Ligand-induced Desensitization of Interleukin 1 Receptor–initiated Intracellular Signaling Events in T Helper Lymphocytes

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Summary

Although interleukin 1 (IL-1) receptor signaling events in T helper lymphocytes are incompletely characterized, events associated with translocation of the transcription factor NF-κB are receptor-proximal assays of ligand-initiated responses. In this report we demonstrate that the transient nature of IL-1–induced NF-κB nuclear translocation occurs as a consequence of ligand-induced receptor desensitization. Other receptor-initiated events including induction of IκBα phosphorylation, expression of c-jun and junB mRNA, and costimulatory effects on IL-2 synthesis also are altered by IL-1 receptor desensitization. IL-1 receptor desensitization is not initiated by tumor necrosis factor, which also stimulates NF-κB translocation, and is not a consequence of alterations in either IL-1 receptor expression or binding affinity. In the absence of IL-1, the effects of desensitization are completely reversed within 18 h. Since IL-1 desensitization is initiated under conditions of low receptor occupancy, it is likely that receptor desensitization results from alterations to a receptor-proximal transducer, rather than from direct modification of the IL-1 receptor. These results suggest that the cyclic nature of the events in the T helper lymphocyte activation program can be controlled, in part, by the reversible desensitization of cell surface IL-1 receptors.

IL-1 receptor- and TCR-initiated intracellular signals have been shown to interact synergistically to increase the expression of multiple lymphokine and lymphokine receptor gene products during the competence phase of the T helper activation program (1–5). The proliferative component of the T cell activation program is augmented because of the increased production of lymphokines (IL-2 or -4) binding in an autocrine or paracrine manner to high affinity lymphokine receptors. Although T lymphocytes express two IL-1 receptor isoforms (types I and II), biological responses elicited by IL-1 are initiated as a consequence of ligation of only type I IL-1 receptors (6–8).

In contrast to the large body of information that has accumulated about TCR-initiated second messenger signaling events, the intracellular events initiated by IL-1 receptor ligation are comparatively ill-defined. The most receptor-proximal events resulting from IL-1 receptor ligation in T lymphocytes are associated with the cytosolic to nuclear translocation of the transcription factor, NF-κB (9). NF-κB can be detected in the nucleus 5–10 min after stimulation by IL-1 or other receptor-initiated events or pharmacologic agents. NF-κB nuclear translocation occurs as a consequence of the NF-κB complex dissociating from its cytosolic inhibitory protein, IκB (10–12). This dissociation is temporally preceded by phosphorylation of IκBα (13–15). However, it is unclear whether the inducible phosphorylation of IκB affects IκB–NF-κB dissociation or the subsequent degradation of dissociated IκB.

The principle transactivating form of NF-κB in T cells, which is comprised of a p50-p65 heterodimer, binds to specific DNA target sequences in the enhancer of a variety of genes (16). In T lymphocytes, NF-κB has been shown to be an important regulator of genes including lymphokines (IL-2 and GM-CSF) (17, 18), the IL-2 receptor α chain (19, 16), and the HIV LTR (20–22). The lymphokine genes are rapidly turned on during the initial phase of the T cell activation program and are subsequently turned off as the cells progress through the later stages of the activation program. Thus, receptor-initiated events that regulate the T cell activation program must have self-limiting control mechanisms. In this report we characterize the intracellular events associated with the transient nuclear translocation of NF-κB induced by IL-1. Results from these analyses demonstrate that in addition to rapidly inducing NF-κB nuclear translocation and the expression of several immediate early response genes, IL-1 also initiates desensitization of IL-1 receptor–initiated intracellular signaling events. The ligand-induced desensitization of IL-1 receptor signaling events likely results from an uncoupling of the IL-1 receptor from a receptor-proximal transducer by a NF-κB independent mechanism, rather than by alterations in receptor expression or binding affinity for IL-1.

Materials and Methods

Cells and Cell Culture. Jurkat T cells expressing the murine type I IL-1 receptor (Ju.1 cells) were produced by sorting cells trans-

fected with a type I IL-1 receptor cDNA-Rep 3 expression vector (Bankers-Fulbright, J., manuscript in preparation). LBRM 33 cells, derived from a murine T cell lymphoma, were obtained from the American Type Culture Collection (ATCC; Rockville, MD). Both cell lines were cultured in spinner flasks in medium containing RPMI 1640, 10% calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-Me, and 10 mM hepes, pH 7.3. Ju.1 cell culture medium also contained 400 μg/ml hygromycin.

