CD83 Expression Is a Sensitive Marker of Activation Required for B Cell and CD4+ T Cell Longevity In Vivo

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*J Immunol* 2007; 179:4550-4562; doi: 10.4049/jimmunol.179.7.4550

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CD83 is a surface marker that differentiates immature and mature human dendritic cell populations. Thymic epithelial cell expression of CD83 is also necessary for efficient CD4+ T cell development in mice. The altered phenotypes of peripheral B and CD4+ T cells, and the reduction of peripheral CD4+ T cells in CD83−/− mice, suggest additional functions for CD83. To assess this, a panel of mAbs was generated to characterize mouse CD83 expression by peripheral leukocytes. As in humans, activation of conventional and plasmacytoid murine dendritic cell subsets led to rapid up-regulation of CD83 surface expression in mice. In primary and secondary lymphoid compartments, a subset of B cells expressed low-level CD83, while CD83 was not detected on resting T cells. However, CD83 was prominently up-regulated on the majority of spleen B and T cells within hours of activation in vitro. In vivo, a low dose of hen egg lysozyme (1 μg) induced significant CD83 but not CD69 expression by Ag-specific B cells within 4 h of Ag challenge. Although B cell development appeared normal in CD83−/− mice, B and CD4+ T cell expression of CD83 was required for lymphocyte longevity in adoptive transfer experiments. Thus, the restricted expression pattern of CD83, its rapid induction following B cell and T cell activation, and its requirement for B cell and CD4+ T cell longevity demonstrate that CD83 is a functionally significant and sensitive marker of early lymphocyte activation in vivo. The Journal of Immunology, 2007, 179: 4550–4562.

A cell surface marker, CD83 is predominantly expressed on mature human dendritic cell (DC) populations including Langerhans cells (1–3). DC maturation results in up-regulation of cell surface CD83 expression, along with the induction of costimulatory molecules (1, 2, 4–8). CD83 is a member of the Ig superfamily with one extracellular Ig V-like domain and largely conserved nucleotide and amino acid sequence homologies between human and mouse (1, 9–11). Cell surface CD83 is up-regulated during the maturation of human DCs (1, 2, 4–6, 8), is expressed by malignant Hodgkin’s cells (12), and is expressed transiently by human monocytes and macrophages (5, 6, 13, 14). Cell surface CD83 is only expressed weakly by a population of human germinal center lymphocytes in vivo (1) and by mitogen-activated lymphocytes in vitro (1, 9, 15), and at low levels by a number of human B and T cell lines (1, 9).

Deletion or mutations of mouse CD83 result in a block in CD4+ T cell development that is characterized by a significant reduction in both CD4 single-positive thymocytes and peripheral CD4+ T cells (16, 17). In the periphery, CD83 expression may also affect lymphocyte function because it is expressed by bone marrow-derived DCs, activated CD4+ T cells, and activated B cells (18–20). Peripheral CD4+ T cells present in CD83−/− mice have an altered phenotype with significantly lower surface expression of TCRβ, CD3, and CD5, and the expansion of CD44high memory cells (16, 21). Additionally, resting B cells from CD83−/− mice have an altered phenotype marked by a ~50% reduction in surface MHC class II (MHC II) expression and impaired up-regulation of CD86 and MHC II expression in response to LPS activation (16, 21). Thus, CD83 expression in the periphery may have functional significance and influence lymphocyte maturation, survival, or function.

Thymic epithelial cell expression of CD83 is clearly essential for normal CD4+ T cell development (16, 17). The presence of CD83 homologs in elasmobranch and teleost fish indicates that CD83 function is also conserved through recent evolution (22). Ligand(s) for CD83 have been described on B cells (18), immature and mature DCs (23), monocytes and CD8+ T cells (24, 25), though its exact expression and molecular characterization remain controversial. However, coimmobilization of rCD83 fusion proteins with anti-CD3 mAbs and the overexpression of CD83 by APCs are reported to enhance T cell proliferation in vitro (25–27). When used in soluble form, rCD83 fusion proteins are reported to block T cell proliferation in vitro (18, 28). Furthermore, the soluble form of the extracellular domain of CD83 abrogates DC-mediated T cell stimulation (23, 29, 30), blocks DC maturation in vitro (8, 23), and prevents experimental autoimmune encephalomyelitis development and progression in mice (28). More recently, reduced alloergic T cell proliferation was reported following down-regulation of DC surface expression of CD83 by RNA interference (27, 31). Although these studies using rCD83 proteins and modulation of CD83 surface expression suggest that CD83 plays an immunostimulatory role in generating immune responses, CD83−/− DCs function normally in MLR assays and in vitro APC assays (16, 17, 25–27).
(21). Suggesting a negative regulatory role for CD83, treatment of mice with a CD83 mAb results in an incremental increase in IgG1 Ag-specific humoral immune responses (20). CD83−/− mice have normal levels of all serum Ig isotypes, and generate normal Ag-specific humoral immune responses, but with significantly delayed kinetics that may result from their severe deficiency in CD4+ T cells (16). However, in the absence of CD83 expression, mouse B cells and other APCs also turn over cell surface MHC II molecules at a higher rate and have decreased CD83 expression following activation, but otherwise appear to function normally (21). Thus, while large discrepancies exist regarding the exact role of CD83 in immune function, the expression pattern of CD83 and its essential role in CD4+ T cell development suggest that it serves important roles during immune regulation in humans and mice.

To further elucidate the role for CD83 in peripheral lymphocyte maturation and function, a thorough analysis of the cellular and kinetic expression patterns of CD83 was conducted. High-level cell surface CD83 expression was rapidly detected on activated mouse B and T cells, in addition to mature conventional (cDCs) and plasmacytoid DCs (pDCs). Expression by B and CD4+ T cells was functionally significant and necessary for normal lymphocyte survival in adoptive transfer experiments. Thereby, CD83 represents a marker for acute lymphocyte activation that was necessary for normal B and CD4+ T cell longevity in vivo.

