TCR-Dependent Cell Response Is Modulated by the Timing of CD43 Engagement

Nora A. Fierro, Gustavo Pedraza-Alva and Yvonne Rosenstein

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A specific immune response is initiated when T lymphocytes recognize Ag peptides through the interaction of the T cell Ag receptor complex (TCR), with processed peptides displayed by MHC molecules on APCs (1). Yet, TCR-mediated signals are not sufficient to induce T lymphocyte activation. While T cells stimulated through the TCR in the absence of costimulatory signals reach a state of functional unresponsiveness referred to as anergy (2), additional signals provided by the interaction of costimulatory receptors with their ligands on APCs promote cellular proliferation and gene expression (3–6). Most studies have shown efficient T cell activation by engaging simultaneously the TCR and costimulatory molecules; however, it is unclear whether the time when T cell costimulatory molecules interact with their counterreceptors with regards to Ag recognition leads to different biological responses.

It has been suggested that the outcome of a response likely depends on which contacts are first established between T cells and APCs as well as on the relative density and structure of the molecules that participate (7, 8). Based on the fact that the coreceptor molecule CD43 is a very abundant molecule evenly distributed on the membrane of naive T cells and that its elongated structure protrudes 45 nm from the cell, we hypothesized that CD43 is one of the first molecules that interacts with the APC and thus modulates TCR activation. We show that engaging CD43 before or simultaneously with the TCR inhibited Lck-Src homology 2 domain containing phosphatase-1 interaction, preventing the onset of a negative feedback loop on TCR signals, favoring high levels of IL-2, cell proliferation, and secretion of proinflammatory cytokines and chemokines. In contrast, the intracellular signals resulting of engaging the TCR before CD43 were insufficient to induce IL-2 production and cell proliferation. Interestingly, when stimulated through the TCR and CD28, cells proliferated vigorously, independent of the order with which molecules were engaged. These results indicate that CD43 induces a signaling cascade that prolongs the duration of TCR signaling and support the temporal summation model for T cell activation.

In addition to the strength and duration of intracellular signals, our data underscore temporality with which certain molecules are engaged as yet another mechanism to fine tune T cell signal quality, and ultimately immune function. The Journal of Immunology, 2006, 176: 7346–7353.

**Materials and Methods**

**Reagents**

L10, a murine IgG1 mAb that recognizes human CD43 (19), and OKT3 (American Type Culture Collection; anti-CD3, IgG2a) were purified from ascites on protein A-Sepharose columns. The anti-CD28 and anti-CTLA-4 mAbs were from Ancell. Rabbit anti-mouse-IgG1 and -IgG2a were from Fisher Biotech. The anti-ζ, anti-pERK, anti-ERK2, anti-JNK, anti-Lck, and anti-CD3 Engagement1

Nora A. Fierro,‡ Gustavo Pedraza-Alva,* and Yvonne Rosenstein ‡*

Binding of Ag by the Ag receptor in combination with other stimuli provided by costimulatory receptors triggers the expansion and differentiation of T lymphocytes. However, it is unclear whether the time when costimulatory molecules interact with their counterreceptors with regards to Ag recognition leads to different T cell responses. Provided that the coreceptor molecule CD43 is a very abundant molecule evenly distributed on the membrane of T cell surface protruding 45 nm from the cell, we hypothesized that CD43 is one of the first molecules that interacts with the APC and thus modulates TCR activation. We show that engaging CD43 before or simultaneously with the TCR inhibited Lck-Src homology 2 domain containing phosphatase-1 interaction, preventing the onset of a negative feedback loop on TCR signals, favoring high levels of IL-2, cell proliferation, and secretion of proinflammatory cytokines and chemokines. In contrast, the intracellular signals resulting of engaging the TCR before CD43 were insufficient to induce IL-2 production and cell proliferation. Interestingly, when stimulated through the TCR and CD28, cells proliferated vigorously, independent of the order with which molecules were engaged. These results indicate that CD43 induces a signaling cascade that prolongs the duration of TCR signaling and support the temporal summation model for T cell activation.

