Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6959–6964, June 1998
Immunology

Interleukin-2 induces β_2-integrin-dependent signal transduction involving the focal adhesion kinase-related protein B (fakB)

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Communicated by Hans H. Ussing, University of Copenhagen, Copenhagen, Denmark, March 31, 1998 (received for review January 6, 1998)

ABSTRACT **β_2 integrin molecules are involved in a multitude of cellular events, including adhesion, migration, and cellular activation. Here, we studied the influence of β_2 integrins on interleukin-2 (IL-2)-mediated signal transduction in human CD4$^+$ T cell lines obtained from healthy donors and a leukocyte adhesion deficiency (LAD) patient. We show that IL-2 induces tyrosine phosphorylation of a 125-kDa protein and homotypic adhesion in β_2 integrin (CD18)-positive but not in β_2-integrin-negative T cells. EDTA, an inhibitor of integrin adhesion, blocks IL-2-induced tyrosine phosphorylation of the 125-kDa protein but not other proteins in β_2-integrin-positive T cells. Likewise, a β_2 integrin (CD18) antibody selectively inhibits induction of the 125-kDa phosphotyrosine protein, whereas cytokine-mediated tyrosine phosphorylation of other proteins is largely unaffected. Immunoprecipitation experiments indicate that the IL-2-induced 125-kDa phosphotyrosine protein is the focal adhesion kinase-related protein B (fakB). Thus, IL-2 induces strong tyrosine phosphorylation of fakB in β_2-integrin-positive but not in β_2-integrin-negative T cells, and CD18 mAb selectively blocks IL-2-induced fakB-tyrosine phosphorylation in β_2-integrin-positive T cells. In parallel experiments, IL-2 does not induce or augment tyrosine phosphorylation of p125^{FAK}. In conclusion, our data indicate that IL-2 induces β_2-integrin-dependent signal transduction events involving the tyrosine kinase substrate fakB.**

Interleukin-2 (IL-2) plays a crucial role in the differentiation and clonal expansion of T lymphocytes. The cytokine exerts its biological effect through specific IL-2 receptors expressed on T lymphocytes. Various combinations of three distinct chains (α, β, and γ) form three classes of IL-2 receptors (reviewed in ref. 1). Low-affinity IL-2 receptors consist of IL-2Rα, intermediate-affinity receptors contain IL-2Rβ and IL-2Rγ, and high-affinity receptors contain all three chains. The IL-2Rα chain has a short cytoplasmic tail consisting of only 13 amino acids, and the function of this chain appears to be limited to increasing the affinity of IL-2 binding (2). In contrast, it is likely that both IL-2Rβ and -γ chains function in IL-2 signal transduction, because deletion of the cytoplasmic tails of these chains abolishes IL-2-induced signaling (reviewed in ref. 3).

Although IL-2R subunits do not appear to contain protein tyrosine kinase (PTK) domains within their cytoplasmic tails, a variety of data are consistent with an important role for PTKs in IL-2 signaling. Thus, IL-2 can induce tyrosine kinase activity (4) and is known to cause tyrosine phosphorylation of a number of substrates, including transcription factors such as Stat3 and Stat5 (5–8). Furthermore, IL-2Rs associate noncovalently with tyrosine kinases belonging to the src family (e.g., p56^{lck}) and Janus kinases (Jak1 and Jak3) (refs. 9–11, reviewed in ref. 3). Serine/threonine kinases such as Raf-1 and MAP kinase, the lipid kinase phosphatidylinositol 3-kinase, and the protein phosphatases PP1/PP2A have also been implicated in IL-2 signaling (12–15), and at least three major signaling pathways are involved in IL-2-induced activation of transcription factors such as c-myc, c-fos, and c-jun (16).

In addition to having a function as a growth factor, IL-2 plays a role as a regulator of T cell adhesion and migration. Thus, IL-2 induces homotypic adhesion among T cells and binding of T cells to and migration across specialized and nonspecialized endothelia (17–20). The adhesion response to IL-2 appears to be mediated primarily by β_2 integrins (CD11a/CD18) (18–20). However, it is not clear how IL-2Rs are functionally linked to the β_2 integrin adhesion pathway and whether crosstalk takes place between β_2 integrins and IL-2R. In polymorph nucleated neutrophils, tyrosine phosphorylation in response to tumor necrosis factor (TNF)α requires β_2 integrins (21, 22), suggesting the possibility that β_2 integrins also play a regulatory role in cytokine receptor signaling in T lymphocytes. To test this hypothesis we took advantage of antigen-specific CD4$^+$ T cell lines from healthy donors and a leukocyte adhesion deficiency (LAD) patient. We show that (*i*) IL-2 induces tyrosine phosphorylation of the focal adhesion kinase (FAK)-related protein B (fakB) in β_2-integrin-positive T cells but not in β_2-integrin-negative LAD T cells, and (*ii*) inhibitors of β_2 integrin function such as CD18 antibody and EDTA selectively modulate IL-2-mediated signaling, suggesting that β_2 integrins play a regulatory role in IL-2-induced signal transduction.