Materials and Methods

Mice

CD83−/− mice (16) were backcrossed with C57BL/6 mice (The Jackson Laboratory) for more than or equal to nine generations and used between 8 and 10 wk of age. CD83−/− mice were crossed with AND mice (provided by Dr. S. M. Hendrick, University of California, San Diego, CA), as described previously (16). CD45.1, BALB/c, B6.MRL-Fas−/− (MRL−/−), and NZB/WF1/J (New Zealand Black/New Zealand White (NZB/NZW)) mice were obtained from The Jackson Laboratory and mice overexpressing CD19 (hCD19Tg) were as described (32). MRL−/−, NZB/NZW, and hCD19Tg mice were used between 6 and 10 wk of age. Mice were housed in a specific pathogen-free barrier facility. All studies and procedures were approved by the Animal Care and Use Committee of Duke University.

Antibodies

Abs used included: FITC, PE or CyChrome-conjugated Abs against CD4 (L3T4), CD8a (53-67), B220 (RA3-6B2), CD21 (76G6), CD5 (53-7.3), CD11c (HL3), anti-mouse T and B cell activation Ag (GL-7, Ly77), CD86 (GL-1), IgM (R6.60.2), Ly6G (Gr-1, RB6-8C5), and IgM (DS-1) all obtained from BD Pharmingen; I-A/I-E (M5/114.15.2) and CD69 (H1.2F3) obtained from eBioscience; and anti-mouse IgM antisera from Southern Biotechnology Associates.

Hybridomas producing CD83-specific mouse mAbs were generated by the fusion of NS-1 myeloma cells with spleen cells from CD83−/− mice immunized with murine CD83-GFP-fusion protein cDNA transfected 300.19 pre-B cells (33). The 300.19 pre-B cell line expressing mouse CD83 protein fused with GFP was generated by transfecting 300.19 cells with cDNA encoding the CD83-GFP fused protein (10). Transfected cells were isolated by fluorescence-based cell sorting based on GFP expression. Hybridomas producing anti-mouse CD83 mAbs reactive with 300.19-CD83 cells and LPS-activated A20 cells were isolated and subcloned twice. mAb isotypes were determined using a Mouse Monoclonal Antibody Isotyping kit (Amersham). Tissue culture supernatant fluid from the MB83-06 mAb producing hybridoma and purified mouse CD83 mAbs were used for immunofluorescence staining. PE-conjugated isotype-specific secondary Abs (Southern Biotechnology Associates) were used to reveal CD83 mAb reactivity with 300.19-CD83-transfected cells. PE-conjugated streptavidin (Southern Biotechnology Associates) was used to reveal biotin-coupled MB83-01 mAb reactivity.

Cell isolation and immunofluorescence analysis

Single-cell leukocyte suspensions were stained on ice using predetermined optimal concentrations of each Ab for 20–60 min as described (32). Cells with the forward and light scatter properties of lymphocytes or DCs were analyzed on FACScan or FACSCalibur flow cytometers (BD Biosciences). Background staining was determined using unreactive control mAbs. In some cases, background staining for the CD3 mAbs was determined using CD83−/− leukocytes. DCs from lymphoid organs were obtained as previously described with minor modifications (34). Briefly, spleens and thymi collected from wild-type and CD83−/− littermates were minced and incubated with collagenase D (1 mg/ml; Roche Applied Sciences) and Dnase 1 (0.2 mg/ml; Sigma-Aldrich) for 30 min at 37°C. Cold EDTA was added to a final concentration of 20 mM and cell suspensions were incubated for 5 min at room temperature before filtering through nylon mesh to remove tissue and cell aggregates. To enrich for cells with a low buoyant density, cellular suspensions were separated over a 30% BSA gradient and cells were collected from the interface (35). Before DC staining, FcRs were blocked with CD16/CD32 mAb (2.4G2; BD Pharmingen) for 15 min at 4°C.

Leukocyte activation assays

DCs (0.5–1.0 × 10^6/mL) isolated from spleen and thymic tissue were incubated for 24 h in RPMI 1640 medium containing 10% FCS, 10 mM HEPES, and 55 μM 2-ME, and were activated with LPS (Escherichia coli 0111:B4, 5 μg/ml; Sigma-Aldrich). pDCs were stimulated with unmethylated CpG oligonucleotides (1 μM; IDT) and recombinant mouse IL-3–10 ng/ml; ebioscience). Spleen B cellswere purifed by removing T cells using Thy-1.2 Ab-coated magnetic beads (Dynal Biotech). For in vitro TNF-α were assayed, B cells were incubated (1 × 10^6/mL) in RPMI 1640 medium containing 10% FCS, 10 mM HEPES, and 55 μM 2-ME at 37°C and LPS (10 μg/mL), CD40 mAb (HM40-3, no azide/low endotoxin (NA/LE) format, 1 μg/mL; BD Pharrmingen) or Fab(‘λ); of goat anti-mouse IgM Abs (40 μg/mL; Cappel/ICN Biomedicals). For in vitro T cell activation assays, splenocytes were incubated (2 × 10^6/mL) in RPMI 1640 medium containing 10% FCS, 10 mM HEPES, and 55 μM 2-ME and activated with CD3 (145-2C11, 10 μg/mL; ebioscience) and CD28 (37.51, 10 μg/mL; ebioscience) mAbs. At various time points during incubation, cells (0.5–1 × 10^6) were removed from the suspensions and assessed for CD83 expression using the MB83-01 mAb.

