stimulation index = stimulated group cpm/unstimulated group cpm.

**Preparation of DCs and flow cytometric analyses of immune cells**

Mouse splenocytes (106 cells) were stained with the appropriate mAbs (see below). Mouse DCs were prepared from the spleens of individual mice (24). Briefly, each spleen was cut into small fragments, and then suspended in 1 ml of RPMI 1640-FCS containing 1 mg/ml freshly dissolved collagenase type I (Sigma-Aldrich) and 0.2 ml of 0.1% DNase I (Roche). Collagenase/DNase digestion was conducted at room temperature for 20 min with gentle pipetting to facilitate digestion. Dissociation of T cell-D C complexes was achieved by adding EDTA (1/10 v. of 0.1 M EDTA (pH 7.2) for 5 min. Residual stromal fragments were removed by passing suspensions through a stainless-steel sieve. Samples were kept on ice in a divalent-metal free medium (EDTA-balanced salt solution-FCS) during FACS analysis.FcRs were blocked by Mouse Fc-Block (BD Pharmingen), and then 1% BSA in PBS with 5 mM EDTA containing appropriate mAbs was added to 106 cells and incubated for 30 min at 4 °C. The following mAbs were used for the analyses (all BD Pharmingen unless noted): R-PE or allophycocyanin/CF-7-conjugated rat anti-mouse CD4 (GK1.5), (FITC)-conjugated rat anti-mouse CD45R/B220 (RA3-6B2), FITC-mouse anti-mouse-CD72 (K10.6), PE-rat anti-mouse CD19 (clone ID3), PE-hamster anti-mouse CD54 (ICAM-1) (3E2), and allophycocyanin-rat anti-mouse CD44 (IM7), FITC-rat anti-mouse CD25 (7D4), PE-hamster anti-mouse CD11c (HL3), and PE-anti-DO11.10 TCR (KJI-26). The rat anti-mouse CD100 (BMA12) (10), anti-mouse MHC class II (MHC II) (M5/114; K. Shortman, Walter and Eliza Hall Institute (WEHI), Parkville, Australia), anti-mouse CD86, and hamster anti-mouse CD80 (GL1 and 16-10A1; both from D. Tarlton, WEHI) hybridomas were cultured, harvested, and labeled with Alexa Fluor-647 (Invitrogen Life Technologies). Annexin V-fluos (Roche) was used to stain apoptotic cells (annexin V+PI−) as previously described (25). Flow cytometry was performed on FACSscan (DakoCytomation). The following negative controls were used: for CD100, CD80, or CD86, splenocytes from unmanipulated CD100+/− or CD80−/− or CD86−/− mice were used. For other markers, isotype-matched irrelevant mAb were used. Cells fluorescing at levels above the negative control were considered positive.

**DO11.10 cell preparation, adoptive transfer, and immunization**

Single-cell suspensions were prepared from lymph nodes of DO11.10 (BALB/c OVA-specific TCR-transgenic) mice (26). In experiments transferring purified CD4+ T cells, the CD4+ T cell population was enriched by positive selection via passage of cell preparations through a magnetic column (MACS Technology; Miltenyi Biotec) according to the manufacturer’s instructions. Cells were then stained with PE-anti-CD11c and PE-anti-CD19 and sorted by flow cytometry to further exclude CD11c+ and CD19+ cells. Samples of 107 cells labeled with allophycocyanin/CF-7-conjugated anti-CD4 and PE-KJI-26 demonstrated that ≥97% of cells expressed the transgenic TCR. For CFSE labeling (27), 2 × 105 cells/ml were resuspended in 0.1% BSA-PBS containing 10 μM CFSE (Invitrogen Life Technologies), incubated (37°C, 10 min), and then washed with 2.5% FCS-RPMI 1640 three times. Recipient mice were injected i.v. with 3–5 × 107 (consistent with our previous experiment) OVA-specific CD4+ KJ1-26 T cells labeled by CFSE (20,000 cpm) in 500 μl of complete medium (10% FCS-RPMI 1640, and then immunized i.p. the next day with 0.25 mg of OVA in PBS. Anti-CD86, anti-CD80, or control Ab was administered at a dose of 0.1 mg, i.p., 1 day before and 1 day after transfer of CFSE-labeled
DO11.10 cells. Three days after immunization, mice were humanely killed, splenocytes were stained, and CD4\(^+\), KJ1-26\(^+\), CFSE\(^-\) cells were analyzed.