MATERIALS AND METHODS

Cells. The alloantigen-specific, CD4-positive, β_2-integrin-positive and -negative human T cell lines have been described previously (23, 24). All T cell lines had been depleted of plastic-adherent cells, frozen in liquid nitrogen until use, and washed extensively prior to testing in adhesion and proliferation assays (see below), and none of the T cells proliferated significantly in response to stimulation with phytohemagglutinin, anti-CD3 mAb in soluble form, or staphylococcal enterotoxins A and B (ref. 24 and unpublished observations).

Antibodies and Reagents. mAb against tyrosine-phosphorylated proteins (4G10) was obtained from UBI (Lake Placid, NY). Rabbit antisera to p125^{FAK} and fakB generated with synthetic peptides conjugated to ovalbumin were described previously (25, 26). Rabbit antisera (K15 and C20) to Stat3

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Abbreviations: IL-2, interleukin-2; IL-2R, IL-2 receptor; LAD, leukocyte adhesion deficiency; FAK, focal adhesion kinase; fakB, FAK-related protein B.

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were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anit-CD54 (BBA4) mAb was from R&D systems (Abingdon, Oxon, U.K.), and anti-CD21 (BL13) mAb and IgG control antibodies were from Immunotech (Marseilles, France). Anti-CD18 (60.3), CD4 (G17–2), and CD28 (9.3) mAbs were generous gifts from Jeffrey A. Ledbetter (CDR Therapeutics, Seattle, Washington). Recombinant IL-2 was a generous gift from Craig W. Reynolds (Biological Response Modifiers Program, National Cancer Institute, Bethesda, MD).

Cell Adhesion and Proliferation Assay. Cells were incubated for up to 4 hr in flat-bottom microtiter wells (Falcon 3072, BD) in culture medium with 10% heat-inactivated filtered pooled human serum with or without cytokines as indicated. The cytokine-induced adhesion was evaluated at different time points, and the percentage adhesion was scored as described elsewhere (27). Proliferation assays were performed in a standard microplate system as described elsewhere (23). Briefly, T cells were cultured (10^4 to 10^5 cells per well) for up to 96 hr with or without lymphokines or reagents as indicated. Twelve hours before harvest, [^{3}H]thymidine [1.0 μCi per well (1 μCi = 37 kBq)] was added, the cells harvested onto glass fiber filters, and [^{3}H]thymidine incorporation was measured. The cytokine-induced proliferation was expressed as median cpm and the percentage inhibition by reagents was calculated as described elsewhere (27).

Protein Extraction and Western Blotting. After treatment with or without cytokines and/or chemical enzyme inhibitors, the T cells (3×10^6 cells per experiment for analysis of total phosphorylation, 20×10^6 cells per experiment for immunoprecipitations) were rapidly pelleted, and the reaction was stopped by lysing the cells in ice-cold lysis buffer [1% Nonidet P-40/20 mM Tris·HCl, pH 8.0/137 mM NaCl/5 mM $MgCl_2$/10% (vol/vol) glycerol and the following inhibitors: 5 mM EDTA, 1 mM Na_3VO_4, 10 μg/ml aprotinin, 4 μM iodoacetamide, and 1 mM phenylmethanesulfonyl fluoride]. Total cell lysate proteins were subjected to SDS/10% PAGE and transferred onto nitrocellulose filters. Immunoblotting and immunoprecipitation were conducted as described earlier (8). Blots were evaluated by using enhanced chemiluminescence (ECL), stripped, and reprobed according to the manufacturer's manual (Amersham).

RESULTS

EDTA and CD18 mAb Inhibit IL-2-Induced β_2 Integrin Adhesion. Fig. 1*A* shows the adhesion response in β_2-integrin-positive T cells after treatment with or without IL-2 and EDTA, a chelator of divalent cations that inhibits integrin-mediated adhesion. IL-2-induced homotypic adhesion in antigen-specific CD4$^+$ T cells and EDTA almost completely blocked the adhesion response (Fig. 1*A*) supporting other data (18–20) that β_2 integrins play a crucial role in IL-2-induced adhesion. Because IL-2 induces tyrosine phosphorylation of an unidentified protein that was hypothesized to play a role in IL-2-induced adhesion (27), we examined whether EDTA modulated IL-2-mediated tyrosine phosphorylation. Accordingly, T cells were treated with or without EDTA and stimulated with medium or IL-2 for different periods of time. The cells were then lysed and IL-2-induced tyrosine phosphorylations were analyzed by SDS/PAGE and immunoblotting with anti-phosphotyrosine mAb. IL-2 induced tyrosine phosphorylation of several proteins, the predominant ones being proteins with molecular masses of ≈125 kDa and ≈80–90 kDa (Fig. 1*B*). Whereas tyrosine phosphorylation of the ≈80- to 90-kDa and other low molecular weight proteins was not or only weakly inhibited by EDTA, tyrosine phosphorylation of the ≈125-kDa protein(s) was strongly inhibited by EDTA. The profound inhibition of the ≈125-kDa phosphotyrosine protein was not because of a delay in kinetics, because tyrosine

