CD43-mediated Signals Induce DNA Binding Activity of AP-1, NF-AT, and NFκB Transcription Factors in Human T Lymphocytes*

Received for publication, June 16, 2000
Published, JBC Papers in Press, July 24, 2000, DOI 10.1074/jbc.M005231200

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Although numerous reports document a role for CD43 in T cell signaling, the direct participation of this molecule in cell activation has been questioned. In this study we show that CD43 ligation on human normal peripheral T cells was sufficient to induce interleukin-2, CD69, and CD40-L gene expression, without requiring signals provided by additional receptor molecules. This response was partially inhibited by cyclosporin A and staurosporine, suggesting the participation of both the Ca$^{2+}$ and the protein kinase C pathways in CD43 signaling. Consistent with the transient CD43-dependent intracellular Ca$^{2+}$ peaks reported by others, signals generated through the CD43 molecule resulted in the induction of NF-AT DNA binding activity. CD43-dependent signals resulted also in AP-1 and NFκB activation, probably as a result of protein kinase C involvement. AP-1 complexes bound to the AP-1 sequence contained c-Jun, and those bound to the NF-AT/AP-1 composite site contained c-Jun and Fos. NFκB complexes containing p65 could be found as early as 1 h after CD43 cross-linking, suggesting that CD43 participates in early events of T cell activation. The induction of the interleukin-2, CD69, and CD40-L genes and the participation of AP-1, NF-AT, and NFκB in the CD43-mediated signaling cascade implicate an important role for this molecule in the regulation of gene expression and cell function.

Successful activation of T lymphocytes requires at least two kinds of signals: those generated by the specific interaction of the TcR with peptides presented by major histocompatibility complex molecules on the antigen presenting cells and those resulting from the interaction of other T cell surface molecules, known as co-receptors, with their ligands on antigen-presenting cells (for reviews see Refs. 1 and 2). Thus, a cell senses not only the presence of an antigen but also its environment, and a particular cellular response will result from the integration of signals delivered through the antigen-specific receptor and the numerous co-receptors.

The CD43 co-receptor is very abundant on T cells surface; it comprises a highly glycosylated extracellular portion of 239 amino acids that protrudes 45 nm from the cellular membrane, a transmembrane region, and a cytoplasmic domain of 123 amino acids (3–5). On T cells, CD43 is differentially glycosylated in two major isoforms: a 113–123-kDa product, mainly present on resting CD4$^+$ T cells and a 125–135-kDa form expressed mostly on resting CD8$^+$ lymphocytes, which is up-regulated both on CD4$^+$ and CD8$^+$ cells, following cellular activation (6). The 125–135-kDa form has been shown to be down-regulated during positive selection of double positive cells in the thymus, involving CD43 in thymic selection events (7). Carbohydrate moieties have an important role in CD43 function, because alterations in the glycosylation patterns of the molecule are associated with severe immunodeficiency such as in the case of patients affected with the Wiskott-Aldrich syndrome or infected with HIV (8–10). Although there is no direct evidence of how the interaction of CD43 with each of its three reported ligands intercellular adhesion molecule 1 (CD54), (galectin 1, or major histocompatibility complex I; Refs. 10–12) regulates T cell function, there is abundant experimental evidence to support the idea that CD43 participates in T cell adhesion and activation.

Because of its high content of sialic acid residues, resulting in a strong negative charge, it has been proposed that CD43 functions as an antiadhesive molecule, providing the cells with a repulsive barrier (13–15). However, CD43 ligation with specific mAbs has been shown to promote the formation of homotypic aggregates, some of which were only partially inhibited with mAbs against lymphocyte integrins, suggesting an active involvement of CD43 in regulating cellular adhesion (16, 17). A participation of CD43 in leukocyte rolling was suggested by experiments where an anti-CD43 mAb inhibited the interaction between T cells and endothelial cells (18). Evidence that CD43 participates in TcR-mediated T cell activation has been provided in different experimental models (for reviews see Refs. 19 and 20). Transfection of a cDNA encoding for human CD43 in a murine T cell hybridoma specific for human major histocompatibility complex II molecules resulted in enhanced IL-2 production upon stimulation with the appropriate antigen presenting cell only if cells expressed the wild type form of CD43, together with the specific major histocompatibility complex II molecules (21). Co-ligating CD43 with the TcR enhanced...
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T cell proliferation as compared with cross-linking the TcR alone, in normal mice\(^2\) as well as in CD28\(^{-}\) mice (22).