## Results

**Endogenous CD100 enhances immune renal injury, systemic Ig production, glomerular Ig deposition, and recruitment of glomerular effectors**

After 14 days, wild-type BALB/c (CD100\(^{+/+}\)) mice had developed renal injury. Proliferation, predominantly mesangial, was prominent in glomeruli with deposition of PAS\(^+\) material and proteinuria (summarized in Table I). Renal injury in CD100\(^{-/-}\) mice was attenuated with reduced accumulation of PAS\(^+\) material, glomerular hypercellularity, and decreased proteinuria. Renal failure is not a feature of this model, and there was no difference in serum creatinine between mice (data not shown). Compared with CD100\(^{+/+}\) mice with GN, CD100\(^{-/-}\) mice had reduced deposition of total Ig, IgG1, and IgG2a in glomeruli (Fig. 1). Changes in glomerular Ig deposition were reflected in systemic Ag-specific total Ig, IgG1, and IgG2a titers that were decreased in sera of glomeruli with deposition of PAS\(^+\) material and proteinuria (summarized in Table I). Renal injury in CD100\(^{-/-}\) mice was reduced in the absence of endogenous CD100, but mRNA expression for CXCL10 (IFN-\(\gamma\)-inducible protein 10), CCL1 (TCA-3), XCL1 (lymphotactin), CCL3 (MIP-1\(\alpha\)), and CCL4 (MIP-1\(\beta\)) were not increased over unimmunized mice (except CD100\(^{-/-}\) CCL3 (MIP-1\(\alpha\)) at 7 days, not increased compared with CD100\(^{+/+}\) mice at 7 days; data not shown).

### Table I. Glomerular inflammation is reduced in CD100\(^{-/-}\) mice with immune complex GN

<table>
<thead>
<tr>
<th></th>
<th>CD100(^{+/+}) (n = 7)</th>
<th>CD100(^{-/-}) (n = 8)</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS(^+) material(a)</td>
<td>1.6 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glomerular cells/gcs(b)</td>
<td>41.8 ± 1.0</td>
<td>34.1 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerular Ig(c)</td>
<td>2.3 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glomerular IgG1(c)</td>
<td>2.4 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerular IgG2a(c)</td>
<td>1.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Complement (C3)(c)</td>
<td>2.7 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL2 (MCP-1)(c)</td>
<td>6.0 ± 1.4</td>
<td>2.9 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CXCL1 (MIP-2)(c)</td>
<td>3.0 ± 1.0</td>
<td>1.1 ± 0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Macrophages/gcs(c)</td>
<td>5.7 ± 0.4</td>
<td>4.3 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(a\) Glomeruli were scored under a microscope by using a 0 – 3 scale, with a score of 0 being equivalent to nonimmunized CD100\(^{+/+}\) mice. The value for the accumulation of PAS\(^+\) material in nonimmunized CD100\(^{+/+}\) mice is 0.27 ± 0.02.

\(b\) Expressed as cells per glomerular cross section (gcs). The value for nonimmunized CD100\(^{+/+}\) mice is 33.2 ± 0.6 cells/gcs and 0.21 ± 0.04 cells/gcs for macrophages.