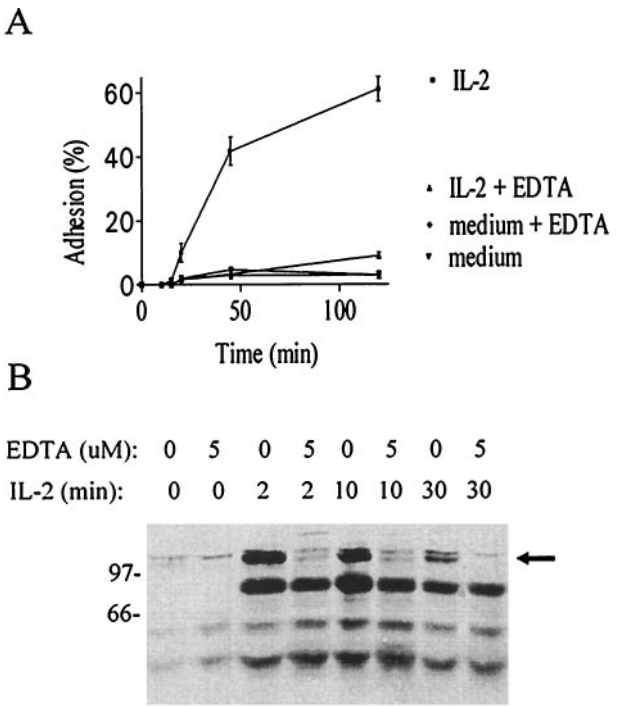

FIG. 1. EDTA blocks IL-2-induced adhesion and tyrosine phosphorylation of p125. (*A*) CD18-positive T cells (10^6 per well) were incubated with medium (▼), EDTA (◆), IL-2 (■), or IL-2 and EDTA (▲) and the percentage adhesion was determined. EDTA was used at 5 μM and IL-2 at 15 ng/ml. (*B*) T cells were incubated with medium and EDTA with or without IL-2 (15 ng/ml) for the periods of time indicated. Cells were lysed and IL-2-induced tyrosine phosphorylation was analyzed by Western blotting with an anti-phosphotyrosine mAb (4G10). The positions of prestained molecular standards (Amersham) are indicated on the left in kDa. These data are representative of three independent experiments.

phosphorylation of the ≈125-kDa protein after 10 or 30 min of IL-2 stimulation was also strongly inhibited by EDTA. Thus, divalent cations such as Ca^{2+} (and Mg^{2+}) appear to play a role in IL-2-induced tyrosine phosphorylation of the ≈125-kDa protein (p125) but not of the ≈80- to 90-kDa and other proteins. Because IL-2 does not induce an influx of calcium in these T cells (28), we hypothesized that the effect of EDTA on IL-2 signaling was indirect—i.e., mediated by a functional inhibition of β_2 integrins. To test this hypothesis, T cells were incubated with antibodies against CD11a/CD18 (LFA-1) β_2 integrins and the CD11a/CD18 ligand, CD54 (ICAM-1). As

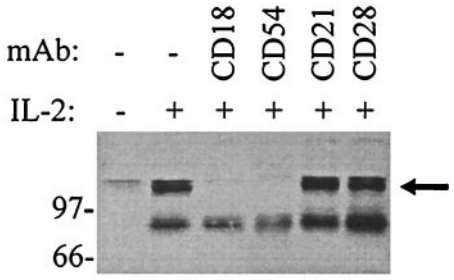

FIG. 2. CD18 and CD54 mAb block IL-2-induced tyrosine phosphorylation of p125. CD18-positive T cells were incubated for 30 min with or without anti-CD18 (60.3), CD54 (BBA 4), CD21 (BL13), or CD28 (9.3) mAb (50 μg/ml) prior to stimulation with IL-2 (15 ng/ml) for 2 min. Cells were lysed and IL-2-induced tyrosine phosphorylation was analyzed by Western blotting with an anti-phosphotyrosine mAb (4G10). The positions of prestained molecular standards (Amersham) are indicated on the left in kDa. These data are representative of four independent experiments.