To date, the molecular mechanisms that transduce CD43-dependent signals from the cell surface to the nucleus are not fully understood. CD43-mediated activation promotes the generation of second messengers like diacylglycerol and inositol phosphates, Ca\(^{2+}\) mobilization, and protein kinase C activation (23). CD43 ligation was shown to induce the association of Fyn and Lck tyrosine kinases to the cytoplasmic domain of CD43 (24, 25) and to lead to the formation of a macromolecular complex which comprises Shc, Grb-2, SLP-76, and the guanine exchange factor Vav (26). Moreover, CD43-specific signals have been found to promote the nuclear translocation of ERK2, suggesting that the MAPKs pathway is involved in the CD43-dependent gene expression (26).

In this study, we provide evidence that CD43-mediated signals are sufficient to regulate gene expression. We show that CD43 ligation on human T cells induced IL-2 gene expression without requiring additional signals provided by other receptor molecules, in opposition to what was previously reported (27). CD43 signals leading to IL-2 mRNA synthesis were partially inhibited by the calciurexin inhibitor cyclosporin A and by the PKC inhibitor staurosporine, suggesting that both the PKC and the calcium pathways participate in the CD43-mediated control of IL-2 gene expression. We also show that CD43 ligation induced the DNA binding activity of the AP-1, NFκB, and NF-AT transcription factors. These data are consistent with a dual pathway of activation through the CD43 molecule because AP-1 and NFκB activation result from a transient high peak of calcium and signals coming from the MAPKs pathway (28–30), whereas NF-AT recruitment depends on a capacitative calcium mobilization, and protein kinase C activation

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Peripheral blood T cells were cultured for 6 h in the presence of 1 µg of two different anti-CD43 mAbs, L10 and MEM-59, alone or in combination. CsA or staurosporine (STAU) were added where indicated 15 min before the mAbs. As positive control, cells were treated with PMA and Iono. Specific IL-2 mRNA was detected by reverse transcription-PCR. As internal control, the expression of the housekeeping gene GAPDH was monitored. Ethidium bromide-stained gels of PCR products (upper panel) were scanned using computer-assisted densitometry (Fluor-S-Multi-imager, Bio-Rad) and the data (IL-2 cDNA/GAPDH cDNA signal) were plotted as the percentages of change (lower panel). Data shown are representative of four independent experiments.

**RESULTS**

**CD43-dependent IL-2 Gene Expression Involves the PKC and the Ca²⁺ Signaling Pathways**—Given the importance of IL-2 in the proliferative and late stages of T cell activation and the fact that simultaneous signaling from CD43 and the TcR had been previously reported to result in IL-2 production (21, 27), we investigated the molecular mechanisms involved in the CD43-dependent IL-2 mRNA induction. As shown in Fig. 1, cross-linking CD43 on the surface of normal human peripheral blood T lymphocytes with two different mAbs, without co-ligating the TcR, was sufficient to generate signals that lead to IL-2 mRNA induction (lanes 1 and 4). When cells were incubated with both anti-CD43 mAbs simultaneously, the amount of IL-2 mRNA increased approximately 7-fold over control cells (compare lanes 7, 10, and 11), although to a lesser extent than when cells were treated with PMA and ionomycin (lane 14). Independent of the anti-CD43 mAb that was used to stimulate the cells, signals resulting in the induction of IL-2 mRNA were blocked by CsA (lanes 3, 6, and 9) and partially blocked by staurosporine (lanes 2, 5, and 8). Cells treated only with CsA or staurosporine but no antibody (lanes 12 and 13), control antibody-treated (lane 11), and untreated cells (lane 10) had equivalent levels of IL-2 mRNA. The graph shown in the lower panel of Fig. 1 represents the percentages of change in the IL-2 cDNA/GAPDH cDNA signal. These data suggest that CD43-mediated signals can induce IL-2 gene expression, recruiting the PKC and the Ca²⁺ pathways.

