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The Src-Protein Tyrosine Kinase Lck Is Required for IL-1-Mediated Costimulatory Signaling in Th2 Cells

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Src-protein tyrosine kinases are intimately involved in TCR-initiated signaling in T lymphocytes. One member of this family, Lck, is also involved in CD28-mediated costimulation in Th1 cells. In Th2 lymphocytes, the costimulatory signal can also be provided by the interaction of IL-1 with type I IL-1R (IL-1RI), culminating in the activation of NF-κB transcription factors. Proximal steps in the IL-1R pathway, however, remain poorly understood, and there is conflicting evidence as to the importance of tyrosine phosphorylation in IL-1R signaling. We have addressed this issue by examining the IL-1 costimulatory pathway in Lck-deficient Th2 cells. Our data demonstrate that, in the absence of Lck, the IL-1 costimulatory pathway is blocked despite the expression of normal levels of IL-1RI. Moreover, the block is associated with a defective degradation of IκBα and an incomplete activation of NF-κB heterodimeric complexes. Protein expression of NF-κB monomers, including p50, p65, and c-Rel, is equivalent in both wild-type and Lck-deficient Th2 cell clones. Finally, we demonstrate that, in normal Th2 cells, stimulation with IL-1 leads to a rapid induction in tyrosine phosphorylation of several substrates including Lck itself. These findings strongly suggest that Lck is required for signaling in the IL-1 costimulatory pathway in Th2 lymphocytes. The Journal of Immunology, 2001, 167: 6827–6833.

Optimal activation of T lymphocytes requires two independent signals. The first is mediated via the TCR following engagement with appropriate peptide-MHC ligands, while the second is delivered through one of a number of costimulatory, T cell surface-expressed, molecules upon binding to their respective ligands on APCs. The functional importance of the different costimulatory molecules appears to vary from one cell type to another. The best understood costimulatory pathway in T cells is the CD28/B7 pathway, which is used for optimal activation of naive T lymphocytes, CD8+ T cells, and cloned Th1 and Th2 cells (1). In addition, Th2 cells can uniquely utilize another costimulatory pathway mediated by the interaction of IL-1 with surface-expressed type I IL-1R (IL-1RI) (2). The exact role of costimulatory signals in the activation of T cells is beginning to be better understood. In naive CD4+ and CD8+ T cells, as well as in Th1 cells, the primary function of the CD28 costimulatory signal is to initiate and stabilize IL-2 transcription (3). In Th2 cells, however, IL-4 gene transcription occurs in the absence of costimulatory signals (2, 4). Evidence to date suggests that the role of costimulatory signals in Th2 cells, whether delivered by anti-CD28 stimulation or through the IL-1R, is to induce responsiveness to IL-4 (5). Furthermore, the costimulatory signal mediated via the CD28 pathway appears to function by inducing the production of IL-1α by Th2 cells, which in turn act in an autocrine fashion to facilitate their proliferation in response to IL-4. Alternatively, IL-1 signaling was recently proposed whereby costimulation of Th2 cells via TCR and IL-1 lead to the production of endogenous IL-1α, which in turn is used by Th2 cells as an autocrine growth factor in an IL-4-independent fashion (6).

Major advances in the identification of the components of the IL-1R signaling pathway have been made in the last few years (for reviews, see Refs. 7–10). The main effectors of this pathway are a group of proteins that belong to the NF-κB family of transcription factors. In resting cells, NF-κB is inactive and sequestered in the cytoplasm as a heterodimer comprising two polypeptides of 50 kDa (p50) and 65 kDa (p65), which are noncovalently associated with the inhibitory IκB proteins, including IκBα. Triggering of the IL-1R leads to the phosphorylation of IκBα on two specific serine residues, an event that signals its ubiquitination and degradation. The released NF-κB protein is then able to translocate to the nucleus and bind to specific DNA regions within the promoter of many genes involved in immune and inflammatory responses.

