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A Novel Mutation in CD83 Results in the Development of a Unique Population of CD4+ T Cells


Using a mouse mutagenesis screen, we have identified CD83 as being critical for the development of CD4+ T cells and for their function postactivation. CD11c+ dendritic cells develop and function normally in mice with a mutated CD83 gene but CD4+ T cell development is substantially reduced. Additionally, we now show that those CD4+ cells that develop in a CD83 mutant animal fail to respond normally following allogeneic stimulation. This is at least in part due to an altered cytokine expression pattern characterized by an increased production of IL-4 and IL-10 and diminished IL-2 production. Thus, in addition to its role in selection of CD4+ T cells, absence of CD83 results in the generation of cells with an altered activation and cytokine profile. The Journal of Immunology, 2004, 173: 2995–3001.

CD83 sequence analysis
The exons of CD83 were amplified from affected and unaffected mice using AmpliTaq gold master mix (Applied Biosystems, Foster City, CA). Amplicons were purified using a mixture of streptavidin-alkaline phosphatase and exconuclease (USB, Cleveland, OH) and sequenced on an ABI3700 capillary electrophoresis instrument (Applied Biosystems).

Contact hypersensitivity model
Mice were sensitized on the shaved dorsal flank with 100 μl of 0.5% FITC (Sigma-Aldrich, St. Louis, MO) on days 0, 1, and 7. On day 12, mice were challenged with 25 μl of 0.5% FITC on the dorsal surface of the ear. Ear thickness was measured before and 24 h posttreatment. One group of mice was treated with dexamethasone (3 mg/kg) delivered by oral gavage.

Ab generation and staining of wild-type and LCD4.1 bone marrow-derived dendritic cells
mAbs to mouse CD83 were generated essentially as described previously (15). To analyze CD83 expression on matured dendritic cells, bone marrow cultures were set up as previously described (16). Cells were stained using 1 μg/ml anti-mouse CD83 and anti-mouse MHC class II (MCHC)-biotin (BD Pharmingen, San Diego, CA) followed by goat anti-rabbit Fc-PE and streptavidin-allophycocyanin. Cells were stained with propidium iodide for live/dead cell discrimination and analyzed on a MoFlo cytometer (Dako-Cytomation, Ft. Collins, CO).
Generation of transgenic mice and rescue of CD83 mutant phenotype

Animal studies were conducted following public health service guidelines. A 35.2-kb XhoI fragment from BAC clone 103P23 (RPCT-23 genomic library; Research Genetics, Huntsville, AL) was subcloned into the SuperCos I vector (Strategene, La Jolla, CA). For oocyte microinjection, this construct was digested with NotI and a 35.2-kb insert was isolated. Microinjections of (C57BL/6 × SJL)F1 hybrid oocytes were conducted by Xenogen Biosciences (Cranbury, NJ). Transgenic animals were bred with LCD4.1 mutant animals to generate LCD4.1 homozygous animals expressing CD83 from the transgene. PBLs were stained with anti-CD4 and anti-CD8 (BD Pharmingen) and analyzed by flow cytometry as above. For immunohistochemistry, sections were cut from frozen mouse thymus, fixed in methanol/acetic, rinsed in TBS, and incubated with serum-free protein block (DakoCytonation). Sections were incubated in anti-mouse CD8 in Ab/Ab diluent (DakoCytomation) and endogenous peroxidase activity inactivated. Slides were rinsed and incubated with a HRP-conjugated secondary Ab (EnVision kit; DakoCytonation). AEC (EnVision kit; DakoCytonation) was used to detect HRP and nuclei were counterstained using Hematoxylin QS (Vector Laboratories, Burlingame, CA).

Lymphocyte functional assays

Responder CD4+ T cells from wild-type, LCD4.1, or LCD4.1/CD83tg animals were isolated using magnetic beads according to the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA). APCs were isolated in a similar manner using magnetic anti-CD11c beads (Miltenyi Biotec). Cells were incubated in RPMI 1640 complete medium with or without recombinant murine IL-2 for the specified times. [3H]Thymidine was added during the last 8 h of these cultures and incorporation was counted. Bone marrow chimeras were constructed essentially as described previously (6).

