Integrin α₂β₁ Inhibits Fas-mediated Apoptosis in T Lymphocytes by Protein Phosphatase 2A-dependent Activation of the MAPK/ERK Pathway*

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The mechanisms by which T lymphocytes escape apoptosis during their activation are still poorly defined. In this study, we elucidated the intracellular signaling pathways through which β₁ integrins modulate Fas-mediated apoptosis in T lymphocytes. In experiments done in Jurkat T cells and activated peripheral blood T lymphocytes, engagement of α₂β₁ integrin with collagen type I (Coll I) was found to significantly reduce Fas-induced apoptosis and caspase-8 activation; Annexin V binding and DNA fragmentation were reduced by ~42 and 38%, respectively. We demonstrated that the protective action of Coll I does not require new protein synthesis but was dependent on the activation of the MAPK/Erk pathway. Furthermore, we found that activation of protein phosphatase 2A (PP2A) by Coll I was required for both Coll I-mediated activation of Erk, and inhibition of Fas-induced caspase-8 activation and apoptosis. Other ligands of β₁ integrins, fibronectin (Fbn), and laminin (Lam), did not sustain significant Erk activation and had no effect on Fas-induced apoptosis. Taken together, these results provide the first evidence of a PP2A-dependent activation of the MAPK/Erk pathway downstream of α₂β₁ integrin, which has a functional role in regulating Fas-mediated apoptosis in T lymphocytes. As such, this study emphasizes the potential importance that Coll I interactions may have on the control of T lymphocyte homeostasis and their persistence in chronic inflammatory diseases.

Integrins are α/β heterodimeric membrane proteins that mediate cell adhesion to the surrounding extracellular matrix (ECM),¹ and can elicit a wide variety of intracellular signals that modulate cell growth and proliferation (1, 2). Normal epithelial and endothelial cells depend on integrin signals for cell cycle progression, and disruption of matrix attachment induces their apoptosis, a process termed anoikis (3). We and others (4–7) have also shown that integrin signaling protects anchored cells from different forms of apoptosis such as serum withdrawal and chemotherapeutic agents. Focal adhesion kinase (FAK), integrin-linked kinase (ILK), and Src kinases have all been involved in connecting integrins to downstream survival signaling pathways such as the phosphatidylinositol 3-kinase (PI 3-kinase), the serine/threonine kinase Akt, and the MAPK/Erk pathways (8–10). In addition to kinases, and although less studied, phosphatases have been involved in integrin-mediated signal transduction (11, 12).

T lymphocytes express several β₁ integrins, the most studied being α₁β₁ and α₂β₁ (13). Both of them mediate cell adhesion to the ECM protein (ECMp) fibronectin (Fbn), and to VCAM-1 in the case of α₂β₁ integrin, and transduce costimulatory signals for T cell receptor (TCR)-mediated proliferation (14–16). The signaling events associated with other β₁ integrins, such as the collagen-binding integrins α₁β₁ and α₂β₁ that are expressed during late stages of T cell activation (17), and the cellular functions modulated by these integrins are still poorly defined.

TCR-mediated apoptosis (also known as activation-induced cell death (AICD)) plays an important role in the regulation of immune response and is largely mediated by the interaction of Fas ligand (Fas-L) with its receptor Fas (18, 19). During activation, Fas-mediated apoptosis must be inhibited for the T lymphocytes to survive and accomplish their task. However, the mechanisms involved in such process are still poorly defined. Some evidence suggests that integrin signaling could modulate apoptosis in T lymphocytes. Recently, we have shown that collagen type I (Coll I) but not Fbn or laminin (Lam) protected Jurkat T cells from AICD (20). In intestinal CD4-positive lymphocytes, activation of β₁ integrins mediates proliferation and inhibition of AICD (21), whereas in antigen-specific T lymphocyte clones, AICD is enhanced by the coligation of the TCR and the α₂β₁ and α₂β₂ (LFA-1) integrins (22). Currently, the exact role of integrin signaling in T lymphocyte apoptosis and the molecular mechanisms by which integrins may accomplish this role are still unclear.

