CD43 Regulation of T Cell Activation Is Not through Steric Inhibition of T Cell–APC Interactions but through an Intracellular Mechanism

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Abstract

CD43 is a large heavily glycosylated protein highly expressed on T cells and actively excluded from the immunological synapse through interactions with ezrin-radixin-moesin proteins. Due to its size and charge, it has been proposed that the CD43 ectodomain acts as a physical barrier to T cell–APC interactions. We have addressed this hypothesis by studying the effect of reconstituting CD43 mutants into the hyperproliferative CD43$^{-/-}$ T cells. Reintroduction of full-length CD43 reversed the CD43$^{-/-}$ T cell hyperproliferation. Interestingly, despite the lack of exclusion from the interaction site, a mutant containing the CD43 ectodomain on a glycosylphosphatidylinositol linkage was ineffective. Additionally, T cell–APC conjugate formation was not affected by this ectodomain-only construct. In contrast, CD43$^{-/-}$ T cell hyperproliferation was reversed by an intracellular-only CD43 fused to the small ectodomain of hCD16. Mutation of this intracellular-only CD43 such that it could not move from the T cell–APC contact site had no further affect on proliferation than the moveable CD43 but did dramatically reduce interleukin-2 production. Thus, the exclusion of the CD43 intracellular region from the immunological synapse is required for CD43 regulation of interleukin-2 production, but the presence of the cytoplasmic tail, independent of its location, is sufficient to reverse CD43$^{-/-}$ T cell hyperproliferation.

Key words: CD43 • CD25 • proliferation • adhesion • SMAC

Introduction

CD43, also known as leukosialin or sialophorin, is a heavily glycosylated transmembrane protein expressed on most hemopoietic cells except erythrocytes (1–3). The extracellular domain (ECD) of CD43 has an elongated, unfolded structure that is predicted to extend 45 nm from the phospholipid bilayer, making it the largest glycoprotein on the cell surface (4), and CD43 has been estimated to cover up to 28% of the surface area on rat thymocytes (5). The extracellular domain of CD43 contains >80 serine or threonine residues, most of which are O-glycosylated, bearing numerous sialic acids residues (4). The cytoplasmic region of murine CD43 consisting of 124 amino acids is highly homologous (70% identity) to human CD43 (6).

The function of CD43 remains elusive (7). Targeted disruption of CD43 in mice and cell lines leads to increased T cell proliferation and adhesion in vitro (8, 9) and increased immune responses in vivo (10). However, cross-linking

Abbreviations used in this paper: DO.CD43$^{-/-}$, DO.11.10.CD43$^{-/-}$; CD16-7, the ectodomain of human CD16 fused to the transmembrane of human CD7; CD16-7-43, the CD16-7 construct fused to the ICD of murine CD43; CD16-7–NGG, CD16-7-43 with the ERM binding KRR motif mutated to the ERM nonbinding NGG motif; CD43FL, full-length murine CD43; CD43GPI, the ectodomain of murine CD43 fused to a GPI anchor; CD43–NGG, the full-length mCD43 with the ERM binding KRR motif mutated to the ERM non-binding NGG motif; CFSE, carboxyfluorescein diacetate succinimidyl ester 5-(and 6-); C-SMAC, central supramolecular activation cluster; ECD, extracellular domain; ERM, ezrin-radixin-moesin; GPI, glycosylphosphatidylinositol; ICD, intracellular domain.

CD43 concurrently with TCR ligation induces a dramatic costimulatory response that is independent of CD28 (11). Recently, Onami et al. have suggested that for in vivo CD8 responses, CD43 is costimulatory early in the response but a negative effector molecule later (12).

The prevailing hypothesis in the literature is that CD43 has a negative function due to an effect on cell–cell repulsion mediated by its extensive sialylated O-glycans (the "barrier hypothesis"). This hypothesis has been seemingly supported by demonstration that TCR signaling induces selective exclusion of CD43 from the T cell antigen–presenting cell contact site via its interaction with the family of cytoskeletal adaptor proteins, ezrin-radixin-moesin (ERM; 13–16). Interestingly, inhibition of CD43 movement leads to decreased IL-2 and IFN-γ production (14, 15). Together, these data suggest that CD43 could represent a novel type of cell surface regulatory protein that dampens T cell responses by its physical presence and is specifically removed upon T cell activation.