In addition to the strength and duration of intracellular signals, our data underscore temporality with which certain molecules are engaged as yet another mechanism to fine tune T cell signal quality, and ultimately immune function. The Journal of Immunology, 2006, 176: 7346–7353.

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2 Address correspondence and reprint requests to Dr. Yvonne Rosenstein, Instituto de Biotecnología and Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico.

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*Instituto de Biotecnología and ‡Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México

1 Abbreviations used in this paper: SHP-1, Src homology 2 domain containing phosphatase 1; TPA, 12-O-tetradecanoylphorbol-13-acetate.
Purified T lymphocytes (2 × 10⁵) were incubated in 0.5 ml of cold RPMI 1640 according to different protocols (all Abs were used at 4 μg/ml).

**L10 or OKT3.** Cells were stimulated only with L10 or OKT3. Abs were added for 10 min at 4°C before addition of the isotype-specific secondary Ab (anti-IgG1 for L10 or anti-IgG2a for OKT3), and cells were activated at 37°C for the indicated periods.

**L10-OKT3.** Cells were stimulated first through CD43, L10 was added for 10 min at 4°C to the cell suspension, cross-linked with isotype-specific secondary Ab (anti-IgG1 for L10 or anti-IgG2a for OKT3), and cells were activated at 37°C for the indicated periods. L10-OKT3 were cells stimulated with L10 and OKT3. Abs were added for 10 min at 4°C before addition of the isotype-specific secondary Ab (anti-IgG1 for L10 or anti-IgG2a for OKT3), and cells were activated at 37°C for the indicated periods.

**OKT3-L10.** When cells were first stimulated through the TCR, we followed the same protocol as described above, except that OKT3 was added as a first Ab and L10 provided the second stimulus.

**L10 + OKT3.** When cells were stimulated simultaneously through CD43 and the TCR, L10 and OKT3 were added at the same time, and cells were incubated for 10 min at 4°C, following which isotype-specific secondary Abs were added and cells were incubated at 37°C for the indicated periods of time.

**Cell proliferation assays**

A total of 2 × 10⁵ T lymphocytes were prestimulated for 2 h (Ab (1 μg/ml) was added for 10 min before addition of the isotype-specific secondary Ab (anti-IgG1 for L10 or anti-IgG2a for OKT3), following which cells were washed to remove excess of the first Ab, and costimulatory signals were applied for 7 days before evaluating cell proliferation. To assess the ability of lymphocytes to respond to secondary stimulation, cells were washed with PBS, adjusted to 2 × 10⁵ cells/well and arrested for 24 h in RPMI 1640, and further stimulated with OKT3 cross-linked with isotype-specific secondary Ab for 5 min at 37°C.

**OKT3-L10.** Cells were resuspended in 0.5 ml of RPMI 1640 and 100 μl of MTT (1 mg/ml) was added for 3 h. The plates were incubated in a 5% CO₂ atmosphere for 4 h at 37°C, and 100 μl of DMSO was added to each well. After 30 min, the absorbance at 570 nm was determined using a microplate reader (Bio-Rad). Absorbance was compared with cells stimulated with PBS buffer and incubated with avidin coupled to FITC for 30 min at 4°C and washed as above. A FACSort (BD Biosciences) was used to analyze the results.

**Cytokine production**

For each of the stimulation protocols described above, cytokines present in the culture medium were measured. Cytokines were measured using a mouse cytokine kit (BD Biosciences). The presence of cytokines was determined using a sandwich ELISA technique, and the results were expressed as picograms per milliliter.

**RESULTS**

**FACS staining**

Cells (1 × 10⁶) were treated with secondary Abs only. Differences were calculated based on levels found in cells subtracted, and the results were expressed as picograms per milliliter. The results were compared with cells stimulated with PBS buffer and incubated with avidin coupled to FITC for 30 min at 4°C and washed as above. A FACSort (BD Biosciences) was used to analyze the results.