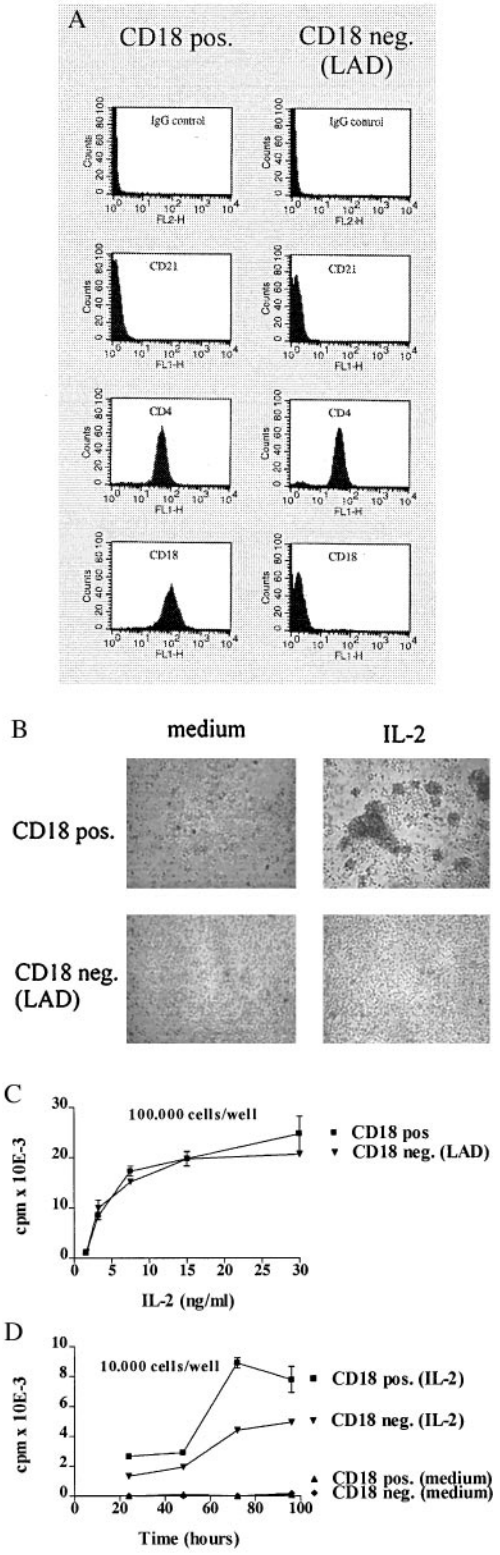

FIG. 3. IL-2 induces mitogenesis but not homotypic adhesion in LAD T cells. (*A*) Alloantigen-specific $CD4^+$ T cell lines obtained from a LAD patient (*Right*) and a healthy donor (*Left*) were labeled with antibodies against CD18 (60.3), CD4 (G17–2), CD21 (BL13), or an IgG control antibody and secondary antibody [goat anti-mouse, fluorescein isothiocyanate (FITC)-conjugated] prior to flow cytometric examination as described (24). (*B*) CD18-positive T cells (*Upper*) from a healthy donor and CD18-negative T cells (*Lower*) from a LAD patient were incubated with medium (*Left*) or IL-2 (*Right*) at 15 ng/ml for 12 hr at 37°C in flat-bottomed microtiter plates in an atmosphere of 5% CO_2. Photomicrographs were taken at ×150. Essentially identical results were obtained in eight independent experiments using two different LAD T cell lines. (*C*) CD18-positive (■) and -negative (▼) T cell lines (10^5 cells per well) were incubated in medium with or without IL-2 as indicated. [^{3}H]Thymidine uptake was measured after 48 hr of culture in an atmosphere as above. The data are expressed as the mean of triplicates. (*D*) CD18-positive (■, ▲) and -negative (▼, ◆) T cell lines (10^4 cells per well) were incubated in medium with or without IL-2 (15 ng/ml) as indicated. [^{3}H]Thymidine uptake was measured after various periods of culture in an atmosphere as above. The data are expressed as the mean of triplicates. Essentially identical results were obtained in three independent experiments using two different LAD T cell lines.

shown in Fig. 2, both CD18 and CD54 mAbs blocked IL-2-induced tyrosine phosphorylation of the ≈125-kDa protein, whereas isotype-matched, anti-CD28 and a nonbinding control (anti-CD21) mAb did not. The inhibition of IL-2-induced tyrosine phosphorylation of p125 was specific, because anti-CD18 and anti-CD54 mAbs had little effect on IL-2-induced tyrosine phosphorylation of the ≈80- to 90-kDa proteins (Fig. 2).

IL-2 Induces Mitogenesis but Not Homotypic Adhesion in β_2 Integrin-Negative T Cells. Fig. 3*A* shows representative flow cytometric profiles of antigen-specific $CD4^+$ T cell lines obtained from a healthy individual (Fig. 3*A Left*) and a LAD patient (Fig. 3*A Right*). Whereas both T cell lines expressed comparable amounts of CD4 molecules, LAD T cells did not significantly express CD18 molecules (Fig. 3*A*). Incubation with IL-2 for up to 12 hr induced a profound adhesion response with formation of large clusters of aggregating CD18-positive T cells (Fig. 3*B*). In contrast, IL-2 did not induce homotypic adhesion in CD18-negative LAD T cells (Fig. 3*B*) despite induction of vigorous proliferation (Fig. 3*C*), supporting the hypothesis that β_2 integrins play a crucial role in the IL-2 adhesion response. These findings also show that at high cell densities (10^5 cells per well), β_2 integrin-dependent adhesion is not a prerequisite for induction of mitogenesis. However, at lower cell densities (10^4 cells per well) mitogenic responses were slower and significantly weaker in CD18-negative T cell lines compared with CD18-positive T cell lines (Fig. 3*D*).

