Activation-induced expression of murine CD83 on T cells and identification of a specific CD83 ligand on murine B cells

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Keywords: CD83, dendritic cells, T cell activation

Abstract

Human CD83 is a cell surface protein expressed predominantly by dendritic cells (DC) and lymphoid cells. So far, there exists no information on the function and distribution of mCD83. Here we demonstrate that mCD83 is moderately expressed on resting T cells and DC, but strongly increases in its expression on T cells following activation with antigenic peptides or T cell receptor-specific mAb. When returning to the resting state, T cells down-regulate CD83 again. Ig fusion proteins which express the extracellular part of the mCD83 molecule (mCD83–Ig) specifically inhibit antigen-specific T cell proliferation and IL-2 secretion in spleen cell cultures from DO11.10 T cell receptor transgenic mice. Staining of spleen cells from BALB/c, XID and µMT (B cell) knockout mice with mCD83–Ig proteins reveals the presence of a CD83 ligand predominantly expressed most likely by B220⁺ cells since spleen cells from µMT knockout mice do not bind mCD83–Ig. CD83, besides its established expression on human dendritic cells, thus, also represents a new marker molecule on activated T cells which with its specific ligand is involved in the regulation of T cell responses.

Introduction

Human CD83 is a 45 kDa glycoprotein which belongs to the Ig superfamily (1,2). Recently the murine cDNA of CD83 has also been cloned, displaying a high degree of conserved amino acids between mouse and man at the level of the expressed protein (3). Human CD83 consists of a single extracellular Ig-like domain, a transmembrane region and a cytoplasmic tail, 39 amino acids long, which lacks any known consensus signaling motifs. In man, CD83 is mainly expressed on the surface of CD11c⁺, interdigitating reticulum cells present in the T cells zones of lymphoid organs and on cells of the dendritic lineage (1). Recent data attribute to dendritic cells (DC) a central role in the activation of naïve T lymphocytes and in the generation of primary T cell responses (4,5). Recent experiments revealed that CD83 on human immature DC is mainly expressed intracellularly and upon maturation of the DC can also be detected on the cell surface (6). CD83, thus, represents a typical surface maturation marker on human DC (7).

To achieve complete activation (8), T cells require a second co-stimulatory signal (9), which can be provided by ligands expressed on the cell surface of professional antigen-presenting cells including DC and B cells. Due to its expression pattern on antigen-presenting cells in T cell-rich areas, hCD83 and its potential ligand might also directly contribute to the activation of T cells. Presently, no data are available, however, that address whether CD83 has a specific ligand on hematopoetic cells and how CD83 or its ligand may influence immune responses. Here we used purified CD83–Ig fusion proteins and an antiserum specific for mCD83 to analyze the expression pattern and kinetics as well as a possible immunological function of CD83 and its putative ligand in cultures containing spleen cells from DO11.10 TCR transgenic mice.

Methods

Cell lines and reagents

All cell lines were maintained in RPMI medium (Gibco/BRL, Eggenstein, Germany) supplemented with gentamicin, gluta-
mine and 10% FCS. A20/7 is a H-2\(^d\) B-cell line and J774 is a monocyctic cell line. CD83-expressing transfectants were generated by electroporation of 20 \(\mu\)g pFM91-CD83 vector, containing the complete mCD83 cDNA, into 1 \times 10^7 TCR\(^+\) 58\(\alpha\)-\(\beta\) T cells (10), as described earlier (11). Neomycin-resistant clones were expanded and analyzed by flow cytometry using a FACSscan (Becton Dickinson, Heidelberg, Germany).

For proliferation assays, spleen cells (2 \times 10^5/well) from 6- to 8-week-old, homozygous DO11.10tg [specific for chicken ovalbumin (OVA)323–339 peptide in the context of H2-A\(^d\)] mice were cultured in 96-well microtiter plates with the indicated, titrated amounts of synthetic, HPLC-purified, chicken OVA323–339 peptide in the presence or the absence of mCD83-Ig fusion proteins, hCD83-Ig, human B7-Ig fusion proteins or purified human Ig at a final concentration of 40 \(\mu\)g/ml. On day 4 of culture 100 \(\mu\)l of each well was removed for IL-2 quantification and the remaining 100 \(\mu\)l was pulsed with \([3H]\)thymidine (0.5 \(\mu\)Ci/ml). IL-2 content in the cultures was assessed by \([3H]\)thymidine uptake of the IL-2-dependent cell line CTLL-2 (5 \times 10^6/well) at 4\(\degree\)C. Cells were fixed and subsequently analyzed in a FACScan (Becton Dickinson, San Jose, Calif.).