Cell stimulation for analysis of cytokine production

CD4+ T cells were purified from three individual wild-type and LCD4.1 mutant mice and cultured in complete RPMI 1640 with plate-bound anti-CD3 and anti-CD28 (BD Pharmingen) or PMA/ionomycin (Calbiochem, San Diego, CA) as described in Table I. After 3 days, 50 μl of supernatant was collected and IL-2, IL-4, IL-10, and GM-CSF levels measured by ELISA (R&D Systems, Minneapolis, MN). For RNA quantitation, CD4+ T cells were stimulated with anti-CD3 and anti-CD28 Abs (1 μg/ml and 0.2 μg/ml CD28, respectively). RNA was isolated using TRI reagent (Sigma-Aldrich) and cDNA was synthesized using SuperScript II (Invitrogen Life Technologies, Carlsbad, CA). Real-time PCR analysis was performed in an ABI7700 instrument. For purposes of relative comparison between samples, the cDNA samples were amplified separately for the housekeeping gene GAPDH and its levels were used for normalization. The GAPDH primers used were 5′-CCTCTCTGGCTTCACTCTTGGT and 5′-CCGGAAGAGATGCGCTTGGA-3′, in conjunction with the TaqMan probe FAMCCTAGCGGTTTGCCTG-3′. IL-4 and IL-10 were tested using the Assays-on-Demand reagents purchased from Applied Biosystems. Using immunohistochemistry (Fig. 2A) we were unable to detect any CD83 expression in the thymus of mutant animals. In a supplemental set of experiments, Chinese hamster ovary cells were transfected with DNA clones containing Flag-tagged full-length wild-type or mutant CD83 genes. From these experiments, we determined that although RNA levels were equivalent for both constructs, there was a severe reduction in the amount of CD83 protein.

Table 1. LCD4.1 mutant CD4+ T cells exhibit altered levels of cytokines after stimulationa

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>CD3/CD28lowb</th>
<th>CD3/CD28highc</th>
<th>PMA/Ionomycind</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt</td>
<td>Mt</td>
<td>Wt</td>
<td>Mt</td>
</tr>
<tr>
<td>IL-2</td>
<td>ND</td>
<td>12 ± 1.1</td>
<td>56.7 ± 15.2</td>
<td>133 ± 29.9</td>
</tr>
<tr>
<td>IL-4</td>
<td>ND</td>
<td>12 ± 11.4</td>
<td>606 ± 126.1</td>
<td>1261 ± 248.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>ND</td>
<td>24 ± 4.6</td>
<td></td>
<td>95 ± 14.4</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>16 ± 8.6</td>
<td>220 ± 55.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values shown are picograms per milliliter.

b One and 0.2 μg/ml, respectively.

t Ten and 2 μg/ml respectively.

f 0.08 and 0.74 μg/ml, respectively.

Wt, Wild type; Mt, mutant; ND, not detected.
detected by Western blot analysis using either an anti-Flag Ab or polyclonal anti-CD83 Abs (data not shown).

To address the effect of this mutation in an inflammatory response, animals were sensitized with FITC in a contact hypersensitivity protocol known to elicit a Th2 response (17) using ear swelling as a readout. As shown in Fig. 1D, wild-type animals responded well to a challenge with FITC, and this response was inhibited by treatment with dexamethasone. The FITC-treated LCD4.1 animals failed to show a significant change in ear thickness over untreated controls. This is similar to the previously reported results in which CD83 knockout mice were treated with oxazalone as Ag (6) in a Th1-dependent contact hypersensitivity assay. Because CD83 has been associated with the maturation of dendritic cells (18), we also evaluated the ability of CD11c⁺ cells to migrate to draining lymph nodes upon exposure to FITC. This response was unaffected in mutant animals (data not shown).

FIGURE 1. Identification and initial characterization of the LCD4.1 mutant mice. A, Percentage of CD4⁺ T cells in peripheral blood from multiple N2 mice. Each diamond represents an individual animal. B, Exon organization and stop codon mutation found in the LCD4.1 mutant animals. C, Bone marrow-derived dendritic cells from mutant mice express dramatically reduced levels of CD83. Bone marrow-cultured cells (day 7) were stained for their cell surface expression of both MHCI and CD83 and were analyzed by flow cytometry. Approximately the same percentage of cells express high levels of MHCI, although only low amounts of CD83 were detected at any time point on mutant cells. D, Mutant mice fail to respond in a contact hypersensitivity model. Ear thickness was measured at 24 h after challenge.