For MLR assays, splenic cells with a low buoyant density were isolated from C57BL/6 mice as described above and cultured overnight in medium (RPMI 1640 medium containing 10% FCS, 10 mM HEPES, and 55 μM 2-ME) or in medium with LPS (1.0 μg/mL) at 37°C. After overnight culture, cells were washed three times in medium, resuspended at 1–2 × 10^6/mL, and incubated with biotinylated MB83-01 mAb or isotype control mAb for 30 min on ice. Following two washes with buffer (PBS containing 0.5% FCS and 2 mM EDTA), MB83-01 and isotype control mAb-treated cells were incubated with rotation at 4°C for 30 min with anti-biotin-conjugated microbeads following the manufacturer’s instructions (Miltenyi Biotec). The cell suspension was washed and applied to LS columns (Miltenyi Biotec) to deplete microbead-labeled cells. Depleted MB83-01 and CTL mAb-treated cells were treated with mitomycin C (50 μg/mL; Sigma-Aldrich) for 30 min at 37°C, washed three times with medium, resuspended at 1 × 10^6/mL, serially diluted as indicated, and used as effector cells. Splenic T cells from BALB/c mice were used as responder cells that were enriched of T cells (100 μl) isolated from spleen and thymic tissue were incubated with serially diluted effector cells (100 μl) in 96-well flat-bottom plates and cultured for 3 days at 37°C. [3H]Thymidine (1.0 μCi/well) was added during the last 16 h of culture. The cells were harvested onto glass fiber filters using a Tomtec Mach Iiww automatic harvester, with radioactivity quantified using a Microbeta TriLux scintillation counter (all obtained from PerkinElmer Life Sciences).

For in vivo B cell activation, LPS (10–50 μg/mouse in 200 μl of PBS) or PBS was i.v. injected into 8–10 wk old, age- and sex-matched C57BL/6 mice. At various times after injection, spleen and peripheral lymph node cells were harvested and subjected to immunofluorescence analysis. To examine CD83 expression on germinal center B cells, wild-type and CD83−/− mice were immunized i.p. with NP (4-hydroxy-3-nitrophenyl acetyl) conjugated to chicken gammaglobulin (100 μg, NP_{acetyl}GGC; Biosearch Technologies) precipitated in alum as described (36). Tissue was harvested on day 8 after injection, with germinal center B cells were analyzed by three-color immunofluorescence analysis.

Adoptive transfer experiments

For adoptive transfer experiments using Ag-specific B cells, MD4-transgenic mice (CD45.2−; 1 × 10^5) were adoptively transferred by s.c. tail vein injection into age- and sex-matched wild-type congenic (CD45.1−) recipients and allowed to equilibrate for 24 h. Twenty-four hours following cell transfer, hen egg lysozyme (HEL; Sigma-Aldrich)
A

B

C

FIGURE 1. Reactivity of anti-mouse CD83 mAbs. A, Immunofluorescence staining of CD83 mAbs (5 μg/ml) with CD83-GFP cDNA-transfected (thick line) or untransfected (dashed line) 300.19 cells. Reactivity of CD83 mAbs was revealed with PE-conjugated isotype-specific secondary Abs with flow cytometry analysis. The isotype of each mAb is indicated. B, CD83 expression by A20 cells. A20 cells were cultured with medium (dashed line) or LPS (solid line) for 24 h and stained with biotinylated MB83-01 mAb as in A and detected with PE-conjugated streptavidin. C, Decreased T cell proliferation in a MLR following depletion of CD83 + stimulator cells. Stimulator cells were cultured overnight in medium (filled) or in medium with LPS (open) and then treated with CTL (square) or MB83-01 (circle) mAb to deplete CD83 + cells. Results represent mean (±SEM) allogenic T cell proliferation as measured by thymidine uptake. **, A significant difference (p < 0.01) in the proliferation induced by CTL-treated and MB83-01 mAb stimulator cells. A–C, All results are representative of those obtained from three independent experiments.

was solubilized in sterile PBS and was i.v. injected at 0.1, 1.0, 10, 100, or 1000 μg/mouse for dose response experiments and at 1.0 μg/mouse for in vivo kinetic experiments. Control mice were treated with PBS alone. Recipient spleens were harvested 4 h after injection for dose response experiments. For in vivo kinetic experiments, spleens were harvested at the indicated times. Splenic B220 + cells were examined for expression of CD45.2, CD83, MHC II, CD86, and CD69 by three-color immunofluorescence analysis.

To assess B and T cell longevity, splenocytes (1.5 × 10^7) from wild-type (CD45.2) or CD83−/− (CD45.2) mice were i.v. injected into age- and sex-matched wild-type congenic (CD45.1) recipients and the frequency and number of donor cells were determined by flow cytometry analysis. To assess the survival of wild-type splenocytes in a CD83−/− environment, splenocytes from wild-type congenic (CD45.1) mice were transferred into wild-type (CD45.2) or CD83−/− (CD45.2) recipients. Donor splenocytes were differentiated by the expression of CD45.2 and assessed for expression of CD4, CD8, and B220 by flow cytometry analysis. To assess the survival of wild-type splenocytes in a CD83−/− environment, splenocytes from wild-type congenic (CD45.1) mice were transferred into wild-type (CD45.2) or CD83−/− (CD45.2) recipients. Donor splenocytes were differentiated by the expression of CD45.1. For short-term migration assays, wild-type and CD83−/− splenocytes (CD45.2, 2 × 10^7) were transferred into wild-type congenic (CD45.1) recipients and the frequency and number of donor cells were determined 16 h after cell transfer by immunofluorescence staining with flow cytometry analysis. To assess CD4 + T cell survival, CD83−/− recipient mice were transplanted with bone marrow or thymocytes from wild-type AND donors or CD83−/− AND donors as described (16). Briefly, recipient mice were irradiated (1200 rad) 12 h before transplantation by i.v. injection with 5 × 10^6 donor bone marrow cells. Vα11 + cells in recipient mice were assessed 4 wk following transplantation. For in vivo kinetic experiments, spleens were harvested at the indicated times. Splenic B220 + cells were examined for expression of CD4, CD83, MHC II, CD86, and CD69 by three-color immunofluorescence analysis.