To directly test whether it is the presence of the CD43–ECD in the T cell–APC interaction site that dampens T cell responses, structure/function studies were done. Additionally, ECD-only and intracellular domain (ICD)-only constructs as well as ICD-only constructs mutated at the ERM-binding site were tested. The CD43–ECD fusion protein failed to be excluded from the immunological synapse, yet did not interfere with T cell–APC conjugate formation or reduce CD43$^{-/-}$ T cell hyperproliferation. However, the ICD of CD43 was sufficient to effectively reverse CD43$^{-/-}$ T cell hyperactivation. Interestingly, mutation of the intracellular region construct so that it could not bind ERM proteins or move from the site of TCR signaling further decreased IL-2 production. Thus, our data definitively demonstrates that it is the ICD of CD43, not the ectodomain, which confers negative regulation of T cell activation.

Materials and Methods

Mice. CD43$^{-/-}$ mice (provided by Dr. Blair Ardman, Tufts University, Medford, MA; reference 8) were backcrossed to BALB/c (The Division of Cancer Treatment, National Cancer Institute) six times and bred to the TCR Tg DO.11.10 as described previously to produce the DO.CD43$^{-/-}$ strain (14). DBA/2NCr were purchased from NCI-FCRDC. All mice housed at the University of Chicago were bred and/or maintained in a specific pathogen-free condition in barrier facilities. The studies reported here conform to the principles outlined by the Animal Welfare Act and NIH guidelines.

Antibodies and Other Reagents. Affinity-purified anti-CD28 (PV-1; reference 17) and anti-CD3 (145–2C11; reference 18) were prepared in our laboratory. mAbs for mouse CD43 (S7), human CD16 (3G8), and mouse CD25 (7D4) were purchased from BD Biosciences. Rabbit anti–PKC-θ (C-18) was from Santa Cruz Biotechnology, Inc. HRP-conjugated goat anti–mouse and goat anti–rabbit Ig were purchased from Bio-Rad Laboratories. FITC or Texas red donkey anti–rat IgG (mouse adsorbed), Texas red donkey anti–rabbit Ig (mouse absorbed), and Texas red donkey anti–goat Ig were all from Jackson Immu-

noResearch Laboratories. The OVA$_{323–339}$ peptide was produced by the University of Chicago Peptide Synthesis Facility.

Production of Primary T Cell Lymphoblasts. LN T cells from DO.11.10 and DO.CD43$^{-/-}$ mice were enriched by nonadherence to nylon wool as previously described (11). The T cells (2 × 10^5 cells/well) were stimulated with irradiated DBA/2 splenocytes (6 × 10^6 cells/well) and chicken ovalbumin peptide (OVA$_{323–339}$) at 0.15 μg/ml in complete media (DME media [Life Technologies] supplemented with 5% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.29 mM L-glutamine, nonessential amino acids, 10 mM Hepes, and 5 × 10^{-5} M β-mercaptoethanol). After 4 d of culture, 1 ml of complete media was added to each well. T cells were harvested and live cells recovered by Ficoll-Hypaque gradient separation 9–14 d after initial stimulation.

Transduction of Retroviral Constructs into Primary T Cells. The following vectors were used: full-length murine CD43 (CD43FL); full-length mCD43 with the ERM binding KRR motif mutated to the ERM nonbinding NGG motif (CD43–NGG); ectodomain of murine CD43 fused to a GPI anchor (CD43GPI); ectodomain of human CD16 fused to the transmembrane of human CD7 (CD16-7); CD16-7 construct fused to the ICD of murine CD43 (CD16-7-43); and CD16-7-43 with the ERM binding KRR motif mutated to the ERM nonbinding NGG motif (CD16-7–NGG). Construction of retroviral vectors and transduction methods have been published previously in detail (14). Briefly, Phoenix packaging cells transfected by calcium phosphate T cells from DO.CD43$^{-/-}$ mice were plated on anti-CD3/CD28–coated plates. At 48 h after transfection, the virus particles and Phoenix packaging were harvested and inactivated. The Phoenix cells and their virus-containing supernatants were recombined and overlaid onto the activated T cell cultures in the presence of hexadimethrene bromide (Sigma-Aldrich) to enhance the infection. The transduced T cells were used in experiments within 7–14 d and sorted for CD43 or hCD16 expression using the MoFlo (Cytomation). Transduced cells consistently expressed the transgene at high levels (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20021602/DC1), and sorting gates were used to ensure similar levels of transgene expression in all populations.