In this study, we investigated the regulation of Fas-mediated apoptosis of T lymphocytes by β₁ integrin signaling. We show that engagement of α₂β₁ integrin by its ligand Coll I inhibits Fas-induced apoptosis by activating the MAPK/Erk survival pathway in a protein phosphatase 2A (PP2A)-dependent manner. In contrast, Fbn and Lam failed to induce PP2A activity or sustain significant Erk activation, and had no effect on Fas-apoptosis-inducing ligand; PBS, phosphate-buffered saline; FTTC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter.

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induced apoptosis. Taken together, these results demonstrate a role for αβ1 integrin and its ligand Coll I in the regulation of Fas-mediated apoptosis in T cells, and underline a differential signaling and functions of the β1 integrins molecules in activated T lymphocytes. These findings may have an important impact on the modulation of immune response and development of inflammatory diseases.

EXPERIMENTAL PROCEDURES

Cell Culture and Preparation of T Lymphocytes—The human Jurkat T-cell line E6–1 was obtained from ATCC and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mmol/l glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin (Gibco). Human peripheral blood T lymphocytes were isolated from Ficoll gradients and were then enriched and purified on human T lymphocyte enrichment columns from R&D Systems (Minneapolis, MN) according to the manufacturer’s instructions. Staining with anti-CD3 antibody and flow cytometry (FACS) analysis indicated that more than 97% of the isolated cells were CD3-positive T cells. Freshly isolated blood T lymphocytes are resistant to Fas-mediated apoptosis and do not express the integrins αβ1 and αβ2. They become sensitive to this form of apoptosis and express both integrins only after 48 h and then cultured in complete medium containing 50 units/ml of IL-2 for 6 days before being used in further experiments.

Antibodies and Reagents—Anti-CD3 monoclonal antibody (OKT3) and isotype-matched control antibodies were purchased from BD Pharmingen (San Diego, CA). The anti-α1 (BD12) and anti-αβ1 (P1E6) integrin antibodies were purchased from Chemicon (Temecula, CA). The agonist mouse anti-human Fas monoclonal antibody (CH11) was purchased from Kamiya Biomedicals (Seattle, WA). The anti-caspase-8 antibody (Ab-1) that recognizes the 18-kDa active fragment of caspase-8 (p18) was from Oncogene Research Products (Boston, MA). The anti-phospho-p44/42 MAPK (E-14), which recognizes the active form of Erk1/2, the anti-Erk1/2 (C-14) and anti-actin (C-2) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The phosho-Akt (Ser-473) antibody, which recognizes AKT1, AKT2, and AKT3 when phosphorylated at Ser-473, and the anti-Akt antibody recognizing total endogenous AKT1, AKT2, and AKT3 were from Cell Signaling Technologies (Beverly, MA). Phorbol 12-myristate 13-acetate (PMA), Collagen type I, mouse laminin, cycloheximide, staurosporine, and etoposide were purchased from Sigma. Human fibronectin and poly-L-lysine were a generous gift from Dr. Kristiina Vuori (The Burnham Institute, La Jolla, CA). The agonist mouse anti-human Fas monoclonal antibody (CH11) was purchased from Kamiya Biomedicals (Seattle, WA). The anti-caspase-8 antibody (Ab-1) that recognizes the 18-kDa active fragment of caspase-8 (p18) was from Oncogene Research Products (Boston, MA). The anti-phospho-p44/42 MAPK (E-14), which recognizes the active form of Erk1/2, the anti-Erk1/2 (C-14) and anti-actin (C-2) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The phosho-Akt (Ser-473) antibody, which recognizes AKT1, AKT2, and AKT3 when phosphorylated at Ser-473, and the anti-Akt antibody recognizing total endogenous AKT1, AKT2, and AKT3 were from Cell Signaling Technologies (Beverly, MA). Phorbol 12-myristate 13-acetate (PMA), Collagen type I, mouse laminin, cycloheximide, staurosporine, and etoposide were purchased from Sigma. Human fibronectin and poly-L-lysine were a generous gift from Dr. Kristiina Vuori (The Burnham Institute, La Jolla, CA). The Mek-1 inhibitor PD98059 and okadaic acid (OA) were pur-
treated with the agonistic anti-Fas antibody (CH11) for 12 h. Treatment with Coll I but not with Fbn, Lam or PLL, a non-integrin binding ligand used as control, protected Jurkat T cells from CH11-induced apoptosis; the percentage of Annexin V-positive cells was decreased by 50% (Fig. 1A) and 42% (Fig. 1B), and DNA fragmentation was inhibited by 38% (Fig. 1C). The strongest protection from Fas-induced apoptosis was observed after 3–4 h of pretreatment with Coll I. Fas-induced apoptosis was reduced by 40 and 20% after 3–4 h and 6–24 h of pretreatment with Coll I respectively (data not shown). Flow cytometry analysis of CH11 binding demonstrated that preincubation of Jurkat T cells with Coll I did not alter the ability of CH11 to bind to the Fas receptor.