Statistical analysis

All data are shown as means ± SEM. The Student t test (two-tailed) was used to determine the significance of differences between sample means.

Results

CD83 mAb reactivity with murine DC subsets

CD83 expression on mouse leukocytes was assessed using a panel of mAbs generated against mouse CD83. Anti-mouse CD83 mAbs were reactive with 300.19 cells transfected with CD83-GFP cDNA and with the mitogen-activated A20 B cell line, but did not react with parental untransfected 300.19 cells or unactivated A20 cells (Fig. 1, A and B, data not shown). However, none of the anti-mouse CD83 mAbs blocked T cell proliferation in Ag and APC assays (data not shown), as also found for anti-human CD83 mAbs (2, 38). Nonetheless, T cell proliferation was significantly decreased when MB83-01 mAb was used to deplete CD83 + cells from either cultured or LPS-stimulated effector cell populations (Fig. 1C), as shown for human CD83 mAbs (2). Consistent with...
this, each of the CD83 mAbs reacted with cell surface CD83 expressed on CD11c⁺ DC subsets from wild-type mice, but not with DCs and other cells from CD83⁻/⁻ littermates (Fig. 2, A–C, and data not shown). Therefore, these mAbs were specific for CD83, with MB83-01 mAb having the highest level of reactivity.

Conventional splenic and thymic dendritic cell (cDC) subsets (CD11c⁺CD8α⁺ and CD11c⁺CD8α⁻) were analyzed for CD83 expression. Freshly isolated splenic cDCs did not express substantial amounts of cell surface CD83 (Fig. 2A), as shown for freshly isolated and monocyte-derived human DCs (1, 6). Freshly isolated
thymic cDC subsets, which have a more activated phenotype (39, 40), expressed higher levels of CD83 than splenic cDCs (Fig. 2B).

However, both splenic and thymic cDCs rapidly up-regulated CD83 expression following LPS activation. CD11c<sup>+CD8α<sup>+</sup></sup> and CD11c<sup>+CD8α<sup>−</sup></sup> DCs, isolated from splenic and thymic tissue, up-regulated CD83 within 4 h of in vitro culture with LPS. Furthermore,
Bone marrow cells that expressed cell surface CD83 were
found to be 19 ± 1% of non-germinal center B cells (19 ± 1% expressed CD83, a similar percentage of non-germinal center B cells as early as 4 h following stimulation (Fig. 4, A and B). The majority (>75%) of activated B220+ cells expressed CD83 at 4 h following activation and maintained CD83 expression throughout the 24-h culture period (Fig. 4, A and B). Although CD83 expression was maximal on LPS and CD40 mAb-stimulated splenocytes at 4 h, CD83 expression by anti-IgM F(ab')2 Ab-stimulated splenocytes continued to increase through 24 h of stimulation. Thus, B cells rapidly up-regulated CD83 expression upon activation, regardless of the stimulus.

CD83 expression was also assessed on T cells from primary and secondary lymphoid organs. CD83 expression was not detected on resting, circulating, splenic, or peripheral lymph node T cells, but was transiently detected on CD4+ and CD8+ splenic T cells within hours of CD3 and CD28 mAb stimulation (Fig. 4, C–F, data not shown). CD3 expression by CD4+ and CD8+ T cells was evident within 1–2 h of stimulation and peaked at 6 h following stimulation (Fig. 4, C–F). CD4+ T cells expressed higher maximal levels of cell surface CD83 when compared with CD8+ T cells (Fig. 4, C and E). CD83 was still expressed by T cells 48 h following stimulation (Fig. 4, C–F). Thus, CD3 surface expression by activated lymphocytes was rapidly induced and sensitive to signals received through cell surface receptors expressed on B cells (TLR4/RP105, CD40, and BCR) and T cells (TCR/CD28).

CD83 expression by activated B cells in vivo

Whether in vivo activation of B cells induced CD83 expression was assessed following injection of mice with LPS (10 μg/mouse). The percentage of B cells from PBS-treated mice that expressed CD83 or the CD69 activation molecule did not change significantly during a 24-h time period (Fig. 5A). CD83 expression by splenic B220+ B cells was induced within 2 h of LPS injection at which time 55 ± 2% of splenic B220+ cells expressed CD83, while only 39 ± 5% of splenic B cells expressed CD69 (Fig. 5B).
Most B cells expressed both CD83 and CD69 by 4 h following LPS treatment and then CD83 expression levels decreased to near baseline levels by 24 h (Fig. 5C). The transient surface expression of CD83 was not a result of a limiting dose of LPS as CD83 was also transiently expressed on B cells from mice injected with five times more LPS (50 μg/LPS/mouse, data not shown). Therefore, CD83 was rapidly induced on B cells activated in vivo and in vitro with similar kinetics.

B cells from autoimmune prone mice were assessed for CD83 expression. Basal CD83 expression by splenic B cells isolated from hCD19Tg, NZB/NZW, and MRL/lpr mice was compared with CD83 expression by C57BL/6J B cells, with B cells from CD83−/− littermates used as controls. Splenic B cells from hCD19Tg, NZB/NZW, and MRL/lpr mice had significantly increased frequencies of CD83+ splenic B cells (34 ± 3%, 15 ± 1%, 17 ± 2%, respectively) when compared with C57BL/6J (9.4 ± 0.4%) mice (Fig. 5D). Resting B cells from hCD19Tg and MRL/lpr mice also had higher basal expression of CD86 and MHC II (Fig. 5D), as described (32, 41–43). The expression of CD83, CD86, and MHC II were up-regulated to a similar extent following LPS stimulation of C57BL/6J, hCD19Tg, NZB/NZW, and MRL/lpr B cells. Thus, CD83 was expressed by activated B cells in vivo and the increased basal expression of CD83 by hCD19Tg, NZB/NZW, and MRL/lpr B cells correlated with the activated phenotype of B cells from these mice (32, 41–43).