Immunofluorescence Staining and Microscopy. T cell lymphoblasts were conjugated with OVA$_{323–339}$–pulsed A20 (2 μg/ml) and stained for immunofluorescence as described in detail in Allenspach et al. (14). Briefly, conjugates were washed, adhered to poly-L-lysine–coated coverslips, and fixed. Fixation of the GPI-anchored CD43 conjugates was done with 4% PFA/0.1% glutaraldehyde/PBS to fix lipid rafts. The samples were quenched, permeabilized, and blocked. The fixed cells were stained with the primary antibodies, washed, and then stained with the fluorochrome-labeled secondary antibodies. Cells were observed using a 63× Planapo objective and photographed using a Carl Zeiss MicroImaging, Inc. Axiovert equipped with a Micromax cooled CCD camera (Roper). Single images were processed by noneighbor deconvolution using SlideBook 3.0 (Intelligent Imaging Innovations) to remove out of focus haze. For en face analysis of the T cell–APC interface, z-series images were processed by nearest-neighbor deconvolution and rendered in three dimension using Slidebook.

Flow Cytometry and ELISA. After sorting, transduced T cells were labeled with 3.33 μM carboxyfluorescein diacetate succinimidyl ester 5-(and 6-) (CFSE; Molecular Probes), and then 10^5 T cells per well were stimulated with mitomycin C–treated antigen-pulsed A20 cells (10^5 cells/well). At day 3, the T cells were

recovered and stained with anti-CD43, and flow cytometric analysis was performed. Data were analyzed using CellQuest and Modfit software to determine the percentage of cells in each generation. Expression of CD25 was determined at 24 h activation. Supernatants were collected 24 h after activation and assayed for IL-2 production by ELISA (BD Biosciences).

Heteroconjugate Adhesion Assay. A20 B lymphoma cells were stained according to manufacturer's instructions with the vital dye PKH26 (Sigma-Aldrich), a membrane marker that fluoresces in the same wavelength as phycoerythrin. The cells were washed, pulsed with OVA peptide as above, and resuspended to 5×10^5 cells/ml. T cells were stained according to manufacturer's instructions with Calcein-AM a cytoplasmic dye (Sigma-Aldrich) that emits in the same wavelength as fluorescein. Prior to mixing the T cells with APCs, the cells were washed once in 5% DMEM and resuspended to 5×10^5 cells/ml. APCs were added at equal volumes to the appropriate T cells. The mixture of cells was centrifuged at 500 rpm for 5 min at 4°C and vortexed for 10 s at setting 4. After agitation, the cell mixtures were incubated in a 37°C water bath for the different times as indicated. The samples were vortexed for 30 s at setting 2 for naive cells and setting 8 for previously activated cells and then immediately fixed with an equal volume of 6% paraformaldehyde in PBS. Conjugates were measured by flow cytometry, and the percent of T cells in conjugates was determined. Triplicate samples were used to determine SDs.

Online Supplemental Material. Fig. S1 shows that the T cell transduced with CD43 mutant constructs expresses equal levels of the control and mutant proteins. Fig. S2 shows a second T cell–APC conjugate similar to Fig. 2 A that demonstrates that CD43GPI is located throughout the immunological synapse including the C-SMAC. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20021602/DC1.