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previously, only when CD43 and the TCR were cross-linked simultaneously or when CD43 provided the first activation signals cells proliferated, whereas cells that had been stimulated with CD43, the TCR, or the TCR before CD43 did not proliferate (data not shown). In addition, and further supporting the fact that CD43-mediated signals prevent anergy when provided previously or simultaneously to TCR engagement, high levels of IL-2 were detected only when CD43 was ligated before or simultaneously with the TCR. On the contrary, providing TCR signals as the first signal or independent stimulation with CD43 or the TCR resulted in low levels of IL-2 (Fig. 1B). The lack of proliferation of cells was not due to cell death and addition of IL-2 at the onset of the second stimulation with OKT3 rescued cells stimulated with the TCR as the first stimulus from anergy as well as those activated only through CD43 or the TCR (data not shown).

Expression of the CTLA-4 molecule on the cell surface as a result of TCR engagement has been related to anergy (21, 22). Consistent with the lack of proliferation in response to a secondary stimulation with OKT3, cells stimulated through the TCR alone (Fig. 1C, upper panel) or through the TCR for 2 h before CD43 costimulation (Fig. 1C, lower panel) expressed CTLA-4. In contrast, ligation only CD43 did not result in CTLA-4 expression (Fig. 1C, upper panel). Concurrent with this, engaging CD43 before or at the same time as the TCR prevented the TCR-induced expression of CTLA-4 (Fig. 1C, lower panel). Altogether, these results suggest that the sequence with which a cell senses receptor-mediated signals may result in different responses.

The TCR-dependent anergic signals can be prevented by CD43 within a narrow time frame

Multiple reports have analyzed the differential responses generated by stimulating T lymphocytes through the TCR and costimulatory molecules, of which CD28 is the most studied (23). Our data indicated that, if provided before or concurrent with TCR engagement, CD43 costimulatory signals led to T cell activation and proliferation, suggesting that the sequence with which signals are perceived influences cell fate. To investigate whether this is a general mechanism for costimulatory molecules or whether it was specific for CD43, we evaluated the costimulatory function of the CD28 molecule under the same experimental conditions used for CD43. CD28 greatly increased TCR-induced cell proliferation, regardless of the order of the stimuli (Fig. 2A). These results suggest that, in contrast to CD43 (Fig. 1A), CD28-dependent signals prevent the TCR-mediated anergizing signals, and that the sequence with which CD28 and the TCR are engaged is not important to drive cell proliferation.

Because we had found that prestimulating the cells through the TCR for 2 h before CD43 costimulation resulted in anergy, we...
investigated whether there was a time frame wherein the TCR signals leading to anergy could be prevented by a subsequent CD43 engagement. Assays were conducted where the TCR was ligated for shorter periods of time before cross-linking CD43. When cells were stimulated with OKT3 for 1 h, subsequent CD43 ligation did not provide proliferative signals. However, decreasing the time of OKT3 stimulation to 30 or 10 min before engaging CD43 resulted in a marked increase of cell proliferation (Fig. 2B), suggesting that there is a critical time window of ~30 min following TCR triggering, where CD43-mediated signals can prevent the TCR-mediated signals leading to anergy. These results indicate that CD43 and CD28 generate costimulatory signals leading to T cell proliferation through different mechanisms. CD28-mediated signals were able to induce cell proliferation even if provided after 2 h of TCR engagement, whereas CD43-specific signals could only prevent cells from becoming anergic if given within the first 10 min of TCR engagement.

**ERK is a key component of CD43-mediated costimulatory signals**

Differences in the duration of intracellular signaling have been implicated in different biological responses (24); specifically, the kinetics and phosphorylation levels of ERK have been associated to the regulatory activation of transcriptional factors (25), modulating cellular functions ranging from survival signals to apoptosis. The TCR (26) and CD43 (12, 27) initiate the MAPK pathway when ligated on the surface of T cells. To evaluate whether ERK activation participates in the CD43-mediated signaling cascade that regulates TCR-induced signals, T lymphocytes were stimulated through cross-linking CD43 and the TCR following the same experimental schemes described above. As soon as 2 min after CD43 pretreatment (L10 – OKT3) or simultaneous costimulation through both molecules (L10 + OKT3), an enhanced ERK phosphorylation that lasted for at least 2 h was observed (Fig. 3A). In contrast, triggering the cells through the TCR and subsequent CD43 engagement (OKT3-L10) did not result in sustained ERK phosphorylation (Fig. 3A). Stimulation through either CD43 or the TCR alone induced a transient ERK phosphorylation, peaking at 2 h for CD43 and at 30 min for the TCR (Fig. 3A). Under our experimental conditions, CD43, the TCR or TCR pretreatment (OKT3-L10) induced preferentially ERK2 (p42) phosphorylation. Similar to TPA, CD43 pretreatment (L10 – OKT3) or simultaneous stimulation through both molecules (L10 + OKT3) resulted in increased ERK1 (p44) and ERK2 (p42) phosphorylation.