Ag-engaged B cells up-regulate CD83 in vivo

Whether B cells up-regulate CD83 expression upon BCR ligation with specific Ag was assessed in vivo using HEL-specific Ig-transgenic splenocytes (MD4, CD45.2). MD4 splenocytes were transferred into wild-type (CD45.1) syngeneic recipients 24 h before the recipient mice were immunized with soluble HEL (Fig. 6A). To determine the relationship between BCR ligation and CD83 expression, recipient mice were injected with increasing doses of HEL, with B cells examined 4 h later. CD83 expression by MD4 and wild-type B cells was compared with CD69, MHC II, and
CD86 expression after Ag and PBS treatments (Fig. 6, B and C). The frequency of CD83-expressing B cells rapidly increased following administration of HEL at a low dose (0.1 μg/mouse) and was sufficient to induce up-regulation of CD83 by a significant portion (58 ± 14%) of MD4 B cells. MHC II and CD86 expression were also increased by HEL injection at 0.1 μg/mouse (Fig. 6, B and C). By contrast, CD69 expression was only significantly induced with a 1000-fold higher dose of HEL (1.0 mg HEL/mouse). The presence of cell surface IgM on MD4 B cells following administration of 0.1 μg HEL/mouse indicated that this dose was not sufficient to induce significant BCR internalization, while all other doses of HEL caused IgM internalization (Fig. 6D). The induced up-regulation of CD83, CD69, MHC II, and CD86 expression was Ag specific given that MD4 B cells from PBS-treated mice, and Ag-nonspecific B cells (gated as shown in Fig. 6A) from HEL-treated mice, did not up-regulate CD83, CD69, MHC II, or CD86 expression at any dose of HEL administered (Fig. 6C). Following injection of recipient mice with 1.0 μg of HEL, CD83 expression was significantly (p < 0.001) up-regulated within 2 h and the majority of MD4 B cells expressed detectable levels of CD83 and CD86 at this time point, while the expression of both CD83 and CD86 peaked at 6 h (Fig. 6, E–G). CD83 expression was rapidly down-regulated at 24 h following Ag administration and the majority of MD4 B cells were no longer expressing CD83 at this time point, while CD86 expression on MD4 B cells was maintained (Fig. 6, E–G). Thus, CD83 expression in vivo is induced by BCR ligation with comparable dose response kinetics to MHC II and CD86 up-regulation. However, CD83 was expressed shortly after Ag ligation and was a more sensitive marker for early B cell activation than CD69 or IgM internalization.

Decreased longevity of CD83−/− lymphocytes in vivo

The effect of CD83 deficiency on B cell subsets was assessed using CD83−/− mice. Similar numbers of pre/pro (IgM−/−B220−), immature (IgM+B220low), and mature (IgM+B220high) bone marrow B cells were present in CD83−/− and wild-type littermates (Table I). There were no significant differences in the percentages or absolute numbers of immature (IgM+B220low), mature (IgM+B220high), or marginal zone (CD21highB220+) spleen B cells isolated from CD83−/− and wild-type littermates (Table I). Furthermore, CD83 deficiency did not alter the percentages or

FIGURE 5. Rapid CD83 expression induced by B cell activation in vivo. A, Representative CD83 and CD69 expression by B220+ splenocytes at the indicated times after LPS treatment. B, Mean (±SEM) percentages of B220+ cells expressing CD83 or CD69 from LPS or CTL treated littermates as in A. C, Time course of LPS-induced CD83 expression. Mean (±SEM) fluorescence intensity values of CD83 expression on gated B220+ splenocytes recovered at the indicated times from LPS or CTL treated littermates. D, CD83, CD86, and MHC II expression on B cells from autoimmune-prone mice. Mean (±SEM) percentage of CD83 or CD86, and mean (±SEM) fluorescence intensities of MHC II expression on resting and activated splenic B220+ cells from wild-type (WT), hCD19Tg, NZB/NZW, and MRLpr mice. Resting or activated splenocytes from CD83−/− mice were used as negative controls. A–D, All results are representative of three independent experiments; asterisks (+ and **+) indicate significantly different frequency or mean fluorescence intensity when compared with resting or activated WT B cells (p < 0.05 and p < 0.01), respectively.
FIGURE 6. BCR engagement induces CD83 expression in vivo. A, Representative gating of MD4 (CD45.2) and host (CD45.2<sup>−</sup>) B<sup>220</sup><sup>−</sup> cells 24 h after adoptive transfer of MD4 splenocytes and 4 h following HEL (10 μg) injection. Frequencies of MD4 and WT B cells are shown as a percentage of total lymphocytes. B, Representative CD83, CD69, MHC II, and CD86 expression by MD4 (CD45.2<sup>−</sup>B<sup>220</sup> thick line) and wild-type (CD45.2<sup>−</sup>B<sup>220</sup> thin line) B<sup>220</sup> cells 4 h following HEL (10 μg) or PBS (CTL) injection. Single-cell splenocyte suspensions were analyzed by three color immunofluorescence analysis. C, Mean (±SEM) percentages of MD4 (■) and wild-type (WT; □) B<sup>220</sup> cells expressing CD83, CD69, or CD86, and the mean (±SEM) fluorescence intensities of MHC II expression following injection with the indicated doses of HEL. Mean percentages were calculated as a percentage of total MD4 or total wild-type B<sup>220</sup> splenocytes, respectively. A–C, Results represent those obtained from more than or equal to four experiments; asterisks (*) and (**) indicate significantly different sample means between MD4 CTL (PBS) treated and MD4 HEL-treated mice (p < 0.05 and p < 0.01), respectively. D, Representative immunofluorescence staining of IgM<sup>+</sup> expression on MD4 B<sup>220</sup> cells following HEL injection; values shown in contour plots represent the frequency of IgM<sup>+</sup> cells among the total lymphocyte population. Bar graphs represent IgM<sup>+</sup> expression as a percentage of total B<sup>220</sup> cells. Results represent those obtained in two experiments. E, Kinetic expression analysis of CD83 in vivo. Representative CD83 and CD86 expression by MD4 B cells (CD45.2<sup>−</sup>B<sup>220</sup>) before injection (Pre) at the indicated time points following injection of 1.0 μg of HEL. F, Mean (±SEM) fluorescence intensities of CD83 and CD86 expression by MD4 B cells at the indicated time points following HEL injection (1.0 μg/mouse).
overall numbers of peritoneal B-1a (CD5⁺B220⁺) or B-1b/B2 (CD5⁻ B220⁻) B cells (Table I). Thus, CD83 expression was not essential for B cell development or tissue localization.