Results and Discussion

CD43 Negatively Regulates T Cell Proliferation and Activation in Antigen-specific Responses. Mitogen or alloantigen stimulation has been shown to induce increased proliferation of CD43-deficient T cells compared with WT T cells (8, 9). To test whether this hyperproliferation would also be found in an antigen-specific model, CD43$^{-/-}$ mice were bred to the DO11.10 TCR transgenic strain (DO.CD43$^{-/-}$). WT and DO.CD43$^{-/-}$ T lymphoblasts were labeled with CFSE dye and cocultured with OVA$_{323–339}$–pulsed A20 cells. We found a significant difference between DO.CD43$^{-/-}$ and WT DO11.10 T cells. The two populations divided similarly on day 2, but on days 3 and 4 the DO.CD43$^{-/-}$ T cells underwent more cell divisions when compared with WT T cells (Fig. 1 A and not depicted). 24 h after activation, CD25 expression on the DO.CD43$^{-/-}$ T cells was also higher than on WT T cells (Fig. 1 B), suggesting that the signals for hyperpro-
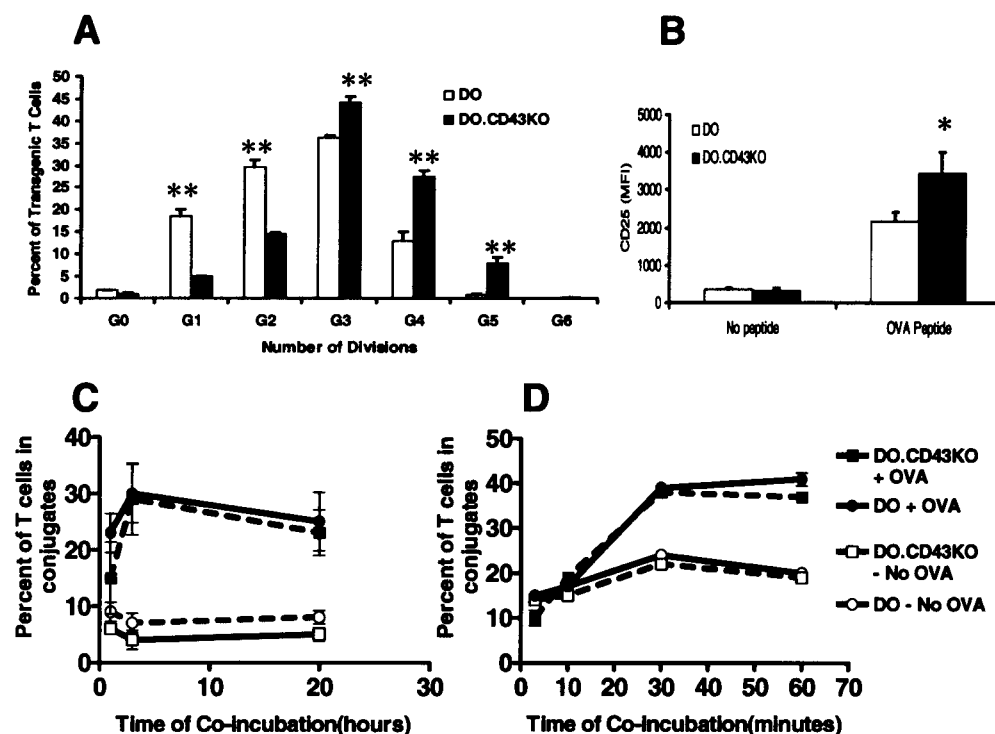

Figure 1. CD43 negatively regulates T cell proliferation and activation but does not affect formation of T cell–APC conjugates. (A) Rested DO.CD43$^{-/-}$ and DO11.10 lymphoblast were dyed with CFSE and stimulated with OVA peptide (0.3 μg/ml)–pulsed A20 B cells. Daughter generations were determined by measuring the CFSE peaks that shift to the right of the parental peak with Modfit software. CD43$^{-/-}$ T cells divide and undergo more divisions compared with CD43 WT T cells on day 3. (B) CD25 expression at 24 h after stimulation with OVA peptide (0.3 μg/ml) pulsed A20 cells is decreased on CD43 WT T cells. *P < 0.05; **P < 0.01. Data are from one representative experiment of a total of three independent experiments. (C and D) A flow cytometric based assay was used to determine adhesion between DO11.10 (circles) or DO.CD43$^{-/-}$ (squares) T cells and OVA-pulsed (filled symbols) or unpulsed (open symbols) A20 cells. No difference was found between CD43$^{-/-}$ and WT murine T cells to form conjugates with 0.3 μg/ml of OVA peptide–pulsed APC. Naive (C) and previously activated (D) T cells were tested. Data are from one representative experiment of a total of three independent experiments.
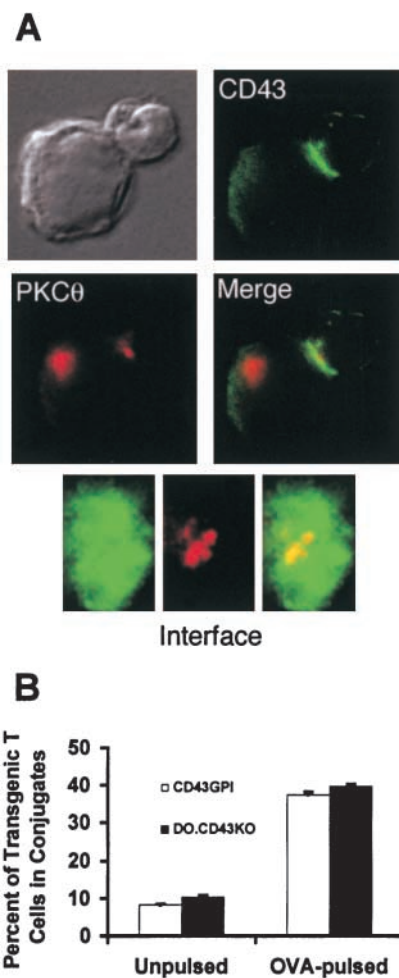
Figure 2. The ectodomain of CD43GPI is present throughout the T cell–APC interaction site, yet has no affect on T cell–APC conjugate formation. (A) DO.CD43GPI T cells (small cells on the right in each picture) were conjugated with antigen-pulsed A20 B cells (large cells on left), fixed, and double labeled for CD43 and PKC-θ as described in Materials and Methods. (A second example of a different CD43GPI-transduced T cell–APC conjugate is found in Fig S2, available at http://www.jem.org/cgi/content/full/jem.20021602/DC1). z-series images were collected, and digital deconvolution and three-dimensional rendering of the cell–cell interface demonstrated that although PKC-θ was focused in the C-SMAC, the CD43GPI molecule was distributed throughout the entire contact area. (B) Using the flow cytometric based adhesion assay described in the legend to Fig. 1, no difference in conjugate formation between CD43$^{-/-}$ and CD43GPI-transduced T cells with 0.3 μg/ml of OVA peptide–pulsed A20 cells was found.