\(c\) Expressed in arbitrary units (AU). Values for nonimmunized mice are as follows: CCL2: CD100\(^{+/+}\), 1.6 AU; CXCL1: CD100\(^{+/+}\), 3.7 ± 1.6 AU; CXCL1: CD100\(^{-/-}\), 1.5 ± 0.3; CD100\(^{-/-}\), 1.7 ± 0.1 AU.

with GN expressed intrarenal chemokine mRNA. In CD100\(^{-/-}\) kidneys, expression of CCL2 (MCP-1) and CXCL1 (MIP-2) were reduced at 14 days, CCL2 being reduced also at 7 days (CD100\(^{+/+}\), 8.4 ± 1.9; CD100\(^{-/-}\), 4.1 ± 0.5 arbitrary units; \(p < 0.05\)). CCL5 (RANTES) mRNA expression was increased in both CD100\(^{+/+}\) and CD100\(^{-/-}\) mice, but mRNA expression for CXCL10 (IFN-\(\gamma\)-inducible protein 10), CCL1 (TCA-3), XCL1 (lymphotactin), CCL3 (MIP-1\(\alpha\)), and CCL4 (MIP-1\(\beta\)) were not increased over unimmunized mice (except CD100\(^{-/-}\) CCL3 (MIP-1\(\alpha\)) at 7 days, not increased compared with CD100\(^{+/+}\) mice at 7 days; data not shown).

**Endogenous CD100 enhances CD4\(^+\) T cell function and survival**

Collectively, these results imply an important role for CD100 in B cell activation and the development of the subsequent Ab response. To understand why humoral responses and therefore renal injury were reduced in the absence of endogenous CD100, immune responses were further assessed in CD100\(^{-/-}\) mice and CD100\(^{-/-}\) mice. Ag-specific lymphocyte proliferation in mice

### FIGURE 1. Attenuated glomerular injury in CD100\(^{-/-}\) mice. a, Proteinuria in CD100\(^{-/-}\) mice with immune complex GN was lower than that of CD100\(^{+/+}\) mice with GN. The dotted line refers to values in nonimmunized CD100\(^{+/+}\) mice. b, Immunofluorescent staining demonstrated strong deposition of mouse Ig in CD100\(^{-/-}\) mice, but there was reduced deposition of Ig in glomeruli of CD100\(^{+/+}\) mice. Magnification, ×400. ***, \(p < 0.001\).

### FIGURE 2. Reduced systemic Ag-specific Ab responses in CD100\(^{-/-}\) mice in immune complex GN. After injection of apoferritin for 14 days, titers of serum total Ag-specific IgG, IgG1, and IgG2a (ELISA) were significantly decreased in CD100\(^{-/-}\) mice (n = 8) compared with CD100\(^{+/+}\) mice (n = 7). *, \(p < 0.05\); **, \(p < 0.01\); ***, \(p < 0.001\).
with GN ([3H]Tdr incorporation) demonstrated decreased lymphocyte proliferation in CD100−/− cells when stimulated with different doses of apoferritin (Fig. 3a). CD100 expressed on T cells has the potential to interact with B cell CD72. However, the reduced humoral response may be at least in part due to alterations in CD4+ T cell activation and function in the absence of CD100. CD4+ T cell responses in wild-type and CD100−/− mice were compared at the end of experiments. IL-10 and IL-4 production by Ag-stimulated CD100+/+ splenocytes were decreased compared with CD100+/+ mice, but IFN-γ production was not significantly reduced (Fig. 3b). Similar proportions of CD4+ cells (CD100+/+, 24.6 ± 1.5%, vs CD100−/−, 22.4 ± 1.1%) and B220+ cells (CD100+/+, 49.2 ± 1.4%, vs CD100−/−, 51.0 ± 1.2%) were present at the initiation of culture. To examine T cell activation and apoptosis, CD54, CD44, and annexin V expression on splenic CD100+/+ and CD100−/− CD4+ T cells were measured (Fig. 3c). CD44 expression was decreased on CD100+/+. CD4+ T cells compared with CD100+/+ mice, but differences in CD54 expression did not reach significance (p = 0.051). The proportion of CD100−/−CD4+ T cells undergoing apoptosis (annexin V PI+) cells) was increased in the absence of endogenous CD100 (Fig. 3d).