The survival of CD83⁻⁻/⁻ and wild-type peripheral lymphocytes was compared following their adoptive transfer into wild-type congenic recipients. Before transfer, the frequencies and numbers of

G. Mean (±SEM) percentages of MD4 B cells that expressed CD83 or CD86 at the indicated time following injection of HEL. Mean percentages were calculated as a percentage of total MD4 B220⁺ cells. E and F. Results represent those obtained from three independent experiments and asterisks (⁺ and ⁺⁺) indicate significantly different mean fluorescence intensity or frequency when compared with preinjection values (p < 0.001 and p < 0.05), respectively.

FIGURE 7. Longevity of CD83⁻⁻/⁻ B cells in vivo. A, Diminished longevity of CD83⁻⁻/⁻ B cells and CD4⁺ T cells. Representative contour plots show wild-type (CD45.2⁺) and CD83⁻⁻/⁻ (CD45.2⁻) donor lymphocytes 1 wk after transfer into wild-type congenic (CD45.2⁻⁻) recipients. The frequencies of recipient (CD45.2⁺) and donor (CD45.2⁻) B220⁺ cells among the total lymphocyte population are shown. The preinjection bar graph represents the absolute number of CD4⁺, CD8⁺, and B220⁺ splenocytes from wild-type and CD83⁻⁻/⁻ donors before injection, with the postinjection bar graph representing the mean (±SEM) numbers of donor CD4⁺, CD8⁺, and B220⁺ lymphocytes recovered from the spleens of wild-type CD45.1⁻⁻ recipients 1 wk following adoptive transfer. **, Significant differences in the absolute numbers of wild-type and CD83⁻⁻/⁻ donor cells recovered; p < 0.01. Results represent those from eight littermate pairs of recipient mice. B, Normal longevity of wild-type lymphocytes in CD83⁻⁻/⁻ recipients. Representative contour plots show wild-type congenic (CD45.1⁺) donor lymphocytes 1 wk following transfer into wild-type and CD83⁻⁻/⁻ recipients. The frequencies of recipient (CD45.1⁻⁻) and donor (CD45.1⁻⁻) B220⁺ cells among the total lymphocyte population are shown. The preinjection bar graph represents the absolute number of CD4⁺, CD8⁺, and B220⁺ wild-type (CD45.1⁺) donor splenocytes before injection. The postinjection bar graph represents the mean (±SEM) absolute number of wild-type (CD45.1⁺) donor CD4⁺, CD8⁺, and B220⁺ lymphocytes recovered from wild-type and CD83⁻⁻/⁻ recipient spleens 1 wk following transfer. Results represent those obtained from five littermate pairs of recipient mice. C, CD83⁻⁻/⁻ and wild-type B cells localize equally following short-term adoptive transfer. The percentage of wild-type or CD83⁻⁻/⁻ B220⁺ cells present before injection (Pre) or recovered from the blood, bone marrow, and spleen 16 h following transfer into wild-type (CD45.1⁺) recipients. Values for preinjection represent the percentage of B220⁺ cells among total donor (CD45.2⁻⁻) splenocytes. Sixteen hours after transfer, the mean percentages (±SEM) of CD45.2⁻⁻ B220⁺ cells recovered among total lymphocytes is shown for blood, bone marrow, and spleen. Results are representative of two independent experiments with similar results. D, Reduced longevity of peripheral CD83⁻⁻/⁻ CD4⁺ T cells compared with wild-type CD4⁺ T cells following bone marrow transplantation into CD83⁻⁻/⁻ recipients. Bar graphs represent the mean (±SEM) absolute number of recovered donor CD4⁺ T cells in the thymus (Thy), blood, spleen, and PLN 4 wk following bone marrow transplant with AND or CD83⁻⁻/⁻ AND bone marrow. Results represent those from six littermate pairs of recipient mice. *, Significantly different sample means (±SEM) between littermate pairs; p < 0.05. E, Reduced longevity of peripheral CD83⁻⁻/⁻ CD4⁺ T cells compared with wild-type CD4⁺ T cells following thymocyte transplantation into CD83⁻⁻/⁻ recipients. Bar graphs represent the mean percentage (±SEM) of donor CD4⁺ T cells recovered from total thymocytes and mean (±SEM) absolute numbers of recovered donor CD4⁺ T cells in the blood, spleen, and PLN 3 wk following transplant with AND or CD83⁻⁻/⁻ AND thymocytes. Results represent those from four littermate pairs of recipient mice. *, Significantly different sample means (±SEM) between littermate pairs; p < 0.05.
B220+, CD4+, and CD8+ lymphocytes isolated from CD83−/− and wild-type donors were determined, with equal numbers of CD83−/− and wild-type B cells injected into congenic recipient mice (Fig. 7A). However, the frequencies and numbers of recovered CD83−/− B cells were significantly lower than for wild-type B cells 1 wk following the cell transfers, indicating either reduced survival or localization within host tissues for CD83−/− B cells. In similar studies, the recovery of CD83+/− B cells was significantly reduced (<0.05) 1 wk following adoptive transfer of pooled, CFSE-labeled, wild-type (CFSElow) and CD83−/− (CFSEhigh) donor lymphocytes into wild-type recipients (data not shown). Alternatively, wild-type congenic lymphocytes gave rise to equal frequencies and numbers of B cells when transferred into either CD83−/− or wild-type recipients (Fig. 7B), indicating that wild-type B cells survive normally in a CD83−/− microenvironment.