liferation may occur within this time period. Similar results were found when naive T cells were used (unpublished data). Thus, similar to mitogenic stimuli, antigen-specific stimulation also induces hyperproliferation and activation of CD43$^{-/-}$ T cells.

DO.CD43$^{-/-}$ T Cells Do Not Display Increased Heteroconjugate Adhesion. A model of T cell activation based on T cell topology and a sorting of surface molecules by size and charge has been proposed (19, 20). In this model, CD43, being one of the largest and most highly charged molecules on the cell surface, would need to be removed from the in-

teraction site in order for the T cell receptor to make contact with the class II molecules on the APC. Thus, it has been suggested that CD43 may regulate cellular interactions by providing a physical constraint and thereby affect the ability of T cells to form the necessary interaction site with APC. In agreement with this model, increased homotypic adhesion has been reported in cultures of cells lacking CD43 (5, 8, 9). However, the presumed interference of CD43 with T cell–APC heterotypic adhesion has never been addressed directly. To determine if CD43 actually affects the formation of heterotypic cellular conjugates, we developed a flow cytometric assay to measure the number of conjugates and the rate of their formation in vitro. Contrary to our expectations, we found that there is no difference between DO.CD43$^{-/-}$ and WT murine T cells with respect to the rate and degree of conjugate formation with APC (Fig. 2). This was true whether the T cells were isolated directly ex vivo or were from lymphoblast cultures. These data suggest that the hyperproliferative response of DO.CD43$^{-/-}$ T cells is not due to a greater ability of these T cells to form conjugates with APC.

The Presence of the CD43 Ectodomain in the T Cell–APC Interaction Site Does Not Affect T Cell Activation or Adhesion. For years, it has been proposed that CD43 negatively regulates T cell activation by acting as a physical barrier to cellular interactions and that its removal is necessary for normal T cell activation. Although we observed no difference in conjugate formation with APC between DO.CD43$^{-/-}$ and WT T cells, we and others have found that TCR signaling induces exclusion of CD43 from the T cell–APC contact site (13–16). Thus, it was still possible that before removal of CD43 from the interaction site there is a short period of steric interference. To address this question, we produced a fusion molecule with the ectodomain of CD43GPI and transduced DO.CD43$^{-/-}$ T cells with either CD43FL or CD43GPI as described previously (14). CD43FL was excluded from the interaction site similar to WT CD43 on T cells, whereas CD43GPI was not excluded (Fig. 2 A). In fact, the CD43GPI tended to move toward the interaction site. This is most likely due to the enrichment of GPI-linked proteins in the cholesterol-rich lipid rafts that are known to coalesce at the interaction site (21). To examine the location of the CD43GPI within the immunological synapse, we performed three-dimensional rendering of the TCR surface at the T cell–APC contact site and determined that the CD43GPI was distributed throughout the entire contact area. Importantly, double labeling with anti–PKC-θ shows that CD43GPI is present in the PKC-θ–rich central supramolecular activation cluster (C-SMAC) region where sustained TCR–MHC interactions are proposed to occur.

