Cutting Edge: CD83 Regulates the Development of Cellular Immunity

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*J Immunol* 2002; 168:2599-2602; doi: 10.4049/jimmunol.168.6.2599

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We recently found that human CD83, a marker of mature dendritic cells, is an adhesion receptor that binds to resting monocytes and a subset of activated CD8+ T cells. We injected CD83-Ig into mice transplanted with the immunogenic P815 mastocytoma and showed that it significantly enhanced the rate of tumor growth and inhibited the development of cytotoxic T cells. In contrast, mice immunized with CD83-transfected K1735 cells, a poorly immunogenic melanoma, could prevent the outgrowth of wild-type K1735 cells. Studies performed in vitro with human PBL showed that coimmobilized CD83-Ig and anti-CD3 enhanced T cell proliferation and increased the proportion of CD8+ T cells. CD83-transfected B lymphoblastoid T51 cells stimulated T cell proliferation more effectively than untransfected T51 cells in MLR cultures and increased the generation of cytolytic T cells. We conclude that CD83 is a functionally important receptor that can regulate the development of cellular immunity by interacting with its ligand(s). The Journal of Immunology, 2002, 168: 2599–2602.

A n inducible glycoprotein member of the Ig superfamily, CD83 is related to the B7 ancestral family. Its cell membrane expression is widely used as a marker of differentiated or activated human dendritic cells (DC)1 (1). The CD83 sequence is conserved in the chimpanzee (2) and the mouse (3). We have recently demonstrated that a human CD83 ligand is expressed on resting monocytes and on a subpopulation of activated T cells (4). A study in TCR transgenic mice has shown that Ig fusion proteins which express the extracellular part of the mCD83 molecule (mCD83-Ig) specifically inhibit Ag-specific T cell proliferation and IL-2 secretion in spleen cell cultures (5). Activated DC and B lymphocytes release a soluble form of CD83, primarily by proteolytic shedding, and sera of normal human donors contain small amounts of circulating CD83 (6). However, the function of CD83 and its ligand remains largely unknown.

To study CD83 function, we have constructed two fusion proteins of the extracytoplasmic domain of human CD83, one with a human IgG1 tail (4) and the other with a mouse IgG2a tail. We also retrotransfected mouse and human cell lines with human CD83. We now show that a soluble CD83-Ig fusion protein is immunosuppressive, both when tested with human cells in vitro and with mouse cells in vivo. We also demonstrate that CD83-Ig is costimulatory when coimmobilized with anti-CD3 and that cells from a mouse melanoma of low immunogenicity (K1735) transfected with CD83 induce a tumor rejection response against wild-type K1735 cells.

Materials and Methods

Experimental animals and protocols

Six- to 8-wk-old normal female C3H and DBA/2 mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN). C3H mice were implanted s.c., on one side of the back, with 2 × 10^6 wild-type K1735 cells (K1735-WT) or with K1735 cells retrotransfected to express CD83 (K1735-CD83). In some experiments, mice were implanted 4 wk later with 2 or 4 × 10^4 K1735-WT cells. Tumor growth was monitored daily and mice were sacrificed when the tumor surface reached 400 mm^2. DBA/2 mice were implanted s.c. on one side of the back with 10^3, 1 × 10^3, or 500 P815 cells and injected i.p. with 100 μg of CD83-murine Ig fusion protein (CD83-mIg), 1, 3, and 6 days after tumor implantation. Tumor growth was monitored daily and animals were sacrificed when the tumor surface reached 100 mm^2. All in vivo experimental protocols were approved by the Pacific Northwest Research Institute Committee for Use and Care of Laboratory Animals (Seattle, WA). The Pacific Northwest Research Institute animal facilities are American Association of Laboratory Animal Care accredited.

Tumors and cell lines

For in vivo experiments, we used the highly immunogenic mouse mastocytoma P815 (DBA/2 origin) and the M2 clone of the poorly immunogenic mouse melanoma K1735 (C3H origin) (7), referred to in this study as K1735-WT. The K1735-WT cells were retrotransfected with human CD83 using previously described techniques (4) and are referred to as K1735-CD83.