Fig. 1. Coll I inhibits Fas-induced apoptosis in Jurkat T cells. Jurkat T cells resuspended in medium A were cultured in the presence or absence of 20 μg/ml of the indicated ECM or PLL for 3 h, after which they were treated or not with 50 ng/ml of anti-Fas mAb (CH11). A, after 12 h of treatment with CH11, apoptosis was determined by Annexin V/7AAD double staining and flow cytometry analysis as described under “Experimental Procedures.” The percentages of Annexin V-positive (lower right) and Annexin V-positive/7AAD-positive (upper right) cells are indicated. One representative experiment is shown. B, average percentages of Annexin V-positive cells from three independent experiments, like the one presented in panel A, are presented graphically with S.E. as indicated. C, apoptosis was also determined by DNA fragmentation analysis as described under “Experimental Procedures.” The results are presented as mean of optical density values from three independent experiments, with S.E. as indicated. Statistical analysis was carried out using Student’s t test: *, p < 0.01 between anti-Fas-treated PLL- and Coll I-stimulated samples.

Fig. 2. Coll I does not affect the binding of CH11 to Fas receptor. Jurkat T cells resuspended in medium A were cultured in the presence or absence of 20 μg/ml of Coll I for 3 h, after which they were treated with 50 ng/ml of CH11 or its isotype control antibody for another 2 h. The cells were then harvested, stained with FITC-conjugated anti-mouse IgM antibody, and analyzed by flow cytometry. Staining of non-treated or Coll I-treated cells after incubation with isotype control antibody (—) and with CH11 (—) is shown. The results are representative of three independent experiments.
bind to Fas receptor (Fig. 2), indicating that the observed inhibition did not result from a reduced CH11 binding. The protective action of Coll I was totally abrogated in the presence of blocking anti-α2β1 integrin antibodies (data not shown), consistent with our previous results and those by others that α2β1 integrin is the functional receptor for Coll I on Jurkat T cells (20, 25, 28). Thus, Coll I induces signals through the α2β1 integrin that modulate Fas-mediated apoptosis.

The modulation of Fas-mediated apoptosis by Coll I signaling also occurs in activated peripheral blood T lymphocytes. Flow cytometry analysis showed that after 6 days of in vitro activation, purified peripheral T lymphocytes express significant levels of α2β1 and α2β1 integrins (Fig. 3A), and their preincubation with Coll I but not Fbn, Lam, or PLL inhibited their Fas-mediated apoptosis (Fig. 3B). This Coll I-mediated inhibition was strongly inhibited by anti-α2 and to a lesser extent by anti-α1 integrin blocking antibodies (Fig. 3B), indicating that the protective effect of Coll I was mediated through its α2β1 and α1β1 integrin receptors. Since AICD in T lymphocytes is mainly mediated through Fas signaling, we examined the effect of Coll
I on anti-CD3-induced apoptosis of activated peripheral blood T lymphocytes. These cells underwent significant apoptosis when cultured in wells coated with anti-CD3 mAb, which was reduced by 38% in the presence of Coll I but not of Fbn, or Lam (Fig. 3C). Similar to the inhibition of Fas-induced apoptosis, Coll I-mediated protection from AICD is also blocked by anti-αv integrin blocking antibodies (data not shown). Together these results indicate that in activated primary T cells, Coll I equally reduces both AICD and Fas-induced apoptosis.