The serine-threonine kinase capable of phosphorylating IκBα was cloned and designated IκB kinase (IKK). This kinase comprises a large molecular mass complex of ~700 kDa, the components of which are not all yet identified (11–13). To date, the complex has been shown to contain at least three subunits: IKK-α, IKK-β, and IKK-γ (14–17). IKK-α and IKK-β act as the catalytic subunits of the serine-threonine kinase, whereas IKK-γ is thought to function as a regulatory subunit. Little is known about the way the activity of the three subunits, or indeed of the larger complex as a whole, is regulated. One aspect that has remained controversial is the potential role of tyrosine phosphorylation in the activation of any of the components of the IL-1R pathway. A previous study by the authors of this paper has shown that, in the absence of Lck, the IL-1 costimulatory pathway is blocked despite the expression of normal levels of IL-1RI. Moreover, the block is associated with a defective degradation of IκBα and an incomplete activation of NF-κB heterodimeric complexes. Protein expression of NF-κB monomers, including p50, p65, and c-Rel, is equivalent in both wild-type and Lck-deficient Th2 cell clones. Finally, we demonstrate that, in normal Th2 cells, stimulation with IL-1 leads to a rapid induction in tyrosine phosphorylation of several substrates including Lck itself. These findings strongly suggest that Lck is required for signaling in the IL-1 costimulatory pathway in Th2 lymphocytes.
study showed that several substrates were tyrosine phosphorylated following IL-1R triggering (18). Additionally, another study showed that tyrosine phosphorylation of IκB-α can lead to NF-κB activation (19). Given that many of the components of the large kinase complex are yet to be identified, it is possible that a tyrosine kinase may be part of this complex. Alternatively, tyrosine phosphorylation may be required for the activation of one or more components of the kinase complex.

To directly assess the requirement for tyrosine phosphorylation in T lymphocyte costimulatory pathways, we used stable T cell transfectants of a Th2 clone in which Lck expression was inhibited by antisense RNA (20, 21). The findings reported here demonstrate that in normal Th2 cells, Lck is tyrosine phosphorylated in response to IL-1 stimulation. Consequently, Lck-deficient Th2 cells are unresponsive to IL-1 stimulation. Moreover, this unresponsiveness appears to correlate with the inability to degrade IκB-α and fully activate NF-κB heterodimeric complexes.

Materials and Methods

Cell lines and immunologic reagents

The parental Th2 clone D10.G4.1 (hereafter referred to as D10) expresses a αβ TCR which specifically recognizes a peptide derived from conalbumin in the context of the murine I-A^k MHC class II molecule (22). Lck-deficient transfectants of D10 were derived by transfecting a vector containing a 400-bp fragment of murine Lck cloned in the antisense orientation. Expression of the antisense RNA is under the control of SV40 promoter and human T cell leukemia virus 1 long terminal repeat enhancer. One of the transfectants, known as D8, which had been extensively characterized previously (20), was used for the present studies (for the sake of clarity, these cells will thereafter be referred to as Lck-deficient T cells). Lck-deficient T cells were maintained by biweekly stimulation with cognate Ag plus syngeneic feeder cells in the presence of IL-1, IL-2, and IL-4 lymphokines. For all experiments, parental as well as Lck-deficient T cells were used in the resting phase at least 10–14 days after the last round of activation. Reconstituent human IL-1α was purchased from Genzyme (Cambridge, MA); mouse rIL-2 and rIL-4 were obtained from BioSource International (Camarillo, CA). The following mAbs were used: H57-597, anti-TCR Cβ (23); 11B11, anti-IL-4 (24); and S4B6, anti-IL-2 (25). All mAbs were affinity purified from hybridoma culture supernatants on protein G-Sepharose (Pharmacia Biotech, Piscataway, NJ).