The production of a mutant CD43 molecule that contains no transmembrane or cytoplasmic region, which through its GPI linkage actually moves the ectodomain of CD43 toward the T cell–APC contact site, allowed for a direct test of the "barrier hypothesis." If this hypothesis is correct, then the CD43GPI mutant that places CD43 in
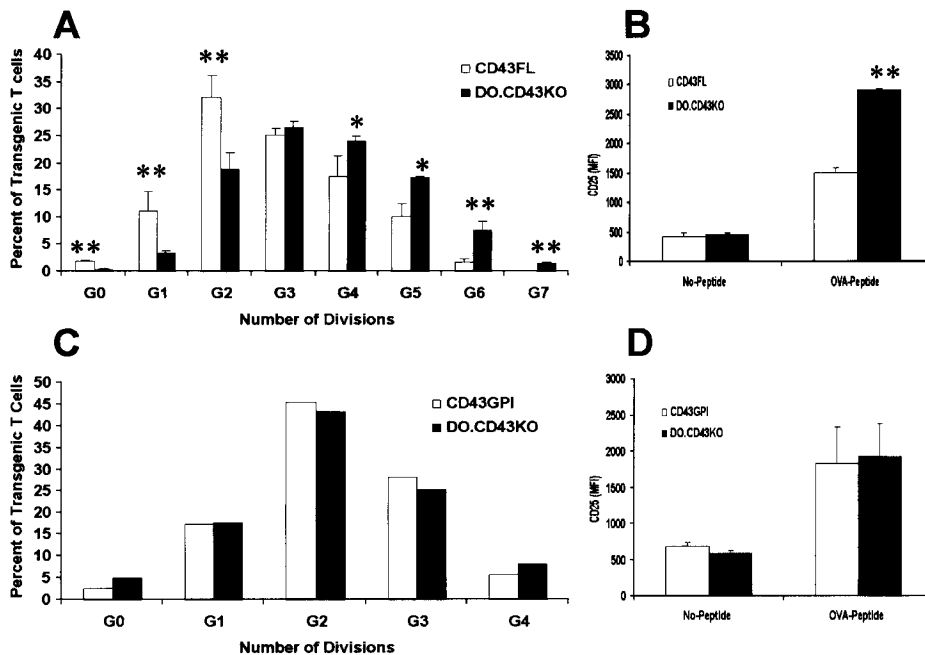
Figure 3. The presence of the CD43 ectodomain in the T cell–APC interaction site is not sufficient to inhibit T cell proliferation or activation. CD43$^{-/-}$ T cells were transduced with either CD43FL or CD43GPI and labeled with CFSE. Unsorted populations containing both transduced and nontransduced cells were stimulated with antigen-pulsed A20 cells. The cells were analyzed for CD25 expression at 24 and 72 h for proliferation. By flow cytometry, the CD4$^+$CD43$^+$ (transduced) cells were compared with CD4$^+$CD43$^-$ (nontransduced) cells from the same culture. (A and B) Reintroduction of CD43FL into CD43$^{-/-}$ T cells inhibited proliferation (A) and CD25 expression (B) to levels similar to CD43 WT T cells. (C and D) Reintroduction of CD43GPI into CD43$^{-/-}$ T cells had no effect on proliferation. The CD43-transduced T cells proliferated as rapidly as CD43$^{-/-}$ T cells at day 3 (C) and had no decrease in CD25 expression (D). *P $<$ 0.05; **P $<$ 0.01. Data are from one representative experiment of a total of three independent experiments.

the T cell–APC contact site should decrease T cell function. Yet, no significant differences between the adhesive capabilities of CD43$^{-/-}$ and CD43GPI-transduced T cells could be discerned (Fig. 2 B). Both populations formed equal numbers of conjugates with OVA peptide–pulsed APC at all time points examined. Thus, the theory that CD43 must be removed from the interaction site to facilitate T cell–APC adhesion is not supported by our data. Further, if the barrier hypothesis was correct, GPI-linked CD43 should have had a greater tendency to inhibit T cell activation than CD43FL which is actively moved to the distal pole complex. Again, our data does not support this hypothesis. Transduction of CD43FL into CD43$^{-/-}$ T cells diminished proliferation and CD25 expression, whereas transduction of the CD43GPI molecule had no effect on the hyperproliferation of CD43$^{-/-}$ T cells (Fig. 3). Thus, we have directly disproven the size-exclusion barrier hypothesis.