When effects of other apoptotic stimuli were evaluated, Coll I also inhibited TRAIL-induced apoptosis, a form of apoptosis that is mediated through death receptor and activation of the caspase-8 apoptotic pathway (29), to a similar degree as it did inhibit Fas-induced apoptosis (40% for Fas versus 35% for TRAIL) (Fig. 4). In contrast, Coll I did not protect Jurkat T cells from staurosporine- and etoposide-induced apoptosis (Fig. 4), two stimuli that activate the mitochondrial death pathway independently from death receptors (30, 31). Treatment with Coll I for longer periods before the addition of staurosporine or etoposide also did not result in any protection from apoptosis (data not shown). These results indicate that in Jurkat T cells, Coll I selectively inhibits death receptor-mediated apoptosis rather than mitochondria-death pathway, and the observed protection does not result from a general inhibitory effect on cell death.

We next investigated whether new protein synthesis is required for the anti-apoptotic effect of Coll I. As shown in Fig. 5, Coll I-mediated protection from Fas-induced apoptosis occurred equally well in the presence or absence of cycloheximide, indicating that the protective effect of Coll I was independent from new protein synthesis.

The αvβ1 Integrin-mediated Protection Against Fas-induced Apoptosis Is MAPK/Erk-dependent—Some evidence indicates that activation of MAPK/Erk and PI 3-kinase/Akt pathways downstream of integrins can block various forms of apoptosis (8, 9). Thus, to investigate the signaling mechanisms underlying the αvβ1 integrin-mediated protection against Fas-induced apoptosis, we analyzed the activation of these pathways by Coll I in Jurkat T cells. Stimulation of the cells with Coll I induced a significant and sustained increase in the phosphorylation levels of Erk1/2 starting at 2 h and lasting up to 24 h (Fig. 6A). Densitometry analysis showed that the increase in Erk1/2 phosphorylation peaked at 4 h, being 3-fold higher than control value (Fig. 6B). In contrast, Akt was found constitutively phosphorylated in Jurkat T cells, which is in agreement with a previous report (32), and treatment with Coll I did not significantly change the levels of phosphorylated Akt (Fig. 6C). No increase in Akt or Erk phosphorylation was detected in cells treated with Coll I for less than 2 h (data not shown).

To demonstrate the implication of MAPK/Erk pathway in the protective action of Coll I, we first ascertained that the stimulation of Erk phosphorylation by Coll I also occurred in the presence of the apoptotic trigger (CH11) (Fig. 7A). Next, we examined the effects of the Mek-1-specific inhibitor PD98059 on αvβ1 integrin-mediated cell survival. Treatment of Jurkat T cells with PD98059 abolished both the ability of Coll I to increase Erk phosphorylation (Fig. 7B) and to protect against Fas-induced apoptosis (Fig. 7C). Treatment of Jurkat T cells with PD98059 had no effect on cell viability, and had only a minor effect on the dose-dependent increase of CH11-induced Jurkat T cell apoptosis (Fig. 7C). These results are in agreement with previous reports, which demonstrated that PD98059 did not sensitize nor did it potentiate Fas-induced apoptosis in Jurkat T cells (27, 33). Importantly, Coll I inhibited apoptosis induced with all tested amounts of CH11 and the presence of PD98059 reversed its effect (Fig. 7C). These results indicate that the effects of PD98059 on apoptosis are not on overall survival in response to Fas ligation but rather on Coll I-mediated survival. Interestingly, Coll I-mediated Erk phosphorylation (see Fig. 6B) and inhibition of Fas-induced apoptosis; which were both optimal after 3–4 h of pretreatment and sustained up to 24 h, are at all time points abrogated in the presence of PD98059 (data not shown). To confirm the implication of the MAPK/Erk pathway in the protective effect of Coll I, we investigated the effects of expressing the dominant negative form of Mek-1 (DN-Mek-1) on the protective action of Coll I. As shown in Fig. 8, expression of DN-Mek-1 in transient transfections also abolishes the ability of Coll I to protect
FIG. 6. Coll I modulates the phosphorylation of Erk1/2 but not of AKT. A, Coll I induces an increase in Erk1/2 phosphorylation. Jurkat T cells resuspended in medium A were stimulated or not with 20 μg/ml of Coll I for the indicated periods of time. Cell lysates were prepared and Erk activation was determined by immunoblot analysis with anti-phospho-Erk1/2 antibody (top). The membrane was stripped and reprobed with anti-Erk2 antibody to confirm equal loading (bottom). B, densitometry quantification of relative increase in total Erk phosphorylation shown in the above blot. The results are expressed as fold increase of the ratio between total phospho-Erk-1/2 and Erk2. C, Coll I does not affect the level of AKT phosphorylation. Jurkat T cells were stimulated as described in A, and AKT activation was determined by immunoblot analysis with anti-phospho-AKT antibody (top). The membrane was stripped and reprobed with anti-AKT antibody to confirm equal loading (bottom). The results are representative of three different experiments.