Functional defect of LCD4.1 animals tracks to CD4⁺ T cells
Prompted by the reported in vitro expression patterns of CD83 in mature dendritic cells and activated CD4⁺ T cells, we examined both dendritic cell and CD4⁺ T cell function from LCD4.1 animals. In an initial series of experiments, we were unable to detect any robust alteration in the ability of CD4⁺ T cells from LCD4.1 mutant animals to respond to direct mitogenic stimulation with Abs to CD3 and CD28 (data not shown). LCD4.1 animals failed to express CD83 in their thymus (Fig. 2A), spleen, and lymph node (data not shown), but this expression was rescued by the CD83 transgene (Fig. 2A). Most important, transgenic expression of CD83 in LCD4.1 mutant mice was able to restore normal CD4⁺ T cell levels in the lymphoid compartment (Fig. 2B).

Genetic rescue of LCD4.1 mutant animals
To confirm that the LCD4.1 mutant phenotype was due to the mutation observed in the CD83 gene, transgenic animals were generated using the full-length CD83 gene. A 35.2-kb genomic DNA fragment containing the full-length CD83 gene and presumptive regulatory elements was used for oocyte microinjection. Four lines were established from these founder transgenic animals and analyzed for transgene expression and stability. Based on transgene stability, one transgenic line was selected for further studies, bred onto the homozygous mutant background, and analyzed for CD83 expression by immunohistochemistry and flow cytometry (data not shown). LCD4.1 animals failed to express CD83 in their thymus (Fig. 2A), spleen, and lymph node (data not shown), but this expression was rescued by the CD83 transgene (Fig. 2A). Most important, transgenic expression of CD83 in LCD4.1 mutant mice was able to restore normal CD4⁺ T cell levels in the lymphoid compartment (Fig. 2B).
In a complementary set of experiments, we characterized the ability of wild-type and CD83 mutant T cells to respond in a MLR. CD4$^+$ T cells were purified from wild-type and LCD4.1 mutant animals (both C57BL/6) and stimulated with purified BALB/c CD11c$^+$ splenocytes. Although wild-type CD4$^+$ T cells responded well in this assay, CD4$^+$ T cells from LCD4.1 mutant animals exhibited a dramatically compromised response (Fig. 3B), and this impaired response was rescued by the expression of a CD83 transgene in the mutant background (Fig. 3B). The decreased responsiveness was seen at all time points examined and thus did not represent a difference in the kinetics of T cell responses (data not shown), nor did it represent a defect in early stimulation since the induction of CD69 proceeded normally (data not shown). Furthermore, this impaired response to allogeneic stimulation was restored by the addition of exogenous IL-2 in a dose-dependent manner, leading to equal proliferative responses in both wild-type and LCD4.1 mutant animals (Fig. 3C).

Aberrant cytokine profile of LCD4.1 mutant cells upon stimulation

As shown in Fig. 1D, LCD4.1 mutant animals fail to respond in a Th2-based contact sensitivity protocol. Furthermore, CD4$^+$ T cells from these animals exhibit an impaired proliferative response in an in vitro setting (Fig. 3B), although this is overcome by the addition of exogenous IL-2 (Fig. 3C). Based on these observations, we characterized the cytokine secretion patterns of LCD4.1 CD4$^+$ T cells. CD4$^+$ T cells isolated from LCD4.1 mutant and wild-type animals were stimulated with a combination of anti-CD3 and anti-CD28 Abs or with PMA and ionomycin. As shown in Table I, under suboptimal stimulation conditions, mutant CD4$^+$ T cells produced greater amounts of IL-2 than did their wild-type counterparts, indicating that signaling via the TCR was intact. This was also supported by expression of activation markers such as CD69 (data not shown). Upon maximal stimulation however, CD83 mutant cells produced significantly less IL-2 than wild-type CD4 cells and this may account for the proliferative defects noted above (Fig.

![Image](http://www.jimmunol.org/Downloadedfrom)
Most significant, as shown in Table I, CD4+ T cells from mutant animals accumulated increased levels of IL-4 and IL-10 under all stimulation conditions and this phenotype was rescued by transgenic expression of CD83 (data not shown). Although increased amounts of IL-4 and IL-10 may account for the decrease in IL-2 production under conditions of maximal stimulation, the ability of mutant cells to produce normal amounts of IL-2 under suboptimal conditions (under which IL-4 and IL-10 are already increased) suggests that the decrease in IL-2 production may be independent of IL-4 and IL-10 concentrations (see below).

To further define the phenotype associated with the mutation in CD83 hereby described, real-time PCR analysis was conducted on samples generated by Ab stimulation of either wild-type or mutant CD4+ T cells and data were normalized using expression of DAD. As shown in Fig. 5, there was a concomitant increase of RNA associated with the increase in cytokine accumulation upon T cell activation. Although these two cytokines are characteristic of the Th2 lineage, there was no correlation between the expression of two transcription factors known to modulate the expression of Th2 and Th1 cytokines, GATA3 and T-bet (19–21) and the cytokine profile of mutant cells (data not shown).