The reduced recovery of CD83−/− B cells was not due to altered tissue localization as wild-type and CD83−/− B cells were recovered at equivalent frequencies and numbers from the blood, bone marrow, spleen, and peripheral lymph nodes at 16 h following cell transfer into wild-type congenic recipients (Fig. 7C and data not shown). Therefore, CD83−/− B cells exhibited either an intrinsic survival defect or inability to compete with wild-type B cells in vivo.

The survival of CD83−/− T cells was also assessed following lymphocyte transfers into wild-type congenic recipients. There was a 75% reduction in the number of CD4+ T cells isolated from CD83−/− donors relative to wild-type donors, while CD8+ T cells were present at similar frequencies and numbers in both donor populations (Fig. 7A). However, 1 wk following lymphocyte transfers, the frequency and number of CD83−/− CD4+ T cells recovered in each recipient was even more drastically reduced (90%) when compared with the recovery of wild-type CD4+ T cells. By contrast, CD8+ T cells from CD83−/− and wild-type donors were recovered at similar frequencies. Likewise, there was no significant difference in the frequencies or numbers of wild-type congenic CD4+ T cells recovered following transfer into either CD83−/− or wild-type recipient mice (Fig. 7B). These results suggest that CD83−/− CD4+ T cells have an intrinsic cell survival defect when compared with wild-type CD4+ T cells.

A requisite for CD83 expression by CD4+ T cells was examined independently using AND mice and AND CD83−/− transgenic mice with fixed Ag receptors for assessment of peripheral CD4+ T cell development following transplantation into CD83−/− mice. Although CD4+ T cell development is severely attenuated in CD83−/− mice due to the absence of CD83 expression by thymic stromal cells, small numbers of peripheral CD4+ T lymphocytes are generated in this environment (16, 17). Bone marrow or thymocytes from wild-type AND or CD83−/− AND mice was transferred into irradiated CD83−/− recipients, with the number of peripheral CD4+ T cells assessed 4 wk later. Bone marrow from CD83−/− AND and AND littersmates gave rise to equivalent numbers of Vα11+ CD4+ single-positive thymocytes and circulating CD4+ T cells (Fig. 7D). However, there was a significant reduction (<0.05) in spleen and lymph node Vα11+ CD4+ T cells in recipient mice transplanted with CD83−/− AND bone marrow. Similar results were obtained when thymocytes from CD83−/− AND and AND donor mice were directly transferred into the thymi of irradiated CD83−/− recipients (Fig. 7E). Three weeks following thymocyte transfer, an equivalent percentage of Vα11+ CD4+ single-positive thymocytes were recovered from recipient mice transplanted with either CD83−/− AND or AND donor thymocytes. However, there was a significant reduction (<0.05) in blood, spleen, and peripheral lymph node (PLN) Vα11+ CD4+ T cells in mice transplanted with CD83−/− AND thymocytes relative to littersmates transplanted with wild-type AND thymocytes. Thus, the longevity of CD83−/− CD4+ T cells in the periphery was significantly reduced relative to the longevity of wild-type CD4+ T cells.

Discussion

These studies reveal that CD83, a known marker of mature DCs, is also expressed at significant levels by activated mouse lymphocyte cells. Although CD83 was expressed by a small population of B cells present in primary and secondary lymphoid organs of naive mice, CD83 surface expression was rapidly induced by the majority of B cells following their in vivo and in vitro activation (Figs. 3, 4A, and 4B, and data not shown). In the bone marrow, CD83 was expressed at low levels by a small subset of IgM*B220low and IgM*B220high B cells, while a small subset of splenic and peri toneal B cells expressed low levels of CD83 (Fig. 3). Splenic B cell expression of CD83 was not specific to germinal center or marginal zone B cells. Similarly, peritoneal B cell expression of CD83 was not specific to B-1a (CD5* B220low) or B-1b/B-2 (CD5* B220high) cells. B cell activation resulted in the rapid up-regulation and high cell surface expression of CD83 in vitro similar to CD69 expression (Fig. 4). Induced CD83 surface expression by peripheral B cells in vivo was also detected within hours of either LPS or Ag administration, with the frequency of CD83-expressing B cells quickly returning to baseline levels following activation (Figs. 5 and 6), in agreement with CD83 regulation by NF-κB induction (15, 44). Increased CD83 expression and an increased frequency of CD83-expressing B cells in autoimmune-prone mice (Fig. 5 and data not shown) also correlated with their increased sensitivity to transmembrane signals and activated phenotypes (41–43, 45). The sensitivity of CD83 induction following B cell activation indicates that CD83 expression may play a relevant role in the in vivo microenvironment when limiting Ag is present or may be directly involved in cellular interactions between APCs and T cells.