FIG. 7. Coll I-mediated inhibition of Fas-induced apoptosis is Erk-dependent. A, increase of Erk1/2 phosphorylation by Coll I occurs in the presence of CH11. Jurkat T cells were stimulated or not with Coll I for 3 h, after which they were treated or not with 50 ng/ml of CH11 for the indicated periods of time. Cell lysates were prepared and Erk activation was determined by immunoblot analysis with anti-phospho-Erk1/2 antibody (top). The membrane was stripped and reprobed with anti-Erk2 antibody to confirm equal loading (bottom). B, densitometry quantification of relative increase in total Erk phosphorylation shown in the above blot. The results are expressed as fold increase of the ratio between total phospho-Erk-1/2 and Erk2. C, Coll I does not affect the level of AKT phosphorylation. Jurkat T cells were stimulated as described in A, and AKT activation was determined by immunoblot analysis with anti-phospho-AKT antibody (top). The membrane was stripped and reprobed with anti-AKT antibody to confirm equal loading (bottom). The results are representative of three different experiments.
against Fas-induced apoptosis. Together, these results demonstrate that αβ1 integrin-mediated activation of the MAPK/Erk pathway is involved in the protective effects of Coll I against Fas-induced apoptosis.

Because Fbn and Lam had no effect on Fas-mediated apoptosis in Jurkat T cells (Fig. 1), we examined the capacity of these ECMp to increase Erk phosphorylation. The results in Fig. 9 demonstrate that Fbn and Lam induce only a slight and transient increase of Erk1/2 phosphorylation that is weaker than that induced by 2 h of stimulation with Coll I. Prolonged stimulations, up to 24 h, with either ECMp, or increasing their concentrations did not result in any further Erk activation, and interestingly, the phosphorylation levels of Akt were also not modulated by these ECMp (data not shown). These results strongly suggest that the differential ability of β1 integrin ligands to modulate Fas-mediated apoptosis in Jurkat T cells may be due to their differential ability to increase Erk phosphorylation.