Because the differential activation of ERK and JNK has been found to regulate the generation of diverse cellular responses (28), the kinetics and intensity of JNK phosphorylation were also assessed in the same experiment. Contrary to ERK phosphorylation, JNK phosphorylation was enhanced by costimulation, regardless of the order of the stimuli. Stimulation through CD43 or the TCR alone resulted in JNK phosphorylation (Fig. 3B). As expected, TPA stimulation induced ERK and JNK phosphorylation revealing an efficient cell-signaling machinery (Fig. 3C). In conjunction, these results suggest that ERK plays a pivotal role integrating and regulating the activation signals driven through CD43 and the TCR.

**CD43 ligation before or simultaneously to TCR engagement induces a sustained ζ-chain phosphorylation**

Tyrosine phosphorylation of the ζ-chain has been considered as an early event of intracellular activation leading to ERK activity in response to TCR (29) as well as of CD43 cross-linking (13). To evaluate whether ERK activation resulting of ligating CD43 before or simultaneously with TCR engagement correlated with ζ-chain phosphorylation, we followed the kinetics of the ζ-chain tyrosine phosphorylation. When cross-linking both molecules simultaneously (L10 + OKT3), the pattern of ζ-chain phosphorylation reflected a combination of what was observed for the OKT3 and L10 stimuli alone, resulting in a more intense (~10-fold as compared with cells stimulated only through the TCR) and a sustained phosphorylation, lasting for up to 30 min (Fig. 4). Engaging CD43 before the TCR (L10 – OKT3) resulted in an early, intense and prolonged ζ-chain phosphorylation, but pre-stimulating through the TCR before CD43 ligation (OKT3-L10), led to transient ζ-chain phosphorylation. Addition of the isotype specific cross-linking reagents, did not result in ζ phosphorylation (Fig. 4). Thus, differences in the intensity and duration of early signals such as ζ-chain phosphorylation and ERK activation reflect variations in the order with which membrane receptors are engaged.

**FIGURE 3.** Engagement of CD43 before or simultaneously with TCR ligation results in sustained ERK phosphorylation. A total of 1 × 10⁷ human T lymphocytes were incubated with the first mAb (L10 or OKT3) for 10 min at 4°C, cross-linked with the isotype-specific secondary Ab (anti-igG1 for L10 or anti-igG2a for OKT3) for the indicated periods of time at 37°C, after washing to remove excess Ab, cells were costimulated for 5 min at 37°C, alternatively, cells were stimulated at the same time with both mAbs, or received a single stimulus. Total cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose, and subjected to immunoblotting with anti-pERK (A) or anti-pJNK (B) mAbs. C: TPA (50 ng/ml) stimulation was used as a positive control. Membranes were probed for total ERK2 or JNK, respectively. Data shown are representative of at least three independent experiments.
CD43 signals modulate the TCR-induced SHP-1-Lck association