**CD43 Induces Binding of AP-1 Transcription Factor to DNA**—To investigate the pathway leading to CD43-mediated regulation of gene expression, we looked for the induction of transcription factors following CD43 ligation, particularly for factors that bind to the IL-2 gene promoter such as AP-1, NF-AT, and NFκB (44). Cross-linking CD43 on the surface of Jurkat cells with either anti-CD43 mAb, L10, and/or MEM-59 promoted the formation of an AP-1 complex over levels of control cells (Fig. 2, lanes 2, 3, 12, and 13); co-stimulation with both mAbs did not result in higher levels of AP-1 binding than those observed when cells were stimulated with MEM-59 alone (lanes 2–4). At this time point, CD43 ligation resulted in higher levels of AP-1 than ligation with optimal amounts of OKT3 (OKT3o) or stimulation with PMA (lanes 8 and 9), probably reflecting a difference in kinetics. As expected, ionomycin did not have any effect on the DNA binding capacity of AP-1 (lanes 10 and 11), because the AP-1 family of transcription factors is activated by the MAPK pathway and not by the sustained calcium plateau characteristic of ionomycin.

To determine the kinetics of the CD43-dependent AP-1 binding activity, human peripheral blood T lymphocytes were activated for different periods of time. Following CD43 cross-linking, AP-1 binding to DNA could be detected as early as 30 min and was maintained for at least 4 h (Fig. 3A, lanes 1, 4, 7, and 10), whereas when cells were stimulated with PMA and ionomycin, the response was slower, as reflected by the fact that AP-1 induction could be observed only 1 h after the onset of the experiment (lanes 2, 5, 8, and 11). When cells were treated with control mAb (3D6), AP-1 binding activity remained constant all through the experiment (lanes 3, 6, 9, and 12).
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Fig. 3. AP-1-DNA complexes induced by CD43 ligation contain c-Jun. A. CD43 induces AP-1 binding activity in a time-dependent manner. Normal human peripheral T lymphocytes were incubated for the indicated times with mAb L10, PMA and Iono, or mAb 3D6, as described. Nuclear extracts were prepared and subjected to EMSAs with the AP-1 probe. The arrow indicates the position of the induced complex. Results were confirmed in three independent experiments. B. AP-1 complexes induced by CD43-mediated signals are specific. Human T lymphocytes were CD43-stimulated for 4 h, and nuclear extracts were used for competition experiments with 50-fold excess of the indicated cold oligonucleotides. The arrow indicates the position of the complex. C. AP-1 complexes induced by CD43 ligation contain c-Jun. Nuclear extracts from anti-CD43 mAb L10-treated normal human T lymphocytes were incubated with the AP-1 probe before adding anti-c-Jun or an irrelevant antibody (Control). The lower arrow indicates the position of the induced complex, and the upper arrow indicates that of the supershifted complex.

Specificity of the DNA-AP-1 complexes formed following CD43 ligation was demonstrated by competition experiments (Fig. 3B), where AP-1 binding to the labeled probe was competed with a 50-fold molar excess of cold AP-1 oligonucleotide (lanes 1 and 2), whereas similar amounts of a nonspecific oligonucleotide, M-CAAT, had no effect (lane 3). Because c-Jun is one of the most common members of AP-1 (45), we investigated whether it was present in the DNA-AP-1 complexes generated through CD43. As shown in Fig. 3C, when nucleoprotein complexes were incubated with anti-Jun antibodies, a partial supershift could be observed (lane 2), thus indicating the presence of c-Jun. Other members of the Jun family, however, might also be present, because the induced complexes did not disappear completely following supercomplex formation. Various members of the AP-1 family of proteins (c-Fos, Fos B, c-Jun, JunB, and Fra1) have been implicated in gene regulation upon T cell activation (46, 47). Altogether these data suggest that AP-1 participates in the CD43-specific signaling pathway leading to gene expression.

We had previously shown that simultaneous stimulation through the CD43 molecule and the TcR-CD3 complex resulted in enhanced IL-2 production (21). Here, we activated normal human peripheral blood T cells with saturating amounts of the anti-CD43 mAbs L10 or MEM-59 alone or in combination with suboptimal concentrations of OKT3 (OKT3s) for 4 h and evaluated the AP-1 binding activity. As shown in Fig. 2, integrating signals generated through the TcR with OKT3s and through CD43 with L10 or MEM-59 did not result in enhanced DNA binding activity of AP-1 as compared with cells activated either through the TcR alone or the CD43 molecule alone (lanes 2, 3, 5, 6, and 7). In fact, at this time point (4 h), an inhibitory effect was observed when OKT3s was used in combination with the anti-CD43 mAbs, suggesting an eventual cross-talk between the TcR and the CD43 signaling pathways.