For in vitro experiments, we used cells from human B lymphoblastoid line T51 (8) and T51 cells that had been retrotransfected with human CD83 as previously described (4) and referred to as T51-CD83. In addition, we used mouse EL4 (9) cells, as well as NK-sensitive human K562 (10) and mouse lymphoma YAC-1 cells (11).

Purification of PBMCs and T cells

A freshly harvested mouse spleen was minced and the suspended cells were filtered through a cell strainer (BD Biosciences, Franklin Lakes, NJ),
after which the splenocytes were separated on Ficoll-Hypaque gradients (Lymphocyte-M; Cedarlane Laboratories, Westbury, NY).

Human PBMC (5 × 10^5) were isolated as previously described (4). For experiments involving T cell activation, PBMCs were resuspended in RPMI medium and T cells were separated from APC by two rounds of adherence to nylon wool columns (12).

Flow cytometry, mAbs, fusion proteins, and CSFE labeling

Labeling for flow cytometry was conducted at 4°C in DMEM (Life Technologies, Grand Island, NY) supplemented with 5% FCS without azide (referred to as DMEM). Anti-human CD3 (64.1) (13), anti-human and mouse CD4, and anti-human and mouse CD8 mAbs were bought from BD PharMingen (Lexington, KY). A CD83-human Ig fusion protein (CD83-hIg) was made as previously described (4). A CD83-mIg was generated similarly to its human counterpart, by cloning a murine tail (14) in the place of the human one. CFSE was bought from Molecular Probes (Eugene, OR) and stored desiccated at −30°C in DMSO. Cells were incubated 15 min at 37°C before they were used for in vitro tests (15).

Proliferation assays

PBMC or spleen cells were cultured using a standard medium (referred to as “RPMI medium”), which consisted of RPMI 1640 (Life Technologies) supplemented with glutamine (1% Life Technologies), penicillin/streptomycin (1%; Life Technologies), and 10% FCS (Atlanta Biologicals, Norcross, GA).

U-bottom 96-well plates (Corning Glass, Corning, NY) were coated with 50 μl of 1 μg/ml of anti-CD3 (64.1), alone or in combination with 10 μg/ml of CD83-Ig or anti-CD28 (9.3) for 2 h at 37°C. Wells were washed with PBS and cells were plated in triplicate at 10^4, 5 × 10^3, 2.5 × 10^3, and 1.25 × 10^3 cells/ml. As controls, cells were incubated with medium only or with PHA 1 μg/ml (Sigma-Aldrich, St. Louis, MO).

After 3 days, the cells were pulsed with 1 μCi of tritiated thymidine for 7 h and the incorporated radiolabeling was counted with TopCount NXT counter (Packard Instrument, Meriden, CT).

Cytotoxicity assays

PBMC were first stimulated for 7 days in the presence of wild-type T51 (T51-WT) or T51-CD83. To prevent the proliferation of the stimulatory cells, both T51-WT and T51-CD83 were incubated with 100 μg/ml of mitomycin C (Sigma-Aldrich) for 1 h at 37°C in PBS. In some experiments, CD83-hlg 10 μg/ml was added after 3 days of incubation. Target cells were labeled 1 h at 37°C with 111Cr, washed two times, and plated at 1 × 10^5 cells/ml in V-bottom 96-well plates (Corning Glass). Effector cells were washed and incubated with the target cells at an E:T ratio of 1:100, 1:50, 1:25, and 1:12.5 for 4 h, in culture medium. Subsequently, 40 μl of supernatant was collected and 111Cr release was measured using chromoluminescence on a TopCount instrument. The percentage of lysis was calculated from the formula: 100 × (E−M)/(M−M), where E is the experimental release, M is the spontaneous release in the presence of medium alone, and T is the maximum release in the presence of 2% Triton X-100.