Expression of immune cell CD100/CD72 in disease, and regulation of DC CD86 by CD100

Serial studies of immune cells at several time points were performed. CD100 was expressed on both naïve CD4+ T cells (60.6 ± 4.6%) and on CD19+ B cells (76.2 ± 4.1%; n = 8). CD100 expression was up-regulated on CD4+ T cells by 72 h after injection of horse apoferritin (Fig. 4, a and b). A higher proportion of CD4+ T cells were CD100 positive at 72 h. The proportion of B cells that were CD100+ was unaltered over the course of the model. Only a small proportion of CD4+ T cells expressed low levels of CD72 (data not shown). CD72 was expressed on most B cells. A marginally higher proportion of B cells were CD72+ 24 h after stimulation (Fig. 4c).

To determine whether impaired CD4+ T cell function is mediated by impaired DC costimulatory function, CD72, CD80, and CD86 expression on CD11c+.MHC II+ cells was assessed. Proportions of CD11c+ cells were similar in both CD100+/+ and CD100−/− mice (data not shown), CD72 was expressed on a small proportion of CD11c+.MHC II+ cells (Fig. 5). Over the first 72 h, this CD11c+.MHC II+ CD72+ population was increased. At 24 h, this increase was evident only in CD100−/− mice. As expected, 24 h following Ag injection, CD86 expression was increased on CD11c+.MHC II+ cells from CD100+/+ mice (Fig. 6, a and b). This increase was attenuated in the absence of endogenous CD100. Expression of CD80 was unchanged in the absence or presence of CD100 (Fig. 6c).
CD100, but not CD80, is important in early T cell proliferation and cytokine production following i.p. immunization

To determine the functional consequences of the selective increase in CD86 expression in the presence of CD100, transgenic OVA-specific CFSE-labeled cells from TCR-transgenic DO11.10 mice were transferred into OVA-immunized BALB/c mice. CD86 expression was important in optimal early proliferation and function (Fig. 7). Proliferation (serial halving of CFSE 72 h after immunization) was reduced in recipients treated with anti-CD86 mAbs, but not after anti-CD80 mAb treatment (Fig. 7, a and b). Compared with OVA-immunized control Ab-treated or anti-CD80-treated mice, in anti-CD86-treated mice more cells remained in an undivided state and fewer cells had reached the fourth division. Ex vivo splenocyte culture demonstrated reduced IFN-γ production in mice treated with anti-CD86 mAbs (Fig. 7c).

CD100 from outside the Ag-specific CD4+ cell population plays a role in T cell activation

Purified CD4+·CD100+·DO11.10 cells were transferred into CD100+/− or CD100−/− recipients. In CD100−/− recipients, the rate of proliferation of DO11.10 cells was reduced (Fig. 8, a and b), demonstrating that endogenous CD100 from sources in addition to Ag-specific CD4+ cells plays a role in early T cell proliferation.

Discussion

Pathogenic Ab responses and immune complexes are features of many nonautoimmune and autoimmune diseases, in which glomeruli are often affected. The current studies demonstrate a role for CD100 in pathological Ab-mediated injury. In these diseases, Ag-specific CD4+ cells, generated by APC-CD4+ cell interactions provide help for B cells that produce pathogenic Abs. The current studies demonstrate that CD100−/− mice developed less severe immune renal injury, both histological and functional, in immune complex GN. Immunized mice developed proliferative GN with mesangial matrix expansion, complement deposition, macrophage accumulation, and proteinuria. However, CD100−/− mice developed only mild GN. Immune responses to the nephritogenic Ag were attenuated at several levels, including DC costimulatory molecule expression, CD4+ cell activation, and Ig production.

Attenuation of injury in CD100−/− mice resulted from reduced T cell-dependent B cell Ab responses to the nephritogenic Ag, horse apoferritin. Reduced serum Ag-specific Ab levels in the CD100−/− mice paralleled glomerular findings. CD100 is up-expressed on activated CD4+ T cells. Because other studies have demonstrated that CD100 promotes B cell activation by the binding of CD100 to CD72 expressed on B cells (6, 7, 10, 11), it is probable that at least part of the reduced Ig production in the current studies in CD100−/− mice relates to uninhibited negative signaling in B cells by CD72. Additional experiments defined a role for CD100 in the early stages of the immune response. CD100 deficiency results in defective T cell activation (7). The current studies show that, in this disease model, in which humoral immunity plays an important role, CD4+ cell activation and function were suppressed. In the absence of CD100, CD4+ cells at day 14 were more prone to apoptosis, exhibited diminished markers of T cell activation, and made less IL-4 and IL-10. In contrast to the findings in CD100−/− mice immunized s.c. with keyhole limpet hemocyanin in Freund’s complete adjuvant (7), IFN-γ production was not reduced. This may be due to the different route of administration and use of adjuvants in the two studies. Reductions in...
humoral responses were not mediated via enhanced CD4+CD25+ regulatory T cells, because CD25 expression on CD4+ cells was decreased in CD100−/− mice (data not shown).