Costimulation assay

To prepare Ab-coated plates, wells of flat-bottom microtiter plates received 50 μl of H57-597 mAb diluted in PBS to a concentration of 3 μg/ml. After incubation for 2 h at 22°C, the plates were blocked for 30 min with 5% FCS-PBS and washed three times before addition of T cells. Resting, viable T cells were isolated from maintenance cultures and activated at 10^6 cells/well in Ab-coated plates in the presence or absence of graded concentrations of human rIL-1α, as indicated. In some assays, neutralizing Abs specific to IL-2 or IL-4 were also added to some cultures at a final concentration of 20 μg/ml. Cell proliferation was assessed after a 72-h incubation period following a pulse with 1 μCi of [3H]thymidine for the last 18 h of culture.

Flow cytometry

The level of expression of lymphokine receptors for IL-1, IL-2, and IL-4 was determined by flow cytometry using a three-step staining procedure. Viable T cells (1 × 10^6 cells/sample) were stained with first-step mAbs on ice for 30 min. The first-step mAbs used were PC61, rat IgG1 specific to IL-2Rα chain (26), anti-CD121α, rat IgG1 specific to mouse IL-1R type I (BD Pharmingen, San Diego, CA), or anti-CD121α, rat IgG2a specific to mouse IL-4R (Genzyme). For positive control staining, cells were stained with YC3-D1 mAb specific to CD3ε (27). As a negative control, TR310 mAb, rat IgG2b specific to TCR Vβ7 (28) was also used (note that D10 cells are Vβ8.2^+). For second-step staining, biotin-conjugated goat anti-rat IgG Ab was used for 30 min on ice. In the third step, cells were stained with PE-conjugated streptavidin (Southern Biotechnology Associates, Birmingham, AL) also for 30 min on ice. After extensive washing, cells were analyzed on a FACSort (BD Biosciences, Mountain View, CA). Data collected from 15,000 cells were analyzed using CellQuest software.

Results

The engagement of the TCR on CD4^+ T cells by MHC class II-peptide ligands can be mimicked by the use of plate-bound anti-TCR Abs. To study the ability of IL-1 to costimulate Th2 proliferation, cells were activated in microrotater wells coated with a suboptimal concentration of TCR Cβ-specific mAb, H57-597, in the presence or absence of variable concentrations of rIL-1α. As shown in Fig. 1A, IL-1α costimulated a strong, dose-dependent proliferative response in wild-type D10 cells. The fold increase in the proliferative response of T cells was 3.5× and 9.3× in the presence of 20 and 200 pg/ml IL-1α, respectively. The addition of IL-1α alone to D10 cells failed to induce any proliferation (Fig. 1A). The ability of IL-1α to costimulate D10 cell proliferation was dependent on endogenously produced IL-4 since the addition of anti-IL-4 mAb almost completely abrogated IL-1 costimulatory activity. In sharp contrast, the combination of immobilized anti-TCR mAb and rIL-1α failed to induce any significant proliferation of Lck-deficient D10 cells (Fig. 1B). The response of both cell lines to stimulation by IL-2 was also evaluated. Normal D10 cells proliferated strongly when stimulated with IL-2 alone, even when anti-IL-4 mAb was also present in the culture (Fig. 1C). It is important to note that the response was almost identical whether anti-IL-4 mAb was present or absent (data not shown). Lck-deficient D10 cells also proliferated in response to IL-2, but at a much reduced rate. When stimulated with 1 or 10 U/ml IL-2, the response of Lck-deficient D10 cells represented only 52 or 28% of the response of normal D10 cells, respectively (Fig. 1C). Moreover, increasing IL-2 concentrations (from 0.1 to 10 U/ml) led to a proportional increase in the proliferative response of D10 cells. In contrast, the response of Lck-deficient D10 cells reached a plateau at 1 U/ml, with no further increase being observed upon stimulation with 10 U/ml IL-2 (Fig. 1C). A rather different picture...
emerged when the cell proliferative response was evaluated upon stimulation with IL-4. In this study, Lck-deficient cells consistently exhibited a stronger proliferative response than normal D10 cells (Fig. 1D). In this case, the response of wild-type D10 cells represented 24 or 48% of Lck-deficient cells when stimulated with 20 or 200 U/ml IL-4, respectively (Fig. 1D). In addition, Lck-deficient cells appeared to have a lower threshold of activation than normal D10 cells, as can be seen from their significant proliferative response when stimulated with IL-4 at 2.0 U/ml. Taken together, these results suggest that Lck-deficient cells are refractory to IL-1-mediated costimulation. Furthermore, however, Lck-deficient cells are perfectly capable of responding to lymphokine stimulatory signals, in particular IL-4, demonstrating that these cells do not have a generalized growth defect.