IL-2 Induces Tyrosine Phosphorylation of the 125-kDa fakB in β_2 Integrin-Positive but Not -Negative LAD T Cells. To address further whether induction by IL-2 of the 125-kDa phosphotyrosine protein is activated by induction of β_2-integrin-dependent adhesion, we compared the tyrosine phosphorylation profiles after IL-2 stimulation of β_2-integrin-positive and -negative T cells. As expected, IL-2 induced tyrosine phosphorylation of several proteins, including the proteins with molecular masses of ≈125 kDa and ≈80–90 kDa, in CD18-positive T cells (Fig. 4*A*, left lanes). IL-2 also induced tyrosine phosphorylation of the ≈80- 90-kDa and other low molecular mass proteins in CD18-negative LAD T cells (Fig. 4*A,* right lanes). However, IL-2 did not induce strong tyrosine phosphorylation of the ≈125-kDa protein in CD18-negative LAD T cells. Thus, only a weak band of ≈125 kDa could be detected 2 min after IL-2 stimulation (Fig. 4*A*, right lanes). The lack of significant tyrosine phosphorylation of the ≈125-kDa protein in CD18-negative T cells was not because of a delay in kinetics, because neither 15 min nor 30 min of IL-2 stimulation induced detectable levels of tyrosine phosphorylation of the protein in question (Fig. 4*A*, right lanes). Likewise, IL-2 stimulation for 1, 2, or 4 hr also did not induce significant tyrosine phosphorylation of the ≈125-kDa protein (data not shown).

Recently, it was reported that extensive crosslinking of β_2 integrins by anti-CD18 mAb and goat-anti-mouse antibody induced tyrosine phosphorylation of a 125-kDa protein designated fakB (26). To address whether the IL-2-induced 125-kDa phosphotyrosine protein was a tyrosine-phosphorylated form of fakB, T cells were treated with or without IL-2, and fakB was immunoprecipitated by an anti-fakB antibody prior

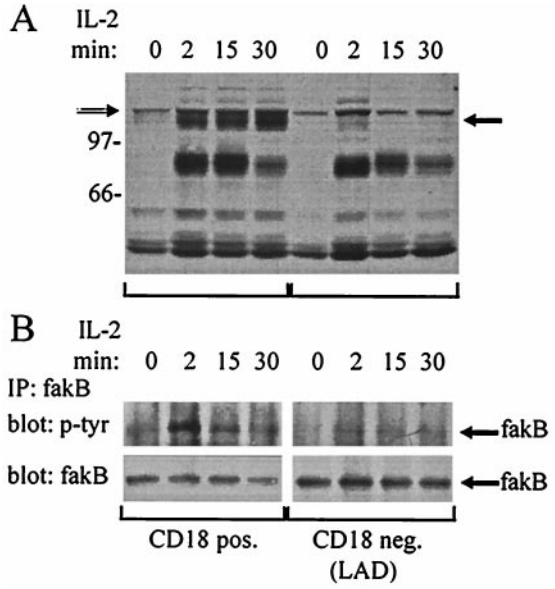

FIG. 4. IL-2 induces tyrosine phosphorylation of fakB in CD18-positive but not in CD18-negative T cells. (*A*) A CD18-positive T cell line from a healthy donor (left four lanes) and a CD18-negative T cell line from a LAD patient (right four lanes) were incubated with medium or IL-2 (15 ng/ml) for the periods of time indicated. Cells were then lysed and IL-2-induced tyrosine phosphorylation was analyzed by Western blotting with an anti-phosphotyrosine mAb (4G10). The positions of prestained molecular standards (Amersham) are indicated on the left in kDa. A solid arrow (←) indicates the IL-2-inducible 125-kDa phosphotyrosine protein (p125) likely to represent fakB (compare below), whereas an open arrow (⇒) indicates a constitutively tyrosine phosphorylated 130-kDa protein likely to represent pp125^{FAK}. (*B*) CD18-positive (*Left*) and -negative (*Right*) T cell lines were stimulated with IL-2 as in *A*. Lysates were immunoprecipitated with anti-fakB antibody and immunoblotted with anti-phosphotyrosine mAb (*Upper*) or anti-fakB antibody (*Lower*). These data are representative of three independent experiments.