Activation of Protein Phosphatase 2A Is Necessary for the Protective Action of Collagen Type I—Recently αβ1 integrin has been shown to promote activation of the protein phosphatase type 2A (PP2A) in osteosarcoma cells (34). PP2A is one of the four major serine/threonine phosphatases that regulate diverse cellular functions such as cell division and transcription, and several reports have shown that PP2A can regulate either positively or negatively the activation of the MAPK/Erk pathway (35–37). Thus, we sought to investigate the role of PP2A in αβ1 integrin-mediated Erk phosphorylation and inhibition of Fas-mediated apoptosis. Since the strongest effects of Coll I on Erk phosphorylation and cell survival were observed after 3–4 h of pretreatment with Coll I, the role of PP2A was also investigated under these conditions. Jurkat T cells were found to express basal levels of active PP2A, and treatment with Coll I, used at the concentration inducing optimal Erk phosphorylation and inhibition of Fas-mediated apoptosis, induced a 2-fold increase in PP2A activity (Fig. 10A). The Coll I-induced increase in PP2A activity was detected after 30 min, peaked after 1 h of stimulation, and remained detectable after 3 h of stimulation. The increase in PP2A activity dropped to basal levels after 4 h of treatment with Coll I and remained so
for up to 24 h (data not shown). Pretreatment of Jurkat T cells with 50 nM of okadaic Acid (OA), a selective inhibitor of PP2A when used at nanomolar concentrations (38), abolished both the basal level as well as the Coll I-induced increase in PP2A activity. Because OA, at high concentrations, can also inhibit protein phosphatase 1 (PP1), the phosphatase reactions were carried out in the presence of OA to distinguish between PP1 and PP2A. In phosphatase assays, OA inhibits PP2A at IC_{50} = 0.1 nM and PP1 at IC_{50} = 10 nM (39). As shown in Fig. 10B, the Coll I-induced increase in phosphatase activity is reduced by ~42 and 75% in the presence of 0.1 nM and 1 nM OA, respectively. These results indicate that the increase in phosphatase activity observed following Coll I stimulation was due to PP2A. Interestingly, neither Fbn nor Lam was able to induce any significant increase in PP2A activity (Fig. 10A).

To determine whether the observed increase in PP2A activity is necessary for the anti-apoptotic effect of Coll I, we have assessed the effects of nanomolar concentrations of OA on both Coll I-mediated increase of Erk phosphorylation and inhibition of Fas-induced apoptosis. As shown in Fig. 11, OA on its own has no effect on Erk phosphorylation (panel A, lane 2) and apoptosis (panel B). However, treatment of Jurkat T cells with OA abolished in a dose-dependent manner the ability of Coll I to increase Erk phosphorylation (Fig. 11A) and to protect the...
cells from Fas-induced apoptosis (Fig. 11B). Importantly, OA had no effect on Fas-induced apoptosis but reversed equally well the effect of Coll I at both low and high concentrations of CH11, indicating that the observed effects of OA are exerted specifically on Coll I-mediated cell survival (Fig. 11B). As a control, OA had no effect on PMA-mediated Erk phosphorylation (Fig. 11C) and inhibition of Fas-induced apoptosis (Fig. 11D), indicating the specificity of the OA treatment, since the effects of PMA on Erk phosphorylation are dependent on protein kinase C. Although Erk phosphorylation, and to a certain extent, cell survival, were sustained for up to 24 h with Coll I stimulation, we found that the observed increase in PP2A activity (between 30 min and 3 h) is necessary for Coll I-mediated Erk phosphorylation and cell survival for up to 8 h of stimulation with Coll I. However, it is not involved in the effects of Coll I on Erk phosphorylation and cell survival after 10–24 h stimulation since these effects were not abolished by OA (data not shown). Together these results indicate that PP2A is involved in the initial protective effect of Coll I, which was the strongest, and not in the late protective action of Coll I.

To further support the role of PP2A/Erk signaling in the protective action of Coll I, we studied the regulation of caspase-8 activation. As shown in Fig. 12, treatment of Jurkat T cells with CH11 results in the generation of the p18 active form of caspase-8, which is reduced in the presence of Coll I but not PLL. Pretreatment of the cells with either PD98059 or OA abolished completely the ability of Coll I to reduce caspase-8 activation. Together these results indicate that ligation of $\alpha_2\beta_1$ integrin inhibits Fas-mediated apoptosis by activating the MAPK/Erk pathway through a mechanism involving activation of PP2A.

**DISCUSSION**

In this study, we analyzed the intracellular signaling by which $\beta_1$ integrins modulate apoptosis in T lymphocytes, and showed that Coll I, but not Fbn or Lam, inhibits Fas-mediated apoptosis in Jurkat T cells and in primary human T lymphocytes through $\alpha_2\beta_1$ integrin signaling. We demonstrated that Coll I can activate the MAPK/Erk pathway by a mechanism that is dependent on the activity of PP2A, and that both PP2A and Erk activation have a functional role in $\alpha_2\beta_1$ integrin-mediated protection against Fas-induced apoptosis in T lymphocytes.