We next investigated whether the differential effects of IL-1 and other growth factors on parental and Lck-deficient D10 cells are related to changes in the level of expression of lymphokine receptors. Fig. 2 shows the results of staining normal (A–D) and Lck-deficient (E–H) D10 cell lines with mAbs specific to IL-1RI (B and F), IL-4R (C and G), IL-2R (D and H), or, as a control, CD3ε (A and E). The results demonstrate that all three lymphokine receptors are expressed on Lck-deficient cells at either comparable (IL-1R), or increased (IL-2R and IL-4R), levels compared with the parent D10 line. The higher expression of IL-2R and IL-4R on Lck-deficient cells is most likely due to the continuous culture of the cells in the presence of these two lymphokines (20). Thus, the refractory response of Lck-deficient cells to IL-1 is not a consequence of altered expression of IL-1RI.

Since costimulatory signal-driven proliferation of T cells is dependent on an intact TCR signaling pathway and given that the latter was previously shown to be partially defective in Lck-deficient cells (20), we sought direct evidence for a block in the IL-1 signaling pathway. As mentioned above, one of the crucial steps in the IL-1 signaling pathway is the serine phosphorylation, ubiquitination, and degradation of IκB-α, thereby allowing NF-κB complexes to translocate to the nucleus. To determine the integrity of the IL-1 pathway, normal as well as Lck-deficient T cells were activated with IL-1 and whole-cell extracts were then analyzed for the expression of IκB-α. As can be seen (Fig. 3), significant levels of IκB-α were observed in resting cells (both parental and Lck-deficient cells). After 30 min of IL-1 stimulation, IκB-α was degraded in D10 cells to an almost undetectable level. The IκB-α protein returned to normal levels by 60 min, presumably due to de novo protein synthesis, as has been described elsewhere (31). This pattern of IκB-α degradation in response to appropriate stimuli is entirely consistent with previous studies (31). Surprisingly, however, no evidence of IκB-α degradation was observed in Lck-deficient T cells (Fig. 3). The lack of IL-1-induced IκB-α degradation is not due to a change in kinetics since no alteration in IκB-α level

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**FIGURE 1.** IL-1 can costimulate the proliferation of Th2 cells via an IL-4-dependent pathway in Lck-expressing but not Lck-deficient T cells. Parental D10 (A) and Lck-deficient (B) T cells were activated with a suboptimal concentration of immobilized anti-TCR mAb (3 μg/ml) in the absence or presence of the indicated concentrations of rIL-1α (▲). Parallel sets of cultures were set up containing 20 μg/ml of a neutralizing anti-IL-4 mAb (○). The dose-response curves of cells stimulated with IL-1 only (in the absence of anti-TCR mAb) are also shown (●). As controls, parental and Lck-de cient (▲) and Lck-deficient (●) D10 cell lines with mAbs specific to IL-1RI (B and F), IL-4R (C and G), IL-2R (D and H), or, as a control, CD3ε (A and E). The results demonstrate that all three lymphokine receptors are expressed on Lck-deficient cells at either comparable (IL-1R), or increased (IL-2R and IL-4R), levels compared with the parent D10 line. The higher expression of IL-2R and IL-4R on Lck-deficient cells is most likely due to the continuous culture of the cells in the presence of these two lymphokines (20). Thus, the refractory response of Lck-deficient cells to IL-1 is not a consequence of altered expression of IL-1RI.

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was seen up 3.5 h following IL-1 treatment in Lck-deficient T cells (Fig. 3 and data not shown).