Because CD72 is expressed on only a small proportion of CD4+ cells, we hypothesized that in vivo CD4+ cell CD100 expression participates in DC activation by switching off CD72-induced negative signals. CD72 is expressed on DCs (9, 28). Following in vitro stimulation, costimulatory molecule expression on DCs was reduced in the absence of CD100. The current studies show that, in vivo, CD100 is required for optimal expression of CD86 on DCs. Although proportions of splenic DCs were similar in the presence and absence of CD100, expression of CD86, but not CD80, was significantly reduced in CD100−/− mice following injection of the disease-initiating Ag. Studies in a DO11.10 TCR transgenic adoptive transfer system demonstrated the functional importance of CD86 expression in early T cell proliferation and cytokine production after i.p. immunization. Following i.v. stimulation, costimulatory molecule expression on DCs was reduced in the absence of CD100. The current studies show that, in vivo, CD100 is required for optimal expression of CD86 on DCs. Although proportions of splenic DCs were similar in the presence and absence of CD100, expression of CD86, but not CD80, was significantly reduced in CD100−/− mice following injection of the disease-initiating Ag. Studies in a DO11.10 TCR transgenic adoptive transfer system demonstrated the functional importance of CD86 expression in early T cell proliferation and cytokine production after i.p. immunization. Although studies showing the functional importance of CD86 in early CD4+ cell activation were performed in a TCR transgenic system, it is likely that CD86 plays a similar role in nontransgenic systems (29), including T cell proliferation in C57BL/6, BALB/c, and NOD strains (30). Transfer of CD100+/+ DO11.10 cells to either CD100+/+ or CD100−/− recipients showed some reduction in proliferation in CD100−/− recipients, suggesting that CD100 from sources other than the Ag-specific CD4+ cells plays a role in early events in T cell activation.

The mechanism underpinning this particular observation is not clear. It is possible that transfer of Ag-specific CD100−/− cells would have a more substantial effect on limiting proliferation.

The current studies focus to a considerable extent on the role of CD100 in affecting the ability of DCs to present Ag to and activate CD4+ cells. B cells are influenced by CD100, express CD72, CD80, and CD86, and have the capacity to present Ag to CD4+ cells. CD100 may influence the Ag-presenting capacity of B cells, particularly in the generation of memory CD4+ cells, because both simulations (31) and experimental studies (32) suggest that B cells may be important in this process.

Renal injury in the current studies is driven primarily by the deposition of immune complexes in glomeruli. Both CD100 and the tissue receptor for CD100, plexin-B1, are expressed in the kidney (6, 33), the former largely in the tubulointerstitial compartment (K. M. O’Sullivan, M. Li, and A. R. Kitching, unpublished observations). Therefore, although intrarenal CD100-plexin B1 interactions may be relevant to the development of other forms of immune renal injury, particularly those with significant tubulointerstitial damage or those involving cell-mediated effector injury (20, 23), they are unlikely to have played any role in this model. Although these studies do not address a potential role for the soluble form of CD100, overexpression of soluble CD100 results in enhanced T cell-dependent Ab production (8). In conclusion, the current studies implicate CD100 in DC activation by expression of CD86, leading to a number of effects on the generation of pathogenetic humoral responses including the activation, cytokine expression, and survival of CD4+ cells.

Acknowledgments

We acknowledge the technical assistance of Alice Wright and Gabrielle Wilson and the assistance of Dr. Paul Hutchinson with flow cytometry.

Disclosures

The authors have no financial conflict of interest.

References