CD4+ secondary T lymphocytes were prepared from BALB/c lymph node plus spleen cells that had been depleted of CD8+ T cells using magnetic column separation (biotinylated 53-6.72 [ATCC, TIB 105] plus strepavidin magnetic beads) (23). Cells were stimulated for 10-14 d with anti-CD3 antibody plus lymphokines (supernatant from Con A–treated rat splenocytes), and subsequently depleted of anti-CD8+ T cells, and class II– and Ig-positive cells using magnetic column separation. The resulting secondary cells, which were >98% CD4+ T lymphocytes by quantitative immunofluorescence analysis, were cultured for 24 h in rIL-2 (Hoffmann La Roche, Nutley, NJ) before experimental manipulation.

Antibodies and Reagents. Anti-IκBα (MAD-3) antibody was prepared by injecting rabbits with an IκBα (MAD-3) glutathione S-transferase (GST-IκB) fusion protein purified by affinity chromatography (24). Affinity-purified rabbit antibodies to IκBα (MAD-3) (sc-203), p50 (sc-114), and p65 (sc-109) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Unless otherwise noted, other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Human recombinant IL-1α was generously provided by Dr. John Sims (Immunex Corp., Seattle WA). The IL-1α had a sp act of 5.7 × 10⁵ U/μg protein (U = EC₅₀ value determined in LBRM 33 cell costimulus assay; 25). Unless noted otherwise, IL-1 was used at a concentration of 200 U/ml. Human rTNF-α (Genentech, Inc., South San Francisco, CA) was used at a concentration of 4 ng/ml. Biotinylated anti-CD3 mAb (ATCC CRL 8001; OKT3) (5-10 μg/ml) was complexed with avidin (10 μg/ml) before being added to cell preparations.

Phosphoprotein Characterization. ³²P-labeled Ju.1 cells were prepared by labeling cells with 0.5 mCi/ml ³²Pᵢ ([³²P]phosphoric acid; ICN Radiochemicals, Irvine, CA) for 3 h at 37°C in phosphate-free RPMI 1640 medium containing 5% dialyzed FCS, 2 mM L-glutamine, 50 μM 2-ME, and 10 mM Hepes, pH 7.3. Labeled cells were divided into aliquots (10⁷/sample) and, after stimulation for varying periods of time, were washed in ice-cold PBS wash buffer containing 400 μM Na₃VO₄, 5 mM EDTA, and 10 mM NaF, pH 7.4. The cells were solubilized for 30 min at 4°C in lysis buffer containing 0.5% Triton X-100, 50 mM Tris hydrochloride, 300 mM NaCl, 5 mM EDTA, 10 mM iodoacetamide, 1 mM sodium orthovanadate, 40 mM β-glycerol phosphate, 10 μg/ml leupeptin, and 10 μg/ml aprotinin, pH 7.6. After centrifugation, lysates were precleared with protein A–Sepharose, and then sequentially immunoprecipitated with normal rabbit IgG and with specific antibodies bound to protein A–Sepharose. Immune complex protein A–Sepharose beads were washed and subsequently eluted with SDS sample buffer. Immunoprecipitates were analyzed on 10% SDS PAGE as previously described (26). Immunoprecipitation experiments were performed a minimum of three times. ³²P-labeled proteins were detected by autoradiography at −70°C for 2-5 d.

Mobility Shift Assays. Nuclear extracts were prepared by the method of Dignam et al. (27), with the following exception: cells were disrupted by incubating in buffer A containing 0.02 or 0.1% NP-40 for 5 min at 4°C. Mobility shift reactions contained 6-15 μg nuclear extract, 0.5-3 μg (poly[dI-dC]) (Sigma Chemical Co.), and 15,000 cpm (0.02-0.5 ng) γ-[³²P]ATP (sp act 3,000 Ci/mmol) end

labeled, double stranded oligonucleotide in a final volume of 15 μl. Protein–DNA complexes were separated by PAGE using a 4.5% nondenaturing gel in a high-ionic strength buffer (40 mM Tris, 384 mM glycine, and 2 mM EDTA, pH 8.3). Specific binding of nuclear proteins to labeled DNA oligonucleotide probes was determined by competition with unlabeled DNA probes that were identical to the labeled probes, and by competition with unlabeled DNA probes that were unrelated to the labeled DNA probes (data not shown for the unrelated DNA probes). Each gel shift assay was done a minimum of three times. The DNA sequence of the IL-2 NF-κB oligonucleotide probe used in this study is as follows (5' to 3', coding strand only): CCCGACCAAGAGGGATTTCAC-CTAAATCCATT.