Results

Exploration of the in vivo role of CD83 in mice

Two sets of mouse experiments were first performed to explore whether CD83 may have an immunoregulatory function in vivo. CD83-hlg and CD83-mlg were tested for their ability to bind to mouse cells by comparing the binding of PBMC, splenocytes, and lymphocytes from lymph nodes by flow cytometry as previously described (4). We found that CD83-Ig bound to 90% of monocytes in peripheral blood, to <5% of lymphocytes from lymph nodes and to ~15% of a non-T cell population of splenocytes.

Based on this observation, two different approaches were taken. First, we implanted immunogenic P815 tumor cells and subsequently injected mice i.p. with CD83-mlg, as described in Material and Methods. Fig. 1A shows that in groups receiving CD83-mlg, tumors were two times larger than those in control mice (p < 0.05). In addition, tumors in mice receiving CD83-mlg grew along the needle trajectory and their draining lymph nodes were enlarged (data not shown). A repeat experiment was performed in which 14 mice were implanted with 10^6 P815 cells, with 7 mice injected with CD83-mlg (3 × 100 μg) and 7 mice injected with PBS as controls. Also in this experiment, tumors grew approximately twice as fast in mice given CD83-mlg. Fig. 1B shows that lysis of P815 cells by splenocytes from CD83-mlg-treated mice, harvested 15 days after the onset of the experiment, was significantly lower (p < 0.05) than lysis by splenocytes from the PBS controls.

Second, we implanted s.c. CD83-retrotransfected poorly immunogenic tumor K1735 (K1735-CD83) in eight C3H mice. K1735-CD83 cells formed small tumor nodules that regressed within a week (data not shown). One month later, four of these eight mice were implanted with 2 × 10^6 K1735-WT cells (Fig. 2A) and four were injected with 4 × 10^5 K1735-WT cells (Fig. 2B); the same amount of K1735-WT cells were implanted in naive control mice. After 40 days, K1735-WT cells grew progressively in all four naive mice. In contrast, five of the eight mice that had rejected the K1735-CD83 cells bore tumors smaller than 10 mm^2 or were tumor-free (Fig. 2, A and B), two developed slow-growing tumors (Fig. 2A), and one had a tumor that grew similarly to those in the naive mice (Fig. 2B). Lymphocytes from mice implanted with K1735-CD83 cells proliferated twice as much against K1735 than
naive lymphocytes when combined with K1735-WT cells in vitro (data not shown).

**CD83-Ig increases proliferation of human T cells when coimmobilized with anti-CD3 mAb**

To test whether immobilized CD83 could affect proliferation of human T cells, fresh human PBMC were incubated at 37°C in plastic wells onto which 10 μg/ml of CD83-Ig was coimmobilized with 1 μg/ml of anti-CD3 mAb. CD83-Ig coimmobilized with anti-CD3 mAb rapidly induced a strong proliferation of the PBMC, while anti-CD3 mAb alone induced a much lower proliferation, and CD83-Ig alone had no effect (Fig. 3A). When adherent cells were removed from the PBMC population by passage through a nylon wool column, PHA proliferation decreased 3-fold, while proliferation in response to anti-CD3 plus anti-CD28 increased. In contrast, no proliferation was observed in the presence of coimmobilized anti-CD3 plus CD83-Ig (Fig. 3B).

**CD83-Ig increases proliferation and activation of CD8+ T cells**

To determine what cell population(s) proliferated at an increased level by coimmobilized anti-CD3 and CD83-Ig, human PBMC were labeled with CFSE before stimulation. Fig. 4 shows that the ratio of CD8+ T cells to CD4+ T cells increased by 2.5 when CD83-Ig was coimmobilized with anti-CD3. In addition, CD8+ T cells were engaged in more cell cycles than CD4+ T cells during an anti-CD3/CD83-Ig stimulation as compared with an anti-CD3 stimulation alone.

**Expression of CD83 on the T51 B cell line increases T cell MLR response and generation of cytotoxicity, and soluble CD83-hlg suppresses this response**

To further explore CD83 costimulatory signals, CD83 was expressed at the surface of cells from the T51 lymphoblastoid B cell line as demonstrated in Fig. 5A; T51-WT cells did not express CD83. Both T51-WT and T51-CD83 expressed high levels of MHC class I and II and CD80 and CD86 (data not shown). T51-WT and T51-CD83 cells were compared for their ability to stimulate allogeneic PBMC in a MLR. Fig. 5B shows that exposure of PBMC to T51-CD83 dramatically increased their proliferation, as compared with exposure to T51-WT cells.