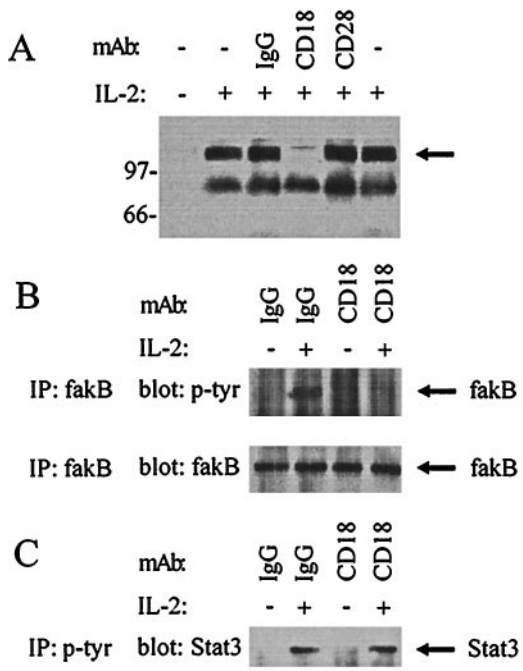

FIG. 5. A selective inhibition of IL-2-induced tyrosine phosphorylation of fakB by CD18 mAb. (*A*) CD18-positive T cells were incubated for 30 min with or without anti-CD18 (60.3), CD28 (9.3), or IgG2a control mAb (50 μg/ml) prior to stimulation with IL-2 (15 ng/ml) for 2 min. Cells were lysed and IL-2-induced tyrosine phosphorylation was analyzed by Western blotting with an anti-phosphotyrosine mAb (4G10). The positions of prestained molecular standards (Amersham) are indicated on the left in kDa. (*B* and *C*) CD18-positive T cells were incubated with anti-CD18 (60.3) or a nonbinding IgG2a control mAb (50 μg/ml) prior to stimulation with medium or IL-2 (15 ng/ml) for 2 min. fakB was immunoprecipitated with anti-fakB antibody and immunoblotted with anti-phosphotyrosine mAb (*B Upper*) or anti-fakB antibody (*B Lower*). In parallel, lysates were immunoprecipitated with anti-phosphotyrosine mAb (4G10) and immunoblotted with anti-Stat3 antibody (C20) (*C*) as above. Similar results were obtained in four independent experiments.

to immunoblotting with anti-phosphotyrosine antibody. Fig. 4*B* shows that fakB isolated from CD18-positive T cells was increased in phosphotyrosine content after IL-2 stimulation. Cytokine-induced tyrosine phosphorylation of fakB was rapid and transient—i.e., peaked at 2 min and declined after 15 to 30 min (Fig. 4*B Left*) similar to the phosphorylation observed with anti-CD3 antibody (25). Equal amounts of fakB were immunoprecipitated before and after IL-2 stimulation, indicating that IL-2 induced an increase in fakB phosphorylation on tyrosine. Fig. 4*B Right* shows that IL-2 induced only a faint tyrosine phosphorylation of fakB in CD18-negative LAD T cells, which was comparable to the faint tyrosine phosphorylation of the 125-kDa protein after IL-2 stimulation (Fig. 4*A*, left lanes versus right lanes). The amount of fakB was comparable in CD18-positive and -negative T cells (Fig. 4*B*), indicating that the lack of significant tyrosine phosphorylation of fakB in CD18-negative T cells was not because of deficient expression of fakB in LAD patients (Fig. 4*B*).

Anti-CD18 mAb Inhibits IL-2-Induced Tyrosine Phosphorylation of fakB. Given that the 125-kDa phosphotyrosine protein is a tyrosine-phosphorylated form of fakB, anti-CD18 antibody would be predicted to inhibit IL-2-induced tyrosine phosphorylation of fakB. Fig. 5*A* shows that an anti-CD18 mAb but not an anti-CD28 or an isotype-matched nonbinding mAb inhibited IL-2-induced tyrosine phosphorylation of the 125-kDa protein, confirming the findings above in another CD18-positive T cell line. Immunoprecipitation of fakB and blotting with anti-phosphotyrosine mAb showed that IL-2-induced tyrosine phosphorylation of fakB was blocked by anti-CD18 mAb (Fig. 5*B*). In contrast, anti-CD18 mAb had no significant effect on IL-2-induced tyrosine phosphorylation of Stat3 (Fig. 5*C*).

IL-2 Does Not Significantly Augment Tyrosine Phosphorylation of FAK. Immunoblotting with anti-phosphotyrosine mAb of cell lysates depleted of fakB indicated that proteins other than fakB (but with a similar molecular weight) might also become tyrosine phosphorylated upon IL-2 stimulation (data not shown). We have previously obtained evidence that the ≈125-kDa phosphotyrosine protein is not a tyrosine-phosphorylated form of the Janus kinases 1 or 3 (ref. 27 and data not shown). Accordingly, we examined whether FAK became tyrosine phosphorylated after IL-2 stimulation. Fig. 6*A* shows immunoblots with phosphotyrosine (*Upper*) or FAK (*Lower*) antibodies of cell lysates immunoprecipitated with FAK antibody. In these cells, FAK was constitutively phosphorylated on tyrosine, and IL-2 treatment did not appear to enhance the level of FAK tyrosine phosphorylation, whereas IL-2 induced profound tyrosine phosphorylation of fakB in parallel samples (Fig. 6*B*). The molecular mass of FAK (≈130 kDa) is slightly higher than that of fakB (25), and, as shown in Fig. 5*A*, a protein of a molecular mass identical to FAK is constitutively phosphorylated on tyrosine in both CD18-positive and -negative T cells. In one CD18-positive T cell line, the background level of FAK tyrosine phosphorylation was low, and a weak (but variable) induction of tyrosine phosphorylation of FAK has been observed in this cell line (data not shown). Therefore, we cannot exclude the possibility that