**Discussion**

Our understanding of the role of CD83 in T cell development has been dramatically enhanced by the seminal studies using CD83...
knockout mice (6). Examination of these mice indicated that lack of CD83 expression on thymic epithelial cells had a profound effect on the development of CD4+ T cells. A role for CD83 in T cell function has also been suggested by studies using recombinant protein (10, 12, 22). In these articles, the authors made use of recombinant CD83 protein and were able to demonstrate modulation of immunological responses both in vitro and in vivo. These conclusions have been further supported by studies using CD83-transgenic animals (13). In this study, we build on these results to demonstrate that a mutation in the CD83 gene, which leads to negligible expression levels of CD83, imprints a novel phenotype on the population of CD4+ T cells that develops.

The defect in CD83 expression observed in LCD4.1 animals results in a very similar developmental phenotype to that described in CD83 knockout animals. In particular, LCD4.1 CD83 mutant mice exhibit low levels of CD4+ T cells in their lymphoid compartment. Furthermore, this phenotype can be rescued in bone marrow chimeras in which mutant LCD4.1 CD4+ T cells are allowed to develop in a normal thymic environment and by the ability of the LCD4.1 thymic environment to imprint the mutant phenotype on wild-type CD4+ T cells. In addition, both the CD83 knockout and the LCD4.1 mice fail to show any functional deficiencies in their CD11c dendritic cell compartment.

To define the function of the CD4+ T cells from LCD4.1 mice, we conducted a series of in vitro studies that allowed us to unveil a novel functional abnormality. In this study, we report that in contrast to the description of the CD83 knockout mouse, the LCD4.1 mutant thymic environment yields a population of CD4+ T cells that fails to proliferate in response to activation by APCs despite normal up-regulation of CD69 on the cell surface. The induction of CD69 on the surface of these cells suggests that the initial components of an allogeneic response are proceeding normally in these animals while the diminished proliferative responses indicate that downstream responses are not functional. This is supported by our observation that exogenously added IL-2 is able to overcome this proliferative defect. The discrepancy between this functional data and that of Fujimoto et al. (6) may be a consequence of the nature of the mutation found in the LCD4.1 mice which results in the extension of the cytoplasmic tail of CD83 by 55 aa. This novel cytoplasmic tail may provide access to an altered signal transduction response. However, CD83 protein levels are severely compromised in these animals and the recessive nature of this phenotype suggests that this is a loss of function mutation. Thus, it seems unlikely that the phenotype hereby described is due to novel functionality associated with the extended cytoplasmic tail of the mutated CD83 protein.

A provocative characteristic of CD4+ T cells that develops under the mutated CD83 environment is their aberrant cytokine expression. These cells, when activated, produce elevated levels of IL-4 and IL-10 cytokines. This may in part account for the decrease in IL-2 production induced by maximal stimulation. Furthermore, our studies using bone marrow chimeras demonstrate that this functional defect is imprinted during thymic development. Notwithstanding this Th2-like profile, we have been unable to show a corresponding alteration in the transcription factors implicated in the transcriptional control of Th1 or Th2 skewing (GATA3 and T-bet) (19–21). Thus, the mechanism by which the absence of CD83 expression in the thymus alters cytokine expression by CD4+ T cells is unclear at present. Although there are no obvious signaling domains within the cytoplasmic region of CD83, it is possible that engagement of CD83 by its presumptive ligand alters the cytokine environment and the differentiation potential of those CD4+ T cells that survive selection. In addition, it is also conceivable that the presence or absence of CD83 alters the affinity of MHCII-restricted TCRs selected during thymic development and preliminary experiments using OT-II-transgenic, LCD4.1 mutant animals have demonstrated an alteration in thymic selection as reflected by a decrease in the number of CD4+ T cells bearing the clonotypic receptor. As a further measure of altered selection, the amount of surface CD5 is decreased on the CD4+ cells from mutant animals, suggesting a change in the affinity of the TCR on those cells that survive thymic selection (data not shown).

Expression of CD83 in the thymus has now been implicated in CD4+ T cell development from both a knockout and a mutated phenotype. In this study, we have obtained further insight into the phenotype of CD4+ T cells matured under a mutated CD83 environment and have determined that they exhibit lowered proliferative responses and aberrant cytokine production profiles. Although this functional change is imprinted during thymic development, the role of CD83 in peripheral T cell function remains to be determined and may represent a new axis of biology as well as a new therapeutic opportunity.

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