To further study the consequences of Lck deficiency on IL-1 signaling, we analyzed the induction of DNA-binding activity of NF-κB complexes. Stimulation of D10 cells with IL-1 leads to the activation of two main NF-κB complexes (Fig. 4A, complexes N1 and N2). Moreover, activation of these complexes appears to occur with slightly different kinetics. Thus, whereas the DNA-binding activity of the N2 complex is apparent at 30 min and increases in strength after 2 h of IL-1 stimulation, the activity of the N1 complex is maximal at 30 min of stimulation and is weaker at 2 h. Surprisingly, IL-1 treatment of Lck-deficient cells led to the specific activation of the N1 complex only. No evidence for the activation of the N2 complex was observed in Lck-deficient T cells (Fig. 4, A and B). To determine the composition of the NF-κB complexes, EMSA was conducted in the presence of Abs specific to p65 and p50 proteins (Fig. 4B). As shown, anti-p65 Abs supershifted the N1 complex completely but had no effect on the N2 complex. In contrast, addition of anti-p50 Ab decreased the binding activity of both complexes and led to the appearance of a new supershifted band (Fig. 4B). Based on these studies, we conclude that the N1 complex corresponds to the p65/p50 heterodimer, while the N2 complex contains p50/p50 homodimer. Interestingly, however, the N2 complex was not supershifted completely by the anti-p50 Ab, suggesting the presence of other NF-κB dimers in that complex. Attempts to supershift the complex with anti-c-Rel Ab have so far been unsuccessful (data not shown).

The differential effect of Lck deficiency on the activation of NF-κB complexes appears to be independent of protein levels of the various monomers. Western blotting of cell extracts using Abs specific to p50, p65, or c-Rel demonstrated that both cell lines express equivalent levels of proteins (Fig. 4C; data not shown). To further confirm the specificity of the observed DNA-binding complexes, an oligonucleotide competition experiment was undertaken. The results demonstrate that both N1 and N2 complexes can be competed specifically by known NF-κB-binding oligonucleotides (Fig. 4D, lanes 3, 4, 10, and 11) but not by unrelated (lanes 8 and 9) or mutated (lanes 5 and 6) oligonucleotides. Interestingly, the fastest migrating band, which is prominent in resting (Fig. 4D, lane 1) as well as IL-1-stimulated (lanes 2 and 7) D10 cells, can be competed by oligonucleotides containing a mutation in the NF-κB motif (lanes 5 and 6), suggesting that this band is most likely in a different family of transcription factors. Taken together, these data strongly indicate that Lck is required for a full activation of IL-1-stimulated NF-κB dimeric complexes.

The above data demonstrate that IL-1 signaling is defective in Lck-deficient Th2 cells. However, how and where in the pathway Lck is involved remains unknown. To shed some light on this, wild-type D10 cells were stimulated with IL-1 and cell extracts were analyzed for any evidence of IL-1-induced tyrosine phosphorylation. As shown in Fig. 5A, IL-1 triggering of Th2 cells led to a significant increase in the tyrosine phosphorylation of at least four protein bands running at a molecular mass of 38, 56, 59, and 116 kDa. The magnitude and kinetics of tyrosine phosphorylation of these bands appear to vary significantly. The most prominent of these bands was the 56-kDa protein which was of great interest to us since this could potentially represent Lck. To further identify this protein, cell extracts were prepared following IL-1 stimulation of D10 cells and immunoprecipitated with a mAb to Lck. The precipitated protein was then run on a 12% gel and blotted with anti-phosphotyrosine mAb. The results show that Lck is indeed tyrosine phosphorylated in response to stimulation by IL-1 (Fig. 5B, middle lane). No Lck band was seen in untreated cells (Fig. 5B, left lane) or in cells treated with a 10× lower concentration of IL-1 (right lane). We conclude that stimulation of cloned Th2 cells with IL-1 can induce the tyrosine phosphorylation of several protein substrates, including Lck. Attempts to determine the IL-1-induced tyrosine phosphorylation pattern in Lck-deficient cells were unsuccessful. This is mainly due to the fact that, in our hands, IL-1-induced phosphorylation could readily be observed when T cells were rested by an overnight incubation in the absence of any growth factors. Such treatment of Lck-deficient cells routinely resulted in a significant degree of death due to their heightened susceptibility to apoptosis, as previously reported (21). Whether Lck is responsible for the observed tyrosine phosphorylation, however, remains to be demonstrated. Whatever the mechanism, our data support a role for Lck in the IL-1 signaling pathway and demonstrate an IL-1-dependent increase in tyrosine phosphorylation in treated Th2 cells.
Discussion