IL-2 Bioassay. IL-2 biologic activity was measured in culture supernatants isolated from Ju.1 cells (10⁵ cells/200 μl/microtiter well) that had been stimulated with various reagents for 24 h. The IL-2 bioassay using HT-2 cells has been described previously (28).

RNA Isolation and Analysis. Expression of immediate early gene c-jun and junB mRNA was measured by Northern blot analysis. Ju.1 cells were stimulated for 30 min and total cellular RNA was isolated by lysis of the cells in guanidium isothiocyanate solution (29), followed by cesium chloride centrifugation (30). The isolated RNA (30 μg/lane) was size fractionated by electrophoresis through a 1% agarose gel containing 2.2 M formaldehyde and was transferred to Hybond M membrane filters (Amersham Corp., Arlington Heights, IL). cDNA probes were radiolabeled with α[³²P]dCTP by random primer extension to a sp act of ∼10⁹ cpm/μg DNA. Hybridization was carried out at 42°C for 18 h in 50% formamide, 5× SSC (1× SSC: 0.15 M NaCl, 0.015 M trisodium citrate), 5× Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 50 μg/ml polyadenylic acid. High stringency posthybridization washes were performed at 23°C in 2× SSC, 0.1% SDS, and at 65°C in 0.1× SSC, 0.1% SDS. The amounts of RNA loaded on different lanes of each Northern blot were shown to be comparable by probing the blots with glyceraldehyde-3-phosphate dehydrogenase cDNA (1.85-kb Pst1 fragment from pIBI 30-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from Dr. S. Kang (Mount Sinai Medical Center, New York). The cDNA probes used include c-jun (1.8-kb EcoRI fragment) (31) and junB (1.8-kb EcoRI fragment) (32), both provided by Dr. D. Nathans (Johns Hopkins University, Baltimore, MD).

¹²⁵I-labeled IL-1 Binding Assays. Radioreceptor assays were performed as previously described (6) with ¹²⁵I-IL-1 that was generously provided by Drs. S. K. Dower and J. Sims (Immunex Corp.) or purchased from New England Nuclear Corp. (Boston, MA).

Results

Previous studies have demonstrated that IL-1 receptor ligation initiates NF-κB nuclear translocation (9). Gel shift analyses performed with type 1 IL-1 receptor transfected human Jurkat T cells (Ju.1 cells) (Fig. 1) and murine LBRM 33 cells (data not shown) have demonstrated that IL-1–initiated NF-κB nuclear translocation is detectable within 5-10 min and is maximal at 30-60 min (Fig. 1). Although cytosolic NF-κB is restored 1 h after IL-1 stimulation because of de novo NF-κB synthesis, preliminary experiments demonstrated that the continued presence of IL-1 does not stimulate nuclear translocation of this newly synthesized NF-κB (data not shown). Consequently, NF-κB DNA binding activity is minimal in nuclear extracts isolated from T cells that
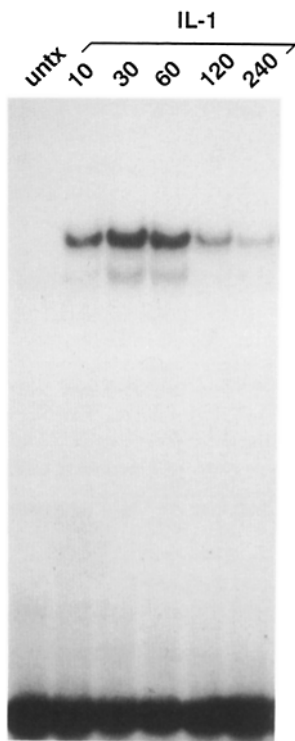
Figure 1. Kinetics of IL-1–induced NF-κB nuclear translocation. Nuclear extracts were prepared from Ju.1 cells stimulated with IL-1 for the indicated times (minutes). A ^{32}P-labeled DNA probe encompassing the NF-κB binding motif from the mouse Il-2 enhancer was incubated with the nuclear extracts and the protein–DNA complexes analyzed using electrophoretic mobility shift assay (EMSA)[1]. Preliminary experiments demonstrated that the upper band consists primarily of p65 and p50 polypeptides whereas the minor lower band consists of p50 polypeptides.
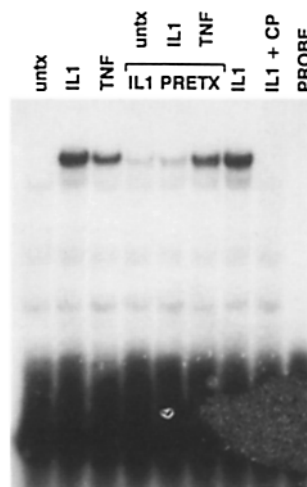