The Cytoplasmic Tail of CD43 Is Sufficient for Diminished T Cell Proliferation. Our results with the CD43GPI construct demonstrate that the negative regulation of T cell activation by CD43 is not due to the presence of the ECD in the T cell–APC interaction site. Since the CD43FL was able to reconstitute the WT phenotype, these data suggest that the ICD is necessary for the negative regulatory function of the molecule. To test this hypothesis, an "intracellular only" construct was used (14). This fusion molecule consists of the ECD of human CD16, the transmembrane region of human CD7, and the ICD of murine CD43 (CD16-7-43). The construct and its control, CD16-7, were transduced into CD43$^{-/-}$ T cells, and the resulting populations were sorted and tested for proliferation and CD25 expression (Fig. 4, A and B). The CD16-7-43–transduced cells proliferated slower and expressed less

CD25 than the CD16-7 cells. Therefore, these data demonstrate that the negative function of CD43 on T cell proliferation is due entirely to an intracellular mechanism.

IL-2 Production Is Inhibited by the Presence of the CD43 ICD in the Immunological Synapse. The role of CD43 exclusion in the regulation of proliferation has been tested by Savage et al. (22). They found that expression of a mutant CD43 construct in which the ERM-binding site was mutated (CD43–NGG) reversed the hyperproliferation of the CD43$^{-/-}$ T cells but did not decrease proliferation further than transduction with the CD43FL construct. In light of these findings, we have investigated whether the reversal of the CD43$^{-/-}$ T cell hyperproliferation by the CD43–NGG mutant was due to the presence of the CD43 ectodomain as suggested by the barrier hypothesis or the ICD as suggested by our studies. When the CD43 intracellular-only construct was also mutated at the ERM-binding site (CD16-7–NGG), the CD16-7–NGG mutant reversed the hyperproliferation of the CD43$^{-/-}$ T cells but did not reduce proliferation further than that seen with CD16-7-43–transduced T cells (Fig. 4 E). Thus, the negative effect of CD43 on T cell proliferation is entirely due to the presence of its ICD, regardless of its location.