The present study demonstrates a novel role for the tyrosine kinase Lck in signaling through the IL-1RI. Th2 cells in which Lck expression has been reduced by >95% exhibit a profound loss of responsiveness to IL-1 stimulation. Evidence is presented that this nonresponsiveness is associated with a defective degradation of IkB-α and, consequently, inhibition of NF-κB activation. Moreover, our study demonstrates that Lck itself is tyrosine phosphorylated in response to IL-1 stimulation in Th2 cells, strongly indicating that Lck may be one of the components of the IL-1RI signaling pathway.

NF-κB is a master transcription factor with key involvement in the control of cellular response to proinflammatory stimuli. The main pathway through which NF-κB is activated involves the induced phosphorylation of two serine residues in the N-terminal region of IkB proteins. In the case of IkB-α, this event is catalyzed by the serine/threonine IKK enzyme complex (11–13). Alternatively, recent evidence had shown that NF-κB can be activated in response to hypoxia and reoxygenation in Jurkat T cells by the phosphorylation of IkB-α on tyrosine 42 (19). This modification, which has been shown to involve Lck and phosphoinositide 3-kinase, does not lead to ubiquitination and degradation of IkB-α but rather induces the dissociation of IkB-α from NF-κB, allowing the latter to translocate into the nucleus (19, 32). Interestingly, this pathway can also be readily activated by treatment with pervanadate, a potent inhibitor of tyrosine phosphatases (19). The present study demonstrates that degradation of IkB-α in response to IL-1 stimulation is also inhibited in Lck-deficient cells. The tyrosine phosphorylation status of IkB-α following IL-1 treatment in our cell lines has not yet been examined. It would also be interesting to test the response of our cell lines to pervanadate treatment. Taken together, experimental evidence suggests the involvement of Lck in both pathways of NF-κB activation in T cells.

The molecular mechanism for the role of Lck in the IL-1 signaling pathway is not understood. We have shown that IkB-α is not properly degraded in Lck-deficient cells following IL-1 stimulation. This points to a dysfunction in the IKK kinase complex, influencing either the activity of IKK itself or one of the hitherto uncharacterized constituents of the large complex. Experimental evidence for the former possibility was recently provided by You et al. (33) who demonstrated that Shp-2, an Src homology 2-containing phosphotyrosine phosphatase, is associated with the IKK complex. A functional Shp-2 was shown to be required for the efficient serine phosphorylation of IkB-α by the IKK complex in response to stimulation by proinflammatory cytokines such as IL-1 and TNF-α. Together with our present findings, these results suggest that tyrosine phosphorylation/dephosphorylation events are essential for proper signaling through the IL-1RI complex.