Our results showed that level of ζ and ERK activation depends on the nature of the first stimulus detected by the T cells. By phosphorylating Lck on Ser59, ERK has been shown to prevent the interaction of Lck with the phosphatase SHP-1. This in turn results in prolonged phosphorylation of Lck Tyr394 and increased enzymatic activity of Lck (30–32), ultimately enhancing TCR signaling. We considered the possibility that by preventing the association of SHP-1 to Lck, CD43 costimulatory signals lead to sustained ERK and ζ-chain phosphorylation. Previous reports (30) assessed SHP-1/Lck association to immunoprecipitated TCR complexes and showed that the presence of SHP-1 in these complexes peaked at 40 min. Under our experimental conditions, the presence of SHP-1 in Lck immune complexes was best detected at 2 h (data not shown). Stimulating the cells through either CD43 or the TCR alone or through the TCR followed by a late costimulation led to sustained phosphorylation of Lck Tyr394 and increased enzymatic activity of Lck (Fig. 5 A, upper panel), resulting in enhanced Zap70 phosphorylation, compared with cells activated through the TCR as the first stimulus, or only through CD43 or the TCR (Fig. 5 A, lower panel). Furthermore, blocking ERK kinase activity reduced Zap70 phosphorylation to levels comparable to those of control cells (Fig. 5 A, lower panel). Interestingly, ζ-chain phosphorylation levels remained elevated for up to 2 h in cells that were first activated through CD43 (L10 + OKT3) or through simultaneous stimulation (L10 + OKT3) as compared with cells activated through CD43 or the TCR (Fig. 5 B). These data indicate that CD43-mediated signals previous or simultaneous to TCR engagement knockdown the SHP-1-Lck association, resulting in sustained ζ-chain and Zap70 tyrosine phosphorylation via an ERK-mediated mechanism.

The costimulatory effect of CD43 on the TCR-mediated signals is ERK dependent

Our results indicate that within a specific time frame (between 0 and 30 min) TCR anergic signals could be rescued by a second CD43-mediated stimulus, and that this response was under the control of the intensity and duration of ERK signals. We reasoned that addition of PD98059 simultaneously with costimulation would prevent the activation of a new pool of ERK molecules, allowing us to discriminate whether the costimulatory signals that hindered the cells from a TCR-induced anergic state were subjegated to a CD43-dependent ERK activation. To ascertain that PD98059 was acting only on the pool of ERK molecules recruited in response to the second stimulus, we evaluated the levels of ERK phosphorylation in total cell lysates from T lymphocytes activated with L10 or OKT3 with or without PD98059.

**FIGURE 5.** ERK and SHP-1 participate in prolonged phosphorylation of Zap70 and ζ-chain. A, A total of 2 × 10^7 human peripheral T lymphocytes were incubated for 15 min at 37°C in the presence or absence of 30 μM PD98059 and stimulated for 2 h at 37°C with the indicated mAbs. After preclearing with Sepharose-protein A, cell lysates were immunoprecipitated (IP) with anti-Lck mAb. Immune complexes were resolved by SDS-PAGE, transferred to nitrocellulose, and blotted with anti-SHP-1, anti-Lck, anti-phosphotyrosine (pY) 4G10 mAb. Membranes were reprobed with anti-ζ mAb. Data shown are representative of at least three independent experiments.

**FIGURE 4.** Engagement of CD43 before or simultaneously with TCR ligation results in sustained phosphorylation of the ζ-chain. A total of 1 × 10^7 human T lymphocytes were stimulated as described under Fig. 3A for the indicated times. Total cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose, and subjected to immunoblotting with the anti-phosphotyrosine (pY) 4G10 mAb. Membranes were reprobed with anti-ζ mAb. Data shown are representative of at least three independent experiments.