Fig. 1. Surface expression of CD83 in CD83 cDNA transfected 58\(\alpha\)-\(\beta\) cells and professional antigen-presenting cells. (A) Shown is the fluorescence of 1 \times 10^4 analyzed 58\(\alpha\)-\(\beta\) cells and two representative CD83\(^+/\) 58\(\alpha\)-\(\beta\) transfectants, the monocytic cell line J774 and bone marrow-derived DC, stained with anti-CD83-specific antiserum or 1:25 to 1:50 dilution preimmune antiserum, since no mAb are available. To demonstrate the binding of the CD83 antiserum to immobilized mCD83–Ig fusion proteins, aliquots (1 \(\mu\)g/lane) of purified hCTLA-4-Ig, mCD83-Ig and hCD83-Ig (containing the extracellular part of the hCD83 molecule) were analyzed in 8% SDS-PAGE followed by an anti-CD83-specific (~1 \(\mu\)g/ml of the immune CD83 serum) immunoblot (anti-CD83). Incubation of the immobilized fusion proteins with the preimmune serum showed no specific protein bands (data not shown). Numbers on the left indicate apparent molecular masses in kDa.

Staining procedures

For FACS staining, Fc receptors of 3 \times 10^5 of the indicated cells were blocked with 10 \(\mu\)g of purified hlg and subsequently incubated (15 min, 4\(\degree\)C) with 1:25 to 1:50 dilution of the anti-mCD83-specific antiserum or 1:25 to 1:50 dilution preimmune serum from the same animal. After washing, CyT2-labeled donkey anti-rabbit Ig (0.5 \(\mu\)g/sample; Dianova, Hamburg, Germany) was added for 10 min at 4\(\degree\)C. Following washing, samples were subsequently stained with phycocyanin (PE)- conjugated anti-CD4-specific mAb (Caltag, San Francisco, CA) or PE-conjugated anti-B220-specific mAb (Caltag) for 10 min at 4\(\degree\)C. Cells were fixed and subsequently analyzed in a FACScan (Becton Dickinson).

Results and discussion

So far nothing is known about the function and cellular distribution of mCD83. Therefore we analyzed the expression pattern of CD83\(^+\) cells by using a CD83 peptide-specific antiserum, since no mAb are available. To demonstrate the specificity of the CD83 antiserum we used CD83 cDNA transfected cell lines which clearly stain with the immune but not with the preimmune serum from CD83 peptide-immunized rabbits (Fig. 1A). Moreover, the CD83-specific antiserum binds to mCD83-Ig in Western blotting but not to hCD83-Ig or hCTLA-4 fusion proteins (Fig. 1B). Flow cytometry analysis further revealed the expression of CD83 on the surface of the monocytic cell line J774 as well as on bone marrow-derived...
CD83 and T cell activation

Fusion molecules to cultures containing freshly isolated spleen cells from DO11.10 TCR transgenic mice. mCD83-Ig inhibited proliferation (Fig. 3A) as well as IL-2 production (Table 1). Especially at lower OVA323–339 peptide concentrations there is a significant reduction of IL-2 secretion in mCD83-Ig-treated cultures. Although mCD83-Ig fusion proteins are not able to completely reduce the proliferation or IL-2 secretion, we take our results as a first hint that CD83 and its ligand may be involved in the process to fully activate T cells. Purified hlg (not shown) as well as hB7–Ig fusion protein used as controls did not inhibit the antigen-specific proliferation and IL-2 secretion. To rule out that mistolding of hB7–Ig or hCTLA-4–Ig fusion proteins is responsible for the observed lack of inhibition in T cell proliferation assays, we tested the individual Ig fusion proteins on control cell lines. As shown in Fig. 3(B), hB7–Ig binds specifically to Jurkat T cells, whereas hCTLA-4–Ig fusion proteins specifically stain the B cell line A20. Taken together, these data argue for a correct folding of the employed hB7–Ig and hCTLA-4–Ig fusion proteins.

Since the inhibition of T cell activation indicates binding to a specific ligand for mCD83, which is not present on long-term cultured A20 or Jurkat cells (Fig. 3B) nor on J774 (murine monocytic cell line), 3D04, DO11.10 (OVA-specific T cell hybridomas) and human Raji B cells (data not shown), we analyzed B220 or CD4 and mCD83-Ig double-stained spleen cells in flow cytometry analysis. As shown in Fig. 4, mCD83-Ig (and hCTLA-4–Ig as a positive control) bound specifically to a B220+ cell population but not to CD4+ T cells. In contrast, hB7–Ig proteins did not stain B220+ B cells. CD11b+ cells showed a high unspecific background staining and complexed hCTLA-4–Ig, hB7–Ig and mCD83–Ig molecules with a comparable affinity (data not shown). B cells from XID mice, which lack B1 B cells, nevertheless displayed a clear staining with mCD83–Ig and hCTLA-4–Ig, but not with hB7–Ig molecules. In contrast, spleen cells from μMT (B cell) knockout mice, which are completely deficient for peripheral B cells, were negative for mCD83–Ig and hCTLA-4–Ig fusion protein binding cells. Thus, the specific ligand for the mCD83 molecule is expressed predominantly on a subpopulation of splenic B cells which do not belong to the class of B1 B cells.