Our results show that in contrast to Coll I, Fbn and Lam had no effect on PP2A activity, induced only a slight transient increase in Erk phosphorylation, and had no effect on Fas-mediated apoptosis. Jurkat T cells attach to Lam and Fbn through $\alpha_2\beta_1$ and $\alpha_4\beta_1$ integrins, respectively (25, 40). In agreement with our results, it has recently been reported that in Jurkat T cells, ligation of $\alpha_4\beta_1$ integrin induced only moderate levels of Erk1/2 phosphorylation (14). Together these results indicate that different members of $\beta_1$ integrin family are connected to different signaling pathways in T lymphocytes, and may thus modulate different T cell functions in the course of immune response. The exact mechanisms by which members of the $\beta_1$ integrin family connect to downstream signaling pathways and modulate T cell apoptosis are not fully elucidated. In this regard, our results strongly suggest that the differential ability of $\beta_1$ integrins to modulate Fas-mediated apoptosis may be due, at least in part, to their ability to activate the MAPK/Erk signaling pathway.

We also show that the PI 3-kinase/Akt pathway is not involved in $\alpha_2\beta_1$ integrin-mediated cell survival against Fas-induced apoptosis. Coll I had no effect on the levels of phosphorylated AKT, and PI 3-kinase inhibitors (wortmannin and LY294002) have no effect on the protective action of Coll I (data not shown). However, our results do not exclude the possible implication of other signaling mechanisms downstream of $\alpha_2\beta_1$ integrin that may cooperate with MAPK/Erk pathway in protecting T cells from Fas-mediated apoptosis.

Although the mechanisms involved in the regulation of this form of death in T cells are not fully defined, activation of the MAPK/Erk pathway has been implicated in the protective action of PMA, TCR/CD3 and concanavalin-A (27, 41, 42). Our results are in agreement with these studies and indicate that $\alpha_2\beta_1$ integrin signaling is an important modulator of T cell survival. We also demonstrated that in Jurkat T cells, Coll I inhibited selectively death receptor-mediated apoptosis rather than the mitochondrial death pathway, which further supports the observed inhibition of caspase-8 processing by Coll I. Because Coll I-mediated protection is independent from new protein synthesis, it is likely that the observed inhibition of caspase-8 was due to post-translational mechanisms. This is in agreement with a report suggesting that in Jurkat T cells, activation of MAPK/Erk downstream of TCR/CD3 inhibited, by post-translational mechanisms, the processing of pro-caspase-8 downstream of DISC formation (27).

Using OA, a selective inhibitor of PP2A (38, 39), we found that the optimal effects of Coll I on Erk phosphorylation and Fas-induced apoptosis require the activity of PP2A. The role of PP2A in Erk phosphorylation and in cell survival downstream of $\alpha_2\beta_1$ integrin is supported by the fact that Coll I-induced activation of PP2A precedes the increase of Erk phosphorylation, and that both OA and PD98059 had similar effects on Coll I-mediated increase in Erk phosphorylation, inhibition of caspase-8 and cell survival. Our findings concur with a recent report showing that $\alpha_2\beta_1$ integrin activates PP2A in osteosarcoma cells (34). However in these cells, activation of PP2A led to an attenuation of AKT phosphorylation. Such alteration in the level of AKT phosphorylation was not observed in our cell model, probably due to the different cell types and/or to the use of three-dimensional collagen in the case of osteosarcoma cells.