To assess whether preventing SHP-1-Lck association by ligating CD43 before or simultaneous to TCR engagement resulted in enhanced Lck activity, we evaluated the tyrosine phosphorylation of Zap70, a substrate for Lck (34), as both CD43 and the TCR induce Zap70 phosphorylation (1, 13). Consistent with data shown above, ligating CD43 before or simultaneous with the TCR engagement resulted in enhanced Zap70 phosphorylation, compared with cells activated through the TCR as the first stimulus, or only through CD43 or the TCR (Fig. 5 A, lower panel). Furthermore, blocking ERK kinase activity reduced Zap70 phosphorylation to levels comparable to those of control cells (Fig. 5 A, lower panel). Interestingly, ζ-chain phosphorylation levels remained elevated for up to 2 h in cells that were first activated through CD43 (L10 + OKT3) or through simultaneous stimulation (L10 + OKT3) as compared with cells activated through CD43 or the TCR (Fig. 5 B). These data indicate that CD43-mediated signals previous or simultaneous to TCR engagement knockdown the SHP-1-Lck association, resulting in sustained ζ-chain and Zap70 tyrosine phosphorylation via an ERK-mediated mechanism.
without the inhibitor or prestimulated with OKT3 for 10 min and then costimulated with L10 in the presence of PD98059. As expected, ERK activation induced in response to CD43 or TCR cross-linking was reduced in the presence of PD98059 (Fig. 6A). When PD98059 was added at the same time as the L10 mA, the enhanced pERK levels that resulted of CD43 engagement 10 min after TCR ligation decreased to levels comparable to those observed by engaging only the TCR (Fig. 6A). Consistent with these data, the proliferative response resulting from engaging CD43 10 min after the TCR was prevented by PD98059 (Fig. 6B). Consistent with the fact that JNK activation levels were comparable in response to the different stimuli, inhibiting JNK with SP600125 did not have a significant effect on cell proliferation in any case (Fig. 6C). SP600125 inhibitory effect, was confirmed by the absence of pJun (data not shown). Together, these data suggest that ERK phosphorylation is essential to mediate the costimulatory effect of CD43 on TCR-signals, fine-tuning responses such as proliferation or anergy.

The temporality of CD43- and TCR-mediated signals modulates the titer of cytokines produced.

To further explore how the temporality with which CD43- and TCR-mediated signals are sensed affected cell response, we analyzed the cytokine profile resulting from the different activation schemes. When CD43 was ligated before or at the same time as the TCR, cells produced equivalents amounts of IL-1α and -β, MCP-2, TGF-β, and TNF-α and -β, as well as of IL-2, -3, and -8. Interestingly, activating the cells first through the CD43 molecule resulted in higher levels (at least 2-fold) of IL-13, IFN-γ, RANTES, and MCP-1, while larger quantities (at least 2-fold) of IL-4 and MIP-1 were detected when CD43 and the TCR were engaged simultaneously. When TCR engagement preceded CD43 ligation, cells produced primarily IL-8 (Fig. 7).

Altogether, these data suggest that stimulating the cells through CD43 as a first or second stimulus results in subtle differences in the duration and intensity of key intracellular signals, which in turn are translated into different responses in terms of cell proliferation and cytokine production.

Discussion

During T cell activation, the plasma membranes of the T cell and APC are apposed next to one another, allowing for the reciprocal engagement of cell surface receptors, including the TCR and costimulatory molecules, with their counterreceptors. However, little is known about the first contacts between a T cell and an APC and the molecules that direct them. It is possible that the cells are brought together first through adhesive molecules and that the shape and structure of these molecules are important to drive the initial interactions. Abundant and elongated molecules, such as CD43, could be suitable candidates to initiate the cross-talk between T lymphocytes and APCs.

To evaluate whether the temporality with which coreceptor molecules become involved modifies their costimulatory function, we assessed the impact of the sequence with which CD43 and the TCR were engaged on cell response. We found that engaging the CD43 molecule before or simultaneously with the TCR resulted in vigorous IL-2 production, robust proliferation, and lack of CTLA-4 expression. In contrast, cells stimulated through the TCR before CD43 ligation resulted in anergy: these cells expressed CTLA-4, failed to produce IL-2 and to proliferate. The fact that CD43 levels did not significantly change 2 h after TCR engagement (data not shown) indicates that anergy was not the result of the lack of CD43 signals. Interestingly, if applied within the first 30 min following TCR engagement, the CD43-dependent signals prevented the anergizing signals resulting of engaging the TCR before CD43 activation and suggest that the CD43-mediated signals are important in the decision-making process of a cell. Consistent with this, the different combinations of costimulatory signals generated in response to CD43 and TCR engagement produced different sets of cytokines. Cross-linking CD43 before or at the same time as the TCR induced comparable levels of an overlapping set of cytokines involved in cell proliferation, anergy prevention, maturation, and differentiation (IL-1β, IL-2, IL-3, IFN-γ, and TNFβ), as well as chemokines up-regulated during inflammation (MCP-1, MIP-1, and RANTES). Interestingly, when preceding the TCR signals, CD43 ligation up-regulated even more (at least 2-fold) the secretion of IL-13 and IFN-γ, whereas when cells were stimulated simultaneously through CD43 and the TCR, IL-4 secretion was increased. As previously described for anergic cells (35), when the first activating signals were provided by the TCR, we found that cells secreted primarily IL-8. Overall, our data support reports showing that CD43 functions as a coreceptor molecule for the TCR (15, 36, 37) and that CD43-mediated signals up-regulate the expression of several proinflammatory genes (27, 38).