Interestingly, the defect in Lck expression resulted in a differential effect on the activation of the various NF-κB complexes. The most pronounced effect, observed in Lck-deficient cells, was on the defective formation of N2 complexes following stimulation with IL-1. The N2 complexes most likely consist of a mixture of p50/p50 homodimers as well as other yet to be identified p50-containing heterodimers. In contrast, the prototypic p50/p65 heterodimers could still be activated, albeit at moderately decreased
levels, in Lck-deficient cells. The fact that this was observed in the absence of efficient IκB-α degradation suggests that 1) a small degree of IκB-α degradation is taking place, which accounts for the observed p50/p65 activity; or 2) other IκB inhibitor proteins may be selectively degraded in the absence of Lck, allowing for the activation of p50/p65 heterodimers. For example, a specific and nonoverlapping role has been previously described for IκB-β in the activation of NF-κB (31). In that study, IκB-β was shown to be specifically activated by a select group of inducers, including IL-1 and LPS, and resulted in persistent activation of NF-κB (31). In contrast, other proinflammatory stimuli, such as TNF-α, induced the degradation of IκB-α, but not IκB-β, leading to the commonly observed transient activation of NF-κB dimers. Thus, it is reasonable to propose that IL-1 could induce the observed p50/p65 dimers via the degradation of IκB-β. Implicit in this argument is the suggestion that the activities of IκB-β and IκB-α may be regulated by slightly different mechanisms, inasmuch as Lck is required for the latter but not the former. At present, no data are available in support of this contention. Previous studies demonstrated that IκB-α regulated NF-κB activity via an autoregulatory feedback loop (reviewed in Ref. 34). NF-κB stimulatory signals achieve their effect through the induced degradation of IκB-α. The active NF-κB dimers can then up-regulate the expression of de novo IκB-α mRNA due to the presence of NF-κB sites in the IκB-α promoter. Replenishment of the IκB-α pool prevents further translocation of NF-κB dimers, effectively shutting down the NF-κB response and ensuring that the responsive genes are activated only transiently. This negative feedback loop is not operative in the control of IκB-β activation since IκB-β is unique in being the only species of IκB whose expression is not regulated by NF-κB. Indeed, the importance of IκB-β activation was highlighted by studies that demonstrated that activation of T cells via the CD28 costimulatory molecule led to a rapid, and selective, degradation of IκB-β and persistent activation of NF-κB (35).

The present data provide strong direct evidence as to the importance of Lck in the IL-2R signaling pathway. Lck-deficient T cells exhibited a profoundly lower capacity to proliferate in response to IL-2. This was seen despite a demonstrated ~5-fold increase in IL-2R expression on Lck-deficient cells when compared with parental D10 cells. The reduction in IL-2-induced cellular proliferation of Lck-deficient cells is most likely a consequence of the noncovalent association of Lck with the β-chain of the IL-2R (36, 37). Furthermore, the role of tyrosine kinases, such as Lck, in the phosphorylation and activation of Janus kinase 1 and Janus kinase 3 kinases, integral components of the IL-2 signaling pathway, is incompletely understood (38). In sharp contrast, IL-4 signaling is unaffected in Lck-deficient D10 cells, demonstrating that the observed effect is specific to the IL-2R pathway. In fact, IL-4-induced proliferation was significantly stronger in Lck-deficient in comparison to normal cells, presumably a consequence of increased surface expression of IL-4R on the former cells. It is interesting to note that, unlike IL-2R, the IL-4R complex consists of only two chains (α and common γ-chain) and lacks a β-chain. Furthermore, no association between any of the T cell-expressed Src-tyrosine kinases and the IL-4R complex has been reported (39). Given that Lck deficiency led to a partial reduction in IL-2R-induced cellular proliferation, our findings indicate that the association between the IL-2R β-chain and Lck is of physiological importance and plays a complementary role in IL-2R-mediated signaling.

In conclusion, our findings demonstrate that a deficiency in the expression of the Src-protein tyrosine kinase Lck in Th2 cells has a profound effect on the IL-1 signaling pathway. The delivery of IL-1 costimulatory signal to Lck-deficient Th2 cells was completely blocked. This was associated with (1) lack of IκB-α degradation and (2) differential activation of NF-κB complexes. Moreover, evidence is presented that Lck itself is tyrosine phosphorylated in response to IL-1 stimulation. Our data provide evidence for an important role for Lck in the IL-1R signaling pathway in T lymphocytes. Whether Lck kinase activity is required for this pathway, or Lck is acting in a kinase-independent manner (40), remains to be determined. Finally, the data suggest that activation of p50-p50 complexes is dependent on the specific degrada- tion of IκB-α, whereas p50-p65 complexes can be activated independently of IκB-α, perhaps through the degradation of other inhibitory proteins, such as IκB-β (31).

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