Our data on T cell proliferation suggest that the ectodomain is not involved in the regulatory function of CD43. However, Delon et al. found that overexpression of the CD43–NGG in WT T cells actually inhibited IL-2 production (15). The authors proposed that this was due to the inability of these cells to move the large highly glycosylated CD43 ectodomain from the T cell–APC contact site. To address the apparent contradiction between our data and those of Delon et al., we investigated the role of CD43 exclusion on IL-2 production. First, we investigated whether, similar to the data in Delon et al., transduction of
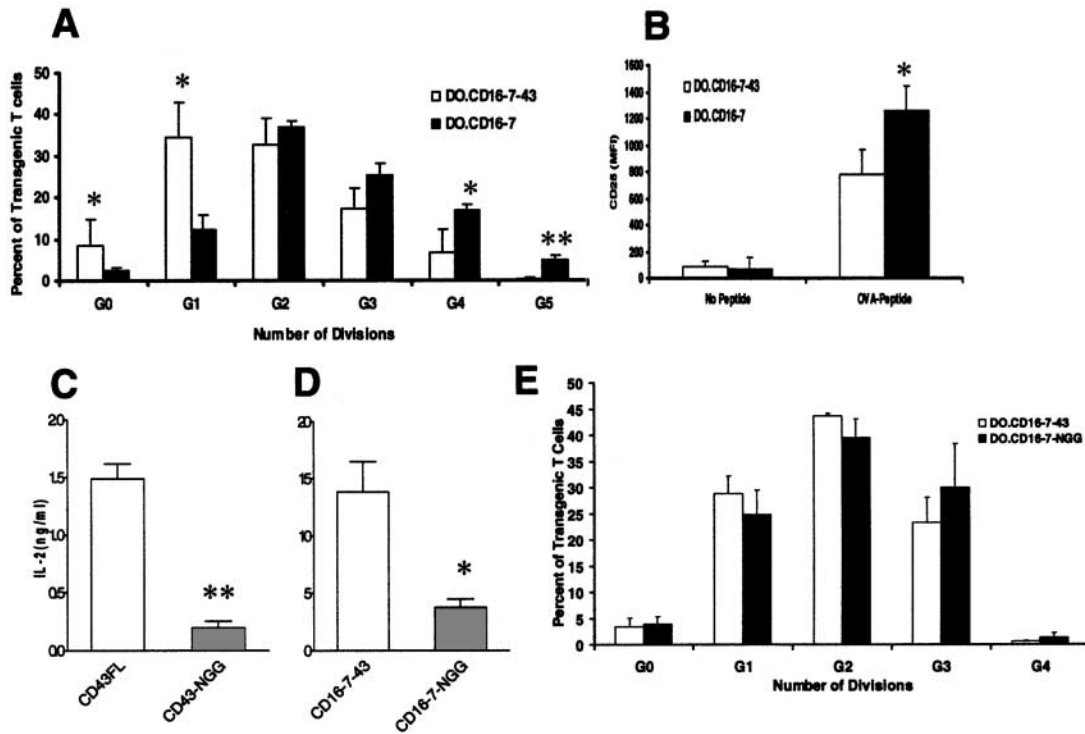
Figure 4. The cytoplasmic domain of CD43 is necessary and sufficient to inhibit T cell proliferation. (A and B) CD43$^{-/-}$ T cells were transduced with the "inside only" construct CD16-7-43 or the control construct CD16-7. (A) The CD16-7-43–transduced T cells had significantly decreased T cell proliferation compared with CD16-7–transduced control T cells. (B) CD25 expression at 24 h after stimulation with OVA peptide (0.3 μg/ml)–pulsed A20 cells is decreased on CD16-7-43–transduced T cells. (C and D) CD43–NGG and CD16-7–NGG mutants decreased IL-2 production compared with their WT controls. Sorted CD43FL and CD43–NGG T cells (C), CD16-7-43 and CD16-7–NGG (D) T cells were stimulated at 24 h with OVA peptide (0.3 μg/ml)–pulsed A20 cells. (E) CD43$^{-/-}$ T cells transduced with CD16-7-43 and CD16-7–NGG constructs were CFSE labeled, and proliferation was measured at day 3. *$P < 0.05$; **$P < 0.01$. Data are from one representative experiment of a total of three independent experiments.

the CD43–NGG mutant into CD43$^{-/-}$ T cells inhibited IL-2 production. As seen in Fig. 4 C, upon restimulation less IL-2 was produced by the CD43–NGG T cells than the CD43FL T cells. However, CD43$^{-/-}$ T cells transduced with CD16-7–NGG also produced significantly less IL-2 upon restimulation than those transduced with moveable CD16-7-43 (Fig. 4 D). Thus, our data does not support the interpretation of Delon and colleagues that CD43 regulation of IL-2 production is due to a steric interference of T cell interactions by the CD43 ectodomain, since decreased IL-2 production required only the presence of the CD43 ICD in the contact site. Further, our findings suggest that although CD43 regulation of T cell proliferation and IL-2 are both mediated through intracellular events, moving the cytoplasmic region from the interaction site is only required for some of its functions.

In conclusion, our study provides definitive evidence against the barrier hypothesis for CD43 function. Our data clearly demonstrate that the CD43 intracellular region is necessary and sufficient for CD43 function, and we could find no evidence for any effect of the extracellular region. It remains to be determined how CD43 actually downregulates T cell activation. Previously, CD43 has been shown to be phosphorylated on resting T cells, and this phosphorylation increases upon T cell activation (23). We

propose that changes in CD43 phosphorylation may lead to signaling events that regulate adhesion and activation. Studies are underway in our laboratory to address this hypothesis. Furthermore, the role of the CD43 ectodomain remains to be explored. Several groups have identified potential CD43 ligands (7, 24, 25), and engagement of these CD43 ligands may provide positive costimulatory signals to the T cell (11, 12). It is also possible that the CD43 ectodomain functions as a soluble molecule. In fact, soluble CD43 ectodomain has been found at substantial levels in human sera (26), and recent work has shown a novel role for soluble CD43 in promoting stable interaction of mycobacteria with receptor on the macrophage, enabling the cells to respond by producing TNF-α (27). Thus, further studies are required to determine the distinct functions of the extracellular, transmembrane, and cytoplasmic domains of CD43.

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