CD43 Cross-linking Induced Two Complexes Recognizing a Composite NF-AT/AP-1 Site—We investigated next whether CD43 signaling would lead to NF-AT binding to DNA when Jurkat cells were activated for 4 h with L10, MEM-59, or both mAbs, with or without suboptimal amounts of OKT3 or with PMA and/or ionomycin. When cells were treated with PMA, a fast migrating complex was observed (Fig. 4, lane 9, complex a) that corresponds to AP-1 binding to the NF-AT site (48, 49). Ionomycin treatment induced the formation of a second DNA-protein complex (complex b, lane 10) that corresponds to NF-AT binding to its cognate site (48, 50). In addition to complexes a and b, treatment with PMA and ionomycin resulted in the formation of a third complex (complex c, lane 11) that contains AP-1 and NF-AT bound simultaneously to the composite NF-AT/AP-1 site (48, 51). Ligation of CD43 with either anti-CD43 mAb resulted in the formation of complex a (lanes 2 and 3). Simultaneous cross-linking of CD43 with MEM-59 and L10 induced the formation of complexes a and b, suggesting an additive effect of signals generated through each epitope (lane 4). Stimulation of the cells with OKT3s promoted also the formation of complexes a and b (lane 8), whereas OKT3s induced only AP-1 binding activity (complex a, lane 5). Co-ligation of CD43 through the MEM-59 mAb with OKT3s resulted in enhanced AP-1 DNA binding activity (complex a) as well as in the translocation of NF-AT (complex b) to the nucleus (lane 7); complex b contained NF-AT because immune band shift experiments showed that complex b was supershifted in the presence of anti-NF-ATc antibody (Fig. 5C) and because this complex was inhibited by cyclosporin A (Fig. 5D). None of the stimuli was capable of inducing the supercomplex formation that re-
The position of the supershifted complex (b) indicates the position of complex a. Nuclear extracts (5 μg) from Jurkat cells co-stimulated with 1 μg/ml of each L10 and MEM-59 for 4 h were used for competition experiments in the presence of either 10- or 50-fold excess of the indicated cold oligonucleotides. The arrows indicate the positions of the induced complexes. B, within the NF-AT/AP-1 composite site, AP-1 complexes comprise c-Jun and c-Fos. Nuclear extracts from MEM-59-stimulated Jurkat cells were incubated with the NF-AT probe as described before, following which DNA-protein complexes were incubated with anti-Jun, anti-Fos, or control antibody (anti-CDC42) and separated as described under "Experimental Procedures." C, complexes induced by CD43 contain NF-ATc. Nuclear extracts from ionomycin-treated Jurkat cells were incubated with the probe as described, prior to the addition of anti-NF-ATc, anti-NF-Atp, or control antibody (anti-CDC42). The lower arrow indicates the position of complex b, and the upper arrow indicates the position of the supershifted complex (b). D, CD43-induced NF-AT complexes formation is inhibited by CsA. Human peripheral blood T lymphocytes were treated with L10 or MEM-59 mAbs, in the presence or absence of CsA for 4 h. EMSA analysis was performed with 2.5 μg of nuclear extracts protein and 32P-labeled NF-AT site containing probe. The arrows indicate the positions of the induced complexes.

resulted from PMA and ionomycin simultaneous addition (Fig. 4, lane 11). When the experiment was done with unstimulated cells or cells treated with the secondary antibody only, none of these complexes were induced (Fig. 4, lanes 12 and 13).

To investigate the nature of the two NF-AT recognizing complexes induced following CD43 cross-linking on Jurkat cells, competition experiments were performed with oligonucleotides containing the composite NF-AT/AP-1-binding site or the AP-1 consensus-binding site. In Fig. 5A, we show that complex a was partially competed with both oligonucleotides, confirming that this complex contained AP-1 (lanes 1–5), whereas addition of an unrelated oligonucleotide had no effect on complex a formation (lanes 6 and 7). A 10-fold molar excess of complex NF-AT oligonucleotide inhibited the formation of complex b (lanes 1–3), whereas comparable amounts of the AP-1 oligonucleotide had no effect (lanes 4 and 5), and MCAAT had only a slightly inhibitory effect (lanes 6 and 7).