To test whether CD83 costimulation improved the generation of cytolytic T cells, PBMC were preincubated for 7 days with T51-WT and T51-CD83 in the presence or absence of soluble CD83-Ig added to the culture medium, after which they were used as effectors against 51Cr-labeled T51-WT and NK-sensitive K562 cells. Fig. 6A shows that lysis of T51-WT cells increased 3-fold after stimulation with T51-CD83 vs T51-WT. Interestingly, lysis of the K562 cells slightly decreased at the same time (Fig. 6B). Addition of soluble CD83-Ig during the preincubation dramatically decreased both the NK and the T cell-mediated cytotoxicity (Fig. 6, A and B).
primarily expressed on resting monocytes, plays an important role in the generation of cell-mediated immune responses. We speculate that this interaction facilitates the differentiation of monocytes into functional APC.

Interestingly, we have noticed in the presence of immobilized CD83-Ig, a down-regulation of CD14 expression by adherent cells and the appearance of collagen-positive, fibroblast-like cells with morphology reminiscent of fibroblasts (19, 20) (N. Scholler, unpublished observations). The regulatory processes that govern the differentiation of blood-borne fibroblasts are still unclear (21) and our data suggest that binding of CD83 to monocytes may facilitate their differentiation into fibroblasts (and maybe other cells that can present Ag, including DC); this will be the subject of further studies.

Discussion
To study the function of CD83 and its interaction with CD83 ligand(s), we used human and mouse tumor cell lines retrotransfected with the full-length human CD83 and two fusion proteins of human CD83 obtained by fusing the extracytoplasmic domain of CD83 to human or mouse Ig tails which had been mutated to prevent nonspecific Fc binding.

We first observed that soluble CD83-mIg significantly enhanced the growth of transplanted P815 tumor cells in mice and decreased the cytotoxicity of spleen cells from mice injected with CD83-mIg against P815 cells. We also found that expression of (human) CD83 at the surface of cells from mouse melanoma K1735, whose immunogenicity is very low, prevented their ability to form tumors and made them capable of inducing an immune response causing the rejection of transplanted K1735-WT cells in five of eight mice and slowing the tumor growth rate in two other mice. We tentatively conclude that interaction between CD83 and its ligand(s) may be involved in regulation of immune response to tumor.

To study the functional aspects of CD83-ligand interaction we also performed a series of in vitro experiments with human PBMC. We first demonstrated that coimmobilization of CD83-Ig with anti-CD3 induced a proliferative response, particularly by CD8+ T cells, and that removal of host cells adherent to nylon wool (most likely monocytes) obliterated this response while it slightly increased proliferation of the T cells in the presence of coimmobilized anti-CD3/anti-CD28 mAbs.

In a second set of experiments, we transfected cells from the B lymphoblastoid T51 line to express CD83 at their surface. This line was chosen because it expresses a high level of MHC class I and II, as well as CD80 and CD86. T51 cells expressing CD83, when compared with T51-WT cells, preferentially induced proliferation of CD8+ T cells and significantly increased the generation of a cytolytic response to T51 cells when combined in MLR cultures. Notably, the addition of soluble CD83-Ig during the incubation of the PBMC with the T51-CD83 abrogated the generation of cytotoxicity.

CD83-Ig does not bind to human resting T cells (4), while it binds to monocytes. Therefore, it is not surprising that when adherent cells were removed from PBMC, the combination of anti-CD3 with CD83-Ig did not induce T cell proliferation, while removal of the adherent cells increased the proliferation triggered by immobilized anti-CD3 and anti-CD28 and decreased the PHA proliferation by a factor of 3, which is in agreement with published data (16–18). The in vitro activity of immobilized CD83 thus depends on the presence of adherent cells, most likely monocytes. We conclude that an interaction between CD83 and its ligand(s),

References