Several studies have shown that OA increases Erk activity in various cell lines (43-45), and have therefore concluded that PP2A is a negative regulator of MAPK/Erk signaling cascade. However, this seems not to be the case in Jurkat T cells. Their treatment with nanomolar amounts (50 nM) of OA inhibited PP2A activity but did not increase basal levels of Erk phosphorylation. In agreement with our results, it is only at high concentration of OA (1 $\mu$M) that treatment of Jurkat T cells with OA resulted in a significant increase of Erk phosphoryla-
the MAPK/Erk pathway. Indeed, recent studies have indicated whether PP2A results in the activation or inactivation of signal development demonstrated that PP2A had both negative and positive effects in the MAPK pathway (48), suggesting that the summation of these effects could dictate whether PP2A results in the activation or inactivation of the MAPK/Erk pathway. Indeed, recent studies have indicated that by activating c-Raf-1, PP2A has had a positive effect on the activation of MAPK/Erk signaling cascade in macrophages and in Caenorhabditis elegans (35, 37). Furthermore, overexpression of PP2A regulatory subunit B by promoted neuronal differentiation by activating the MAPK pathway (50). Thus, our results provide another example of positive regulation of MAPK signaling by PP2A. A model by which PP2A can activate Raf-1 has recently been proposed (51). PP2A can promote Raf-1 activation by dephosphorylating its p-S259 that in turn will result in the dissociation of Raf-1 from 14-3-3 proteins allowing Ras to fully activate Raf-1 (51). Interestingly, αβ1 integrin signaling in Jurkat T cells may also be recognized as a substrate, which can be due to a differential subcellular localization of PP2A and Erk1/2.

On the other hand, genetic studies in Drosophila photoreceptor development demonstrated that PP2A had both negative and positive effects on multiple substrates in the MAPK pathway (49), suggesting that the summation of these effects could dictate whether PP2A results in the activation or inactivation of the MAPK/Erk pathway. Indeed, recent studies have indicated that by activating c-Raf-1, PP2A has had a positive effect on the activation of MAPK/Erk signaling cascade in macrophages and in Caenorhabditis elegans (35, 37). Furthermore, overexpression of PP2A regulatory subunit B by promoted neuronal differentiation by activating the MAPK pathway (50). Thus, our results provide another example of positive regulation of MAPK signaling by PP2A. A model by which PP2A can activate Raf-1 has recently been proposed (51). PP2A can promote Raf-1 activation by dephosphorylating its p-S259 that in turn will result in the dissociation of Raf-1 from 14-3-3 proteins allowing Ras to fully activate Raf-1 (51). Interestingly, αβ1 integrin signaling in Jurkat T cells may also be recognized as a substrate, which can be due to a differential subcellular localization of PP2A and Erk1/2.

PP2A has been involved in both cell growth and apoptosis (48). Our results indicating that PP2A is associated with cell survival and probably cell growth downstream of αβ1 integrin signaling are further supported by a recent study showing the potent costimulatory effect that Coll I had on TCR-mediated proliferation of human effecter T lymphocytes (54). These studies identify the αβ1/PP2A/Erk signaling complex as one important modulator of T cell survival and growth. Since PP2A is not involved in the late effects of Coll I on Erk phosphorylation (data not shown), additional signaling events are likely to be involved, which underscores the complexity of Coll I signaling in T lymphocytes.

AICD in T lymphocytes is mediated by activating the Fas/Fas-L apoptotic pathway in response to TCR/CD3 complex signaling. We have previously shown that Coll I reduces anti-CD3-induced Fas-L expression in Jurkat T cells (20). Thus, with the results presented in this study, it appears that αβ1 integrin signaling blocks AICD not only by interfering with the expression of Fas-L, but also by directly inhibiting the Fas signaling death pathway. The expression of αβ1 integrin on T lymphocytes is associated with late stages of activation that occur in peripheral tissues (17). Thus, our results suggest that in vivo, Coll I can protect activated T cells from AICD in tissues rich in collagen, such as connective tissues of skin and in the course of chronic inflammation such as rheumatoid arthritis (RA). Indeed, RA synovial T lymphocytes do have increased expression of αβ1 and αβ2 integrins (55, 56) and show resistance to Fas-mediated apoptosis (57). In this context, further elucidation of the signaling events activated by αβ1 integrin in T lymphocytes is likely to provide novel insights into the role of αβ1 integrin signaling in the regulation of immune response and in chronic inflammatory diseases.

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