The rapid induction of CD83 expression by activated B cells following cell surface receptor ligation may have functional significance in vivo because longevity was significantly decreased for CD83−/− B cells compared with wild-type B cells. Specifically, there was a significant reduction in the number of CD83−/− B cells recovered following adoptive transfer into wild-type recipients compared with wild-type B cells (Fig. 7). This defect was intrinsic to CD83−/− B cells because equivalent numbers of wild-type B cells were recovered following their transfer into either CD83−/− or wild-type recipients. The failure of CD83−/− B cells to survive in comparison with wild-type B cells was not due to migration defects as CD83−/− B cells migrated normally to the bone marrow, blood, spleen, and peripheral lymph nodes, and the development of all B cell subsets was normal in CD83−/− mice (Fig. 7E and Table I). Thus, in vivo, CD83−/− B cells had impaired survival when placed in competition with wild-type lymphocytes. In addition, CD83−/− B cells also have enhanced MHC II turnover and decreased CD86 expression following activation (16, 21), further supporting an important functional role for peripheral B cell expression of CD83. Additionally, recent evidence indicates that treatment of mice with a CD83 mAb results in altered IgG responses to a T-independent Ag (20). Our current appreciation of the diverse effects of CD83 deficiency and the lack of known CD83 ligands, it is difficult to attribute CD83 function to a single outcome. Moreover, CD83−/− B cells proliferate normally in vitro in response to B cell-activating stimuli (16), and are capable of inducing efficient T cell proliferation in vitro in response to Ag (21). Thus, CD83 is likely to mediate or initiate important Bcell interactions.
cell functions that overlap in part with those of other cell surface receptors.

Although cell surface CD83 expression was not detected on circulating or resting T cells present in primary and secondary lymphoid organs, CD83 expression was rapidly induced on CD4+ and CD8+ T cells after activation in vitro (Fig. 4 and data not shown). Kinetic analysis of CD83 expression by CD4+ and CD8+ T cells demonstrated that maximal CD83 expression was detected between 4 and 6 h of activation (Fig. 4, C–F), in contrast to earlier studies where low-level CD83 expression was detected on Ag-specific CD4+ T cells at 1, 3, and 7 days following the addition of Ag in vitro (18, 19). In addition, the current study is the first to report CD83 expression by activated peripheral CD8+ T cells, although CD4- T cells had higher levels of cell surface CD83 expression than mouse CD8+ T cells (Fig. 4, C–F). Nonetheless, these results contrast markedly with previous studies of CD83 expression by human B and T cells, and mitogen- or MLR-activated T cells, which only express very low levels of cell surface CD83 when compared with DCs (2, 15, 27, 46). Although the differences in lymphocyte expression of CD83 are significant, it remains possible that high-level CD83 expression may be induced on rare human lymphocytes in vivo following Ag-specific activation, although this has not been suggested by in vitro activation studies or immunohistochemistry assessment (1). Thereby, it is likely that human and mouse lymphocytes express cell surface CD83 at intrinsically different levels. CD83 expression by peripheral CD4+ T cells in the mouse is functionally significant as evidenced by the reduced recovery of CD83+/−CD4+ T cells following splenectomy, bone marrow, and thymocyte transplantation (Fig. 7). Peripheral T cells from CD83+/−mice and transgenic mice that overexpress CD83 also have altered phenotypes and cytokine production (16, 17, 47). Thus, CD83 expression is not only essential for the development of CD4+ T cells in the thymus, but is required for the normal survival or expansion of peripheral CD4+ T cells in vivo.

Mouse bone marrow DCs up-regulate CD83 expression in response to LPS stimulation (18, 19), and as shown here, CD83 was also expressed and up-regulated by tissue-resident DCs in the mouse (Fig. 2). Splenic cDCs and thymic cDCs expressed low levels of CD83 upon isolation and up-regulated CD83 with a similar kinetic expression pattern upon maturation. However, freshly isolated and activated thymic cDCs had higher cell surface expression of CD83 than splenic cDCs. Increased cell surface CD83 expression by thymic cDCs correlates with the more mature phenotype associated with thymic cDCs, including increased expression of MHC II, and costimulatory molecules (40). CD83 surface expression was not detected on immature pDCs (CD11c<sup>low</sup>B220<sup>+</sup>Gr-1<sup>+</sup>) but was up-regulated following pDC maturation, although at lower levels than found on mature cDCs. Because DC subsets have distinct roles in the regulation of T cell-mediated adaptive immunity through their unique expression patterns of coregulatory molecules (48), the differential cell surface expression of CD83 on DC subsets may prove to have functional significance. Regardless, CD83 expression by mouse DC subsets correlated closely with CD83 expression on human DC subsets, being predominantly restricted to DCs with a more mature phenotype (1–3).

In conclusion, CD83 expression was rapidly induced on mature DC populations and acutely activated B and T cells in the mouse (Figs. 2 and 4–6), similar to other molecules associated with the regulation of cellular activation. Thereby, CD83 was a sensitive marker of B cell activation in vivo, suggesting that CD83+ B cells represent preactivated, primed, or recently activated B cells. Although CD83 was expressed with similar kinetics and on essentially the same cell populations as many coregulatory or costimulatory molecules (i.e., CD80, CD86, and B7h), only CD83+/− mice have a remarkable defect in T cell development (16, 17, 49–51). Thus, these studies indicate a non-redundant role for CD83 in B and T cell development, survival, and function during the initiation of immune responses.

Acknowledgments

We thank Ann Miller and Glicerio Ignacio for their help with these studies and Drs. Karen M. Haas, Jonathon Poe, and Susan Smith for their helpful suggestions and critical review of this manuscript.

Disclosures

T. F. Tedder is a paid consultant for MedImmune and Angelica Therapeutics.

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