To demonstrate that complex a induced by CD43 contained AP-1, DNA-protein complexes were allowed to react with anti-Fos, anti-Jun, or a nonspecific antibody (Fig. 5B). Addition of either anti-Fos or anti-c-Jun resulted in partial inhibition of complex a formation (lanes 2 and 3), whereas the nonspecific antibody had no effect (lane 4). The presence of NF-ATc in complex b was demonstrated by the fact that incubation of DNA-protein complexes from ionomycin-treated Jurkat cells with anti-NF-ATc antibody induced a supershift (Fig. 5C, lane 3), whereas anti-NF-ATp antibody had no effect (lane 2). Moreover, in normal human T lymphocytes, induction of complex b but not complex a was inhibited when cells were treated with cyclosporin A prior to CD43 cross-linking (Fig. 5D, lanes 2 and 4).

Co-ligation of CD43 with the L10 mAb and suboptimal amounts of anti-CD3 OKT3 mAb resulted also in reduction of the AP-1 binding activity (complex a) to both the composite NF-AT/AP-1 site (Fig. 4, lanes 2 and 6), and the AP-1 consensus site (Fig. 2, lane 6). On the contrary, co-ligation with the anti-CD3 mAb MEM-59 and suboptimal amounts of anti-CD3 OKT3 mAb resulted in higher AP-1 binding activity (Fig. 4, lanes 3 and 7). Under these conditions, NF-AT binding (complex b) was also induced.

Together, these results show that CD43-mediated signals induce AP-1 and NF-AT DNA binding activity to the distal NF-AT site of the IL-2 promoter, that different anti-CD3 mAb generate slightly different intracellular signals, and that, depending on the anti-CD3 mAb, TCR-mediated signals modulate the DNA-binding pattern to the composite NF-AT/AP-1 site of the IL-2 promoter.

**CD43 Signaling Induces NFκB**—The NFκB family of transcription factors controls the expression of numerous cytokine genes involved in T cell function (for review see Ref. 52). We investigated whether CD43 signaling would induce NFκB activity. As shown in Fig. 6, cross-linking CD43 on the surface of normal human T cells with the L10 mAb induced NFκB binding activity in a time-dependent manner. NFκB induction could be observed within 1 h, reaching a maximum at 2 h and remaining constant for up to 4 h after stimulation (lanes 2, 6, 10, and 14). Interestingly, induction of NFκB DNA binding activity following CD43 ligation with equivalent amounts of MEM-59 mAb was detected only 4 h after stimulation (lanes 3, 7, 11, and 15). The kinetics of NFκB binding activity induced by PMA and ionomycin treatment was faster, reaching a maximum as early as 30 min and decreasing slowly thereafter (lanes 4, 8, 12, and 16). In all cases, the NFκB activity mediated through the CD43 molecule was weaker than that observed for PMA- and ionomycin-treated cells. Incubation with control mAb (3D6) did not result in induction of NFκB binding activity (lanes 5, 9, 13, and 17).

Specificity of the NFκB-DNA complexes resulting of CD43 ligation was demonstrated by competition assays where the addition of 50-fold molar excess of cold NFκB abolished DNA binding (Fig. 7A, lane 3). No competition was observed when the same amount of the unspecific oligonucleotide MCAAT was added (lane 2). Additional proof that NFκB was present in these complexes came from immune band shift experiments. When the NFκB-DNA complexes induced after CD43 engagement (Fig. 7B) were treated with anti-p65 antibody, a supershift was observed correlating with the disappearance of the specific NFκB complex (lane 3), demonstrating that the CD43-mediated NFκB binding activity involves p65. Addition of a control antibody had no effect on the electrophoretic mobility of the DNA-protein complexes (lane 2). Altogether, these data show clearly that NFκB binding activity is induced by CD43-dependent signals.