The ERK family members have emerged as pivotal regulators in the translation of signals mediated by receptor engagement. The duration and intensity of ERK1/2 activation differentially regulates diverse cellular functions (28, 39). During successive TCR triggering, accumulation of the activated form of ERK1/2 is determined by the level of TCR engagement, and it is more notorious when costimulatory signals are provided (40). ERK1 and ERK2 have been implicated in proliferation and differentiation; however, the differences between ERK1 and ERK2 function remain unclear (41). Our data show that the intensity of ERK1 and ERK2 phosphorylation was dependent on the order with which costimulatory signals were applied. CD43 signals prior or simultaneous with
Interestingly, CD43 has been shown to compensate for CD28 function and will favor differential cell responses. The sequence with which cell surface molecules encounter their ligand can generate differences in the early signaling events, which in turn can establish an intracellular loop, contributing to increased Zap70 and SHP-1-Lck association and a diminished ERK phosphorylation, a sustained SHP-1-Lck association and a diminished ERK phosphorylation. These fluctuations in the duration and intensity of activation were not detected for JNK, where the kinetics and intensity of phosphorylation were comparable, independent of the activation protocol. Moreover, inhibiting JNK did not result in inhibition of cell proliferation. Thus, the sequence with which cell surface molecules encounter their ligand can generate differences in the early signaling events, which in turn will favor differential cell responses.

CD28 is considered the hallmark of coreceptor molecules, and interestingly, CD43 has been shown to compensate for CD28 functions in the CD28−/− mouse (45). We investigated whether the temporality with which the TCR and CD28 were engaged also influenced the quality of T cell response. Surprisingly, under our experimental conditions, we found that independent of the sequence, ligation of the TCR and the CD28 coreceptor molecule resulted in cell proliferation. These data suggest that the costimulatory functions of CD43 and CD28 are mediated through distinct mechanisms, allowing for a fine regulation of cellular responses generated by means of differences in timing.

Multiple and diverse ligands have been described for CD43, ICAM-1 (46), MHC-I (47), galectin-1 (48), human serum albumin (49), and the macrophage adhesion receptor sialoahesin (siglec-1) (50). ICAM-1, MHC-I, and siglec-1 could play a role during T cell activation through their interaction with CD43, because they are expressed on the cell surface of different APCs. However, because these molecules are also the ligands for other cell surface molecules on the T cell, the significance of the interaction of CD43 with ICAM-1, MHC-I or siglec-1 on the coreceptor functions of CD43 during T cell activation is not clear. Moreover, it is not known whether the sequential interaction of CD43 with its ligands modulates its biological functions. Interestingly, CD43 has been shown to be a costimulatory molecule that can modulate HIV expression in T lymphocytes, potentiating HIV-1 promoter-driven activity and virus production that result of engaging the TCR (51). In addition, sera from HIV-infected individuals have shown to contain auto-antibodies specific for CD43 (52). Whether these Abs are capable of ligating CD43 on the surface of lymphoid cells, and are responsible for the costimulatory function of CD43 remains to be demonstrated.

Our results highlight the role of CD43 both as a coreceptor and as a molecule that participates in proliferation, inflammation, and cell migration/adhesion, modulating immune responses. Moreover, our data support the temporal summation model (53) where by interacting with its counterreceptor(s) on the APC at the same time or before Ag recognition, CD43 induces a signaling cascade that prolongs the duration of TCR signaling. In addition to the strength and duration of intracellular signals, our data underscore temporality with which certain molecules are engaged as yet another mechanism to fine-tune T cell response, and ultimately immune function.

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