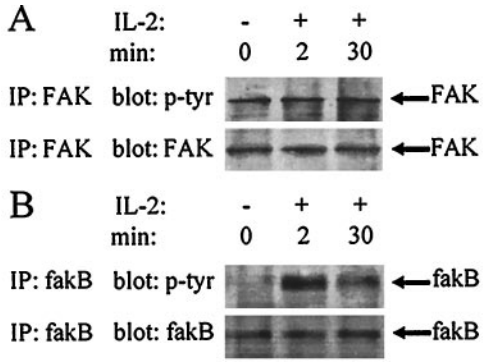

FIG. 6. IL-2 selectively enhances tyrosine phosphorylation of fakB. A CD18-positive T cell line was incubated with medium or IL-2 (15 ng/ml) for 2 or 30 min. Lysates were immunoprecipitated with antibody against FAK (*A*) or fakB (*B*) and immunoblotted with anti-phosphotyrosine mAb or the antibody used for immunoprecipitation as indicated. Similar results were obtained in three independent experiments.

limited levels of tyrosine phosphorylation of FAK may be modulated after IL-2 stimulation in some T cells.

DISCUSSION

In the present study we show that an anti-CD18 mAb, which inhibits IL-2-induced adhesion (20), almost completely blocked cytokine-induced tyrosine phosphorylation of a 125-kDa protein in β_2-integrin-positive T cells. An antibody against the β_2 integrin ligand, CD54, also blocked IL-2-mediated induction of the 125-kDa phosphotyrosine protein. Because both mAbs inhibit IL-2-induced homotypic adhesion (18–22), our data suggest that β_2-integrin-dependent adhesion was involved in cytokine-induced tyrosine phosphorylation of the 125-kDa protein. This conclusion was supported by our findings that EDTA, an inhibitor of integrin-dependent adhesion, almost completely blocked both the IL-2 adhesion response and the induction of the 125-kDa phosphotyrosine protein. Tyrosine phosphorylation of proteins other than the 125-kDa protein was largely unaffected by inhibitors of adhesion, suggesting that β_2 integrins play a selective role in IL-2 signaling. In support of this hypothesis, we observed that IL-2 did not induce homotypic adhesion and significant tyrosine phosphorylation of the 125-kDa protein in β_2-integrin-deficient T cells obtained from a LAD patient. In fact, IL-2 induced a tyrosine phosphorylation profile in β_2-integrin-deficient T cells that was very similar to the profile observed in anti-CD18-treated, β_2-integrin-positive T cells. Collectively, these findings indicated that IL-2 induced tyrosine phosphorylation of the 125 kDa protein via a β_2-integrin-dependent signal pathway. Moreover, they strongly suggested that β_2-integrin-dependent tyrosine phosphorylation was triggered by an induction of homotypic adhesion. Thus, these data provide evidence that IL-2 induces signal transduction by means of multiple cell surface molecules, including β_2 integrins, and that IL-2 signaling is not confined to the "classical" signal pathways involving the IL-2R β and γ chains.

To identify the 125-kDa phosphotyrosine protein, we focused on a recently identified 125-kDa protein, fakB, which has been implicated in β_2-integrin-induced signaling (26). It was recently reported that extensive crosslinking of CD18 induced tyrosine phosphorylation of fakB (26). Accordingly, immunoprecipitation experiments were carried out, and our data clearly showed that IL-2 induced rapid tyrosine phosphorylation of fakB. The tyrosine phosphorylation of fakB and the unidentified 125-kDa protein peaked within 2 min after IL-2 stimulation, suggesting that fakB may be identical to the 125-kDa protein. This conclusion was supported by two lines of evidence: (*i*) CD18 mAb blocked IL-2-induced tyrosine phosphorylation of fakB; and (*ii*) IL-2 did not induce a significant tyrosine phosphorylation of fakB in β_2-integrin-deficient T cell lines despite high levels of fakB expression in these T cells. Thus, the present data reveal that IL-2 induced tyrosine phosphorylation of fakB and indicate that fakB phosphorylation was mediated via a β_2-integrin-dependent signal pathway. Immunodepletion of fakB suggested that other proteins with a molecular mass of 125–130 kDa might also become phosphorylated on tyrosine in response to IL-2 stimulation. Because FAK ($p125^{FAK}$) has also been implicated in signal transduction in T cells (29), we examined whether FAK became phosphorylated on tyrosine in response to IL-2. FAK was, however, constitutively phosphorylated on tyrosine and (with one possible exception) IL-2 did not augment the content of tyrosine-phosphorylated FAK in our T cell lines. The recent cloning of a FAK-related kinase, PYK2/CadTK/RAFTK/CAKβ, with a molecular mass of 120 kDa (30–32) and involved in integrin-mediated adhesion (33), suggests the possibility that this kinase might be involved in the IL-2 adhesion response. Our preliminary findings suggest that IL-2 may induce a rapid and transient (<5 min) tyrosine phosphorylation of PYK2/RAFTK/CadTK/CAKβ in some T cells (data not shown).