**CD43 Ligation Induces the Expression of CD69 and CD40-L in T Lymphocytes**—The induction of AP-1, NF-AT, and NFκB by CD43-mediated signals implicated a potential role for this molecule in the expression of genes with recognition sites for these transcription factors in their promoters. To test this possibility, we investigated the effect of CD43 cross-linking on CD69 expression in human peripheral blood T cells. CD69 is an early marker of T cell activation whose expression is mediated by NFκB activation in response to tumor necrosis factor α (33) or by the proximal AP-1 site of the CD69 promoter region in response to TCR/CD3 cross-linking or PMA treatment (34). As shown in Fig. 8A, ligation of CD43 with the L10 or MEM 59
Fig. 6. CD43 ligation induced specific NFκB binding activity in a time-dependent manner. Human T lymphocytes were incubated with 1 μg/ml of L10, MEM-59, or 3D6 mAbs or with PMA and ionomycin for 0.5, 1, 2, or 4 h. Nuclear extracts were used for EMSA with a 32P-labeled probe containing the consensus NFκB site. The arrow indicates the induced DNA-protein complex. A representative experiment out of three is shown.

Fig. 7. The CD43-dependent NFκB complex contains p65 (Rel A). A, CD43-induced NFκB complexes are specific. Competition assays with 50-fold excess of cold NFκB or MCAAT oligonucleotides were done with nuclear extracts from human T lymphocytes isolated from peripheral blood, treated for 4 h with 1 μg/ml L10 mAb. The arrow indicates the position of the induced complex. B, CD43-induced NFκB complexes contain p65. Nuclear extracts from human T lymphocytes, stimulated for 2 h in the presence of mAb L10, were used for supershift assays with an anti-p65 antibody. The lower arrow indicates the induced complex, and the upper arrow indicates the p65 supershifted complex (NFκBp65).

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an important role in CD40-L expression (35). Because CD43 signaling involves Ca2+ mobilization and NF-AT DNA binding activity, we tested whether CD43 signaling induced CD40-L expression in human peripheral T cells. As shown in Fig. 8C, CD40-L expression in human peripheral T cells is clearly dependent on Ca2+ signaling, because 95 ± 8% of the cells were CD40-L+ after 8 h treatment with ionomycin, whereas PMA treatment resulted only in 2.7 ± 0.7% of CD40-L+ cells. When CD43 was cross-linked with L10 or MEM 59 mAbs, 12.86 ± 2 and 16.62 ± 1.5%, respectively, of the cells were CD40-L+. Cells treated with 3D6 (isotype control) showed basal CD40-L expression (3.6% ± 0.8). When cells were activated through the TCR, 45 ± 3% of the cells were CD40-L+. These results show that CD43 signaling induces the expression of CD40-L, most probably through Ca2+ mobilization and the induction of NF-AT binding activity to the proximal NF-AT site of CD40-L. All together the data provided here show that CD43 signaling can positively regulate the expression of different genes containing AP-1, NFκB, or NF-AT responsive elements in their regulatory regions.

DISCUSSION

Reports regarding the role of CD43 involve this co-receptor molecule in multiple aspects of cell function, yet the molecular basis of CD43 signaling is only partially understood. The participation of CD43 in T cell function (13–22) prompted us to search for the CD43 signaling pathway. The ideal way to evaluate the signaling capacity of a molecule would be to use natural ligands; however, in the case of CD43, this is particularly difficult because all the ligands that have been described so far are also ligands of other T cell surface molecules (10–12). To mimic the interaction with its ligands, CD43 was cross-linked on the cell surface with two different anti-CD43 monoclona antibodies, L10 and MEM-59. Independent of the antibody used, CD43 signals were sufficient to induce IL-2 mRNA, and an additive effect was observed when both anti-CD43 mAbs were used simultaneously (Fig. 1). Consistent with previous reports showing that CD43 ligation resulted in both PKC activity and Ca2+ flux (23, 25), we found that CD43-mediated IL-2 mRNA induction was partially inhibited by cyclosporin A and staurosporine, regardless of whether the cells were activated with either mAb alone or in combination (Fig. 1). This dual pathway of activation was further confirmed by the CD43-dependent induction of CD69 and CD40-L (Fig. 8), whose expression depend on the MAPKs and the Ca2+ pathways, respectively (33–35).