In this study we analyzed a possible immunological function of CD83 as well as of a so far putative CD83 ligand. Using an antiserum specific for the mCD83 molecule as well as employing a panel of Ig fusion proteins we could show for the first time the cellular distribution and regulation of the mCD83 molecule and the involvement of a CD83 ligand which is dominantly expressed on B220+ B cells. Because of its expression kinetics and its cellular distribution, CD83 and its ligand apparently play an important role in the antigen-specific activation of T cells. Since B cells seem to represent the major cell pool of CD83 ligand-positive cells, it will be interesting to learn how these different cell types interact and how CD83-dependent interactions modulate the immune response. Due to our in vitro experiments one could speculate that the interaction of CD83 and its putative ligand induces signaling cascades as already known T cell-expressed co-receptors like CD2 or CD28. The lack of conventional signaling motifs in the cytoplasmic tail of CD83, however, makes it unlikely that CD83 itself is transducing T cell stimulatory signals but rather that the CD83 ligand is triggered by binding of the
Table 1. Inhibition of IL-2 secretion by mCD83-Ig fusion proteins

<table>
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<tr>
<th>OVA 323–339 (ng/ml)</th>
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<td>4848</td>
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<td>4150</td>
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<td>3400</td>
<td>1600</td>
<td>925</td>
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The IL-2 content in supernatant containing the indicated Ig fusion proteins at a concentration of 40 µg/ml of two independent experiments was determined in a standard CTLL assay as described in Methods. Numbers indicate the mean c.p.m. value of triplicates. SD < 15% in all experiments.

Fig. 3. mCD83-Ig fusion proteins inhibit proliferation. (A) Spleen cells (2×10⁵) from DO11.10tg mice were incubated (triplicates) with titrated amounts of OVA323-339 synthetic peptide in RPMI medium ('medium'), together with 40 µg/ml mCD83–Ig ('mCD83Ig') or hB7–Ig ('hB7Ig') fusion proteins. On day 4 of culture proliferation was determined by [³H]thymidine uptake (c.p.m.). Shown is a typical representative of four independent experiments. Error bars indicate SD of triplicate values. The unspecific background of cultures containing no peptide was <500 c.p.m. (B) Binding of Ig fusion proteins to murine A20 B cells and human Jurkat T cells. A20 and Jurkat cells (2×10⁵) were incubated with the indicated Ig fusion proteins (1 µg/ml) for 30 min at 4°C, and were subsequently stained with an anti-human IgG1-specific, FITC-labeled mAb (1:50 dilution) and analyzed in the FACScan (open peaks). Controls were incubated without any fusion proteins (filled peaks). Shown is the relative fluorescence intensity (x-axis) versus the mean cell number (y-axis).

Fig. 4. mCD83-Ig fusion proteins bind to B220⁺ spleen cells. Spleen cells (2×10⁵) from BALB/c, XID or µMT knockout mice were stained (BALB/c) with the indicated Ig fusion proteins (1 µg/ml). Control ('neg.') were incubated without any Ig fusion protein. The diagrams show the mean fluorescence-1 intensity of cells stained with the indicated fusion protein (FITC) (x-axis) versus the mean fluorescence-2 intensity (PE) (y-axis, B220 (A) or CD4 (B)) of 10,000 (BALB/c and µMT⁻/⁻) and 20,000 (XID) analyzed cells.

CD83 molecule and in consequence these signaling events lead to the modulation of a CD4⁺ T cell response. In vivo, CD83 might be also involved in directing or 'homing' activated T cells to immune competent organs like the spleen or lymph...
nodes. The frequently observed localization of mCD83-Ig
binding cells in close contact to blood vessels (unpublished
observations) supports this idea and makes it promising to
look further in this direction. In this respect, the cloning and
molecular characterization of the specific CD83 ligand is a
major focus of ongoing experiments. Taken together our data
reveal an important immunological function of the CD83
molecule having direct consequences for the stimulation of
CD4+ T cells. In this respect the usage of gene manipulated
animals might provide new insights for the role of CD83
also during T cell development, since in humans, CD83 is
also expressed on cells in the thymic epithelium (1).

Abbreviations

DC dendritic cell
DO11.10tg DO11.10 TCR transgenic mice
h/mCD83–Ig human/mouse CD83–Ig fusion protein
PE phycoerythrin
OVA ovalbumin

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