We previously observed that tyrosine phosphorylation of the 125-kDa protein was inhibited by herbimycin A, an inhibitor of tyrosine kinases (27). It remains to be seen whether fakB itself is a tyrosine kinase with the potential for autophosphorylation. A series of other tyrosine kinases are activated by IL-2, and fakB might be a substrate for one or more of these kinases. Upon TCR/CD3 stimulation, fakB becomes associated with the tyrosine kinase ZAP-70. Because ZAP-70 is also activated by IL-2 (reviewed in ref. 34), it is possible that fakB is a substrate for IL-2-activated ZAP-70. We were, however, unable to detect a co-association between fakB and ZAP-70 in IL-2-stimulated or unstimulated T cells (data not shown). Studies are in progress to elucidate whether fakB becomes physically associated with other IL-2-activated kinases.

At low cell densities, mitogenic responses were slower and significantly weaker in CD18-negative T cell lines than in CD18-positive T cell lines, suggesting that β_2-integrin-dependent adhesion and fakB tyrosine phosphorylation might play an important role in cytokine-driven proliferation. This conclusion is in keeping with our preliminary findings that anti-CD18 mAb inhibits IL-2- induced tyrosine phosphorylation of fakB and mitogenesis in parallel (data not shown) and the findings by others (18) that some anti-CD11a antibodies inhibit both IL-2-induced adhesion and proliferation. Surprisingly, at high cell densities IL-2 induced comparable levels of proliferation in β_2-integrin-positive and -negative T cells, indicating that under these experimental conditions, β_2-integrin- dependent adhesion and tyrosine phosphorylation of fakB were not a prerequisite for induction of proliferation. Because β_1 integrin molecules are also involved in IL-2-induced adhesion (18–20) and expressed by LAD T cell lines (data not shown), it is possible that at high cell densities, β_1 integrins can provide comitogenic signals in β_2-integrin-negative LAD T cell lines.

The present findings that IL-2 induces rapid T–T cell adhesion support the idea that IL-2, in addition to its well established role as a T cell growth factor, has a function in the regulation of direct cell–cell contact. Because other immunocompetent cells—e.g., monocytes, and NK (natural killer) and B cells—express IL-2 receptors, it is likely that adhesion between these cells and T helper cells is also up-regulated by IL-2. However, the effect on cellular adhesion is not limited to interactions between immunocompetent cells. Thus, IL-2 activates β_2- as well as β_1-integrin-dependent adhesion of T lymphocytes and NK cells to and migration across specialized and nonspecialized epithelium (17–19). Therapeutic adminis-

tration of IL-2 in cancer patients induces homing and perivascular infiltration of activated T lymphocytes in the dermis (35). Taken together, these data suggest that IL-2 has a specialized function in T cell adhesion, homing, and migration. Our results strongly support this hypothesis by showing that IL-2 triggers a β_2-integrin-dependent signal pathway involving fakB. Thus, IL-2 signaling may not be confined to the "classical" IL-2R β and γ signal pathways, suggesting that the current models for IL-2 signaling might have to be reevaluated to encompass the role of surface molecules such as β_2 integrins.

We thank Dr. Craig W. Reynolds (Biological Response Modifiers Program, National Cancer Institute, Bethesda, MD) for the generous gift of recombinant IL-2 and Jeffrey A. Ledbetter (Seattle, Washington) for the generous gift of antibodies to CD18 (60.3), CD4 (G17-2), and CD28 (9.3). We gratefully thank Gitte Kølander for technical assistance and Anne-Mette Borre Carlsen for making the figures. This work was supported in part by The Danish Research Councils, Danish Biotechnological Center for Cellular Communication, Danish Allergy Research Center, Novo Nordic Foundation, Becketts Fond, the Danish Medical Associations Research Foundation, The Danish Cancer Research Foundation (Dansk Kræftsforsknings Fond), The Danish Cancer Society (Kræftens Bekæmpelse), Købmand i Odense Johann og Hanne Weimann født Seedroff's Legat, and the Bristol Myers–Squibb Pharmaceutical Research Institute.

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