Initial steps of T cell activation are orchestrated through the recruitment of several transcription factors, including NF-AT, AP-1 and NFκB (2, 44, 53). Cross-linking CD43 with either L10 or MEM-59 mAbs on Jurkat cells resulted in the induction of AP-1 DNA binding activity (Fig. 2). Complexes between the AP-1 probe and nuclear proteins were formed as early as 30 min following CD43 ligation and were still present after 4 h.

3 H. Lindgren, personal communication.
These complexes were specific and contained c-Jun as evidenced by the supershift induced in the presence of anti-c-Jun antibody (Fig. 3) and the inhibition of the AP-1-NF-AT composite site by anti-Fos or anti-Jun antibodies. The differential behavior of anti-Jun antibody on the complex formation between c-Jun and the AP-1 or NF-AT/AP-1 sites could be due to the different affinity of c-Jun for either site. The fact that not all protein-DNA complexes supershifted when incubated with the anti-c-Jun antibody suggests that other members of the Jun family may be recruited in response to CD43 ligation. In T cells, AP-1 activity is tightly regulated by the MAPKs pathway at the transcriptional and posttranslational levels, increasing the amounts and enhancing the stability and binding activity of AP-1 proteins through phosphorylation (for review see Ref. 45). The CD43-mediated AP-1 activity reported here may result of signals generated through the MAPK pathway because we have shown that CD43 signaling leads to ERK2 activation and nuclear localization and to Fos-SRE-dependent transcription (26).  

Four different NF-AT proteins expressed in lymphoid cells as well as in other cell types have been described (54). Upon dephosphorylation by the calmodulin-dependent phosphatase calcineurin, NF-AT proteins become activated and translocate to the nucleus. The NF-AT transcription factors can bind to DNA directly or in combination with AP-1 family members (for review see Ref. 31). We report here that CD43-specific signals induced two specific NF-AT DNA-protein complexes (Fig. 4). One of the complexes, similar to that generated by incubating the cells with PMA, contained AP-1 proteins and was CsA-resistant. Interestingly, CD28 and PMA signaling have been shown to result in activation of the IL-2 promoter by NF-AT proteins in a CsA-resistant manner. The complex formed under such conditions was also competed by AP-1 sequences (49). This is particularly relevant considering the fact that CD43 has been shown to be a substitute for CD28 function in CD28 resistant. Interestingly, CD28 and PMA signaling have been shown to result in activation of the IL-2 promoter by NF-AT proteins in a CsA-resistant manner. The complex formed under such conditions was also competed by AP-1 sequences (49).

Transient peaks of intracellular calcium result in AP-1 and NFκB activation (28–30). Data reported here show that the molecular events following CD43 cross-linking involved AP-1 and NFκB induction, correlating with the transient peak of intracellular calcium reported upon CD43 ligation on HPB-ALL or Jurkat cells with L10 or MEM-59 mAbs on Jurkat cells. However, in peripheral blood T lymphocytes, signals generated through either mAb were sufficient to induce that nucleoprotein complex, in a CsA-sensitive way.

The NFκB family proteins regulate the transcription of a wide range of genes, some of which play a central role in immunological processes. When inactive, these proteins reside in the cytoplasm, associated with specific IκB inhibitors that are degraded upon serine phosphorylation, allowing translocation of NFκB factors to the nucleus. MAPKs and PKC participate in the regulation of IκB kinases activity (29). We found that cross-linking CD43 on the surface of peripheral T lymphocytes resulted in a time-dependent induction of NFκB-DNA complexes containing p65, as determined by supershift formation in the presence of anti-p65 antibody (Figs. 6 and 7). Similar experiments performed with an anti-c-Rel antibody indicated that c-Rel was not present in those complexes (data not shown), consistent with the fact that the presence of c-Rel in NFκB complexes is a late response and that p65 recruitment precedes and is required for c-Rel expression. p65 translocation to the nucleus during inflammatory and immune responses is well documented (for review, see Refs. 55 and 56). Thus, our results suggest that CD43 participates actively in immune response modulation. However, not all CD43-mediated signals result in NFκB recruitment and T cell activation. In a recent report where Jurkat cells were activated through the CD43-specific mAb J393, cells were found to undergo apoptosis. The
molecular mechanisms underlying this event were associated with NFκB down-regulation (57), because this transcription factor has been linked with an anti-apoptotic function (58–60). Consistent with this, the CD43-dependent NFκB activity we report in this study did not correlate with apoptosis. The fact that ligation of CD43 with two different monoclonal antibodies resulted in opposite effects on NFκB activity (this report and Ref. 57) supports the idea that different anti-CD43 monoclonal antibodies generate different signals and biological responses. Altogether, data shown here, in combination with other reports where CD43 has been described as a signaling molecule (13–22), suggest that by regulating the activation of several transcription factors, CD43 has an important participation in cell signal and function. To further test that CD43-mediated induction of AP-1, NFκB, and NF-AT transcription factors could result in the induction of T cell activation genes, we tested the effect of CD43 ligation upon the induction of CD69, whose transcription depends upon AP-1 or NFκB (57). CD43 ligation slightly but significantly increased the amount of CD69 positive cells and cooperated with PMA signals by further increasing both the amount of cells that were CD69+ and the density of CD69 molecules/cell. CD43 signals also resulted in an increased CD40-L expression, presumably through the activation of NF-AT transcription factors. The low percentage of CD40-L+ and CD69+ cells resulting from CD43 ligation suggested that a particular subset of cells was able to express these molecules. However, these cells did not correspond exclusively to a memory cell phenotype nor to the γδ lineage (data not shown). Considering that CD43 expression and signals might target specific subsets of memory and effector T cells (61), further experiments are needed to characterize these cells. In a recent report it was shown that CD43 ligation on the surface of dendritic cells induces the expression of IL-1β, tumor necrosis factor α, IL-6, IL-12, and IL-10, leading to macrophage activation (62). The promoters of IL-1β and tumor necrosis factor α are regulated by AP-1 (63, 64), functional roles for AP-1, CREB, NFκB, and NF-IL-6 have been described for the IL-6 promoter (65), and NFκB-mediated induction of IL-12 has been reported (66), further suggesting the participation of these transcription factors in the CD43-dependent gene expression.

T cell activation is the result of a series of intracellular events initiated through the specific interaction of multiple cell surface molecules with their counter-receptors. We have shown here that the AP-1 binding activity, either to the consensus site or to the AP-1 site of the NF-AT composite sequence upon ligation CD43 alone on the cell surface with the L10 mAb was diminished by minimal concomitant TcR signaling (Fig. 3). This down-modulation correlated with a reduction in IL-2 mRNA when cells were co-stimulated under the same experimental conditions for the same length of time (data not shown). On the other hand, co-stimulation with the anti-CD43 mAb MEM-59 rather than L10 (Fig. 5), resulted in inhibition of AP-1 binding activity to the consensus site but increased binding activity to the AP-1 site of the NF-AT composite sequence. Furthermore, co-ligation with this mAb also resulted in NF-AT translocation to the nucleus, probably because of a longer calcium flux and to the switch from a MAPK-dependent to a Ca2+-dependent response (28). However, when cells were co-stimulated with either L10 or MEM-59 and OKT3 for 24 h, IL-2 mRNA production and IL-2 secretion to the medium were enhanced. Thus, cross-linking CD43 with different mAbs results in different signals that in turn will be regulated differentially by concomitant TcR ligation. This cross-talk modulates the AP-1 binding activity to different sequences, suggesting that different sets of genes could be turned on or off, depending of the epitope of the CD43 molecule that is being recognized by a given ligand and whether the TcR is being activated or not. Additional examples of cross-talk between CD43 and other cell surface molecules have been described. Enhanced LFA-1-dependent homotypic aggregation was shown following up-regulation of CD43 expression by retinoic acids (67), correlating with previous reports where, upon activation with anti-CD43 mAbs, integrin-mediated cell adhesion and movement were up-regulated (68, 69). A mutual activation of CD43 and CD2 adhesion function has also been described (12), further documenting the cross-talk between co-receptors.

The CD43-dependent induction of IL-2 mRNA, CD69, and CD40-L expression and activation of DNA binding activity of AP-1, NF-AT, and NFκB implicate an important role for this molecule in the regulation of gene expression. Further work on CD43 signaling and control of gene expression in different scenarios will further understandings of the multiple regulatory effects of CD43.

Acknowledgments—We thank Dr. Leonor Pérez-Martínez and Dr. Roberto González-Amaro for helpful discussions and suggestions, Dr. Eduardo Huerta for leukocyte concentrates and Erika Melchy for technical help.

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