Figure 2. IL-1 pretreatment of Ju.1 cells initiates desensitization by homologous but not heterologous stimuli. Ju.1 cells were stimulated for 30 min with IL-1 or TNF after being cultured for 16 h in either medium or in medium containing IL-1. Nuclear extracts were prepared from the cells, incubated with a ^{32}P-labeled NF-κB probe, and the protein–DNA complexes were analyzed by EMSA. Samples in lanes 2 and 7 are duplicate samples using the same cell extract. IL-1 plus cold probe (IL-1 + CP) is the nuclear extract isolated from Ju.1 cells stimulated for 30 min (lanes 2 and 7 sample) incubated with the ^{32}P-labeled probe in the presence of excess unlabeled NF-κB probe. (*PROBE*) ^{32}P-labeled NF-κB probe only.

have been stimulated by IL-1 for 2–4 h (Fig. 1). These kinetic analyses of IL-1–initiated NF-κB DNA binding activity suggest that IL-1 receptor ligation initiates a rapid, but transient NF-κB nuclear translocation stimulus in T lymphocytes. To characterize the mechanism responsible for the transient IL-1–initiated NF-κB nuclear localization, we evaluated whether IL-1–pretreated cells could respond to homologous (IL-1) or heterologous (TNF-α) receptor-initiated signals with NF-κB nuclear translocation. Ju.1 cells were cultured with IL-1 for 16 h, washed, and then restimulated for 30 min with either Il-1 or TNF-α. Gel shift analysis (Fig. 2) demonstrated that TNF stimulates comparable levels of NF-κB translocation in either untreated or IL-1–pretreated Ju.1 cells. These results demonstrate that heterologous cell surface receptor-initiated signals can stimulate normal levels of NF-κB nuclear translocation in IL-1–pretreated cells. However, IL-1–induced NF-κB translocation is largely absent in cells pretreated with IL-1 for either 4 h (data not shown) or 16 h (Fig. 2) as compared with the IL-1–initiated response in cells that were not pretreated. Comparable results also have been obtained in gel shift analyses of extracts prepared from LBRM 33 cells (data not shown).

In this Ju.1 model system, signals initiated through the IL-1 receptor interact synergistically with antigen receptor-initiated signals to upregulate the production of IL-2. NF-κB is the predominant IL-1–responsive transcription factor in the IL-2 enhancer (Kalli, K., manuscript in preparation). If un-

coupling of the IL-1 receptor–induced NF-κB response occurs in vivo, we would expect IL-1 pretreatment to affect the IL-2 response elicited by the combination of anti-CD3 plus IL-1. Ju.1 cells were stimulated with varying concentrations of anti-CD3 antibody in the presence or absence of exogenous IL-1 (Fig. 3). In the Jurkat model system, anti-CD3 antibody treatment alone is insufficient to stimulate detectable IL-2 release. However, IL-2 production is increased in a concentration-dependent manner in response to stimulation by anti-CD3 and IL-1. In contrast, Ju.1 cells that have been pretreated with IL-1 do not produce a detectable IL-2 response when stimulated with anti-CD3 and IL-1.

One of the most receptor-proximal events detectable after stimulating Ju.1 cells with IL-1 is the phosphorylation of the cytosolic NF-κB inhibitor, IκBα (13). This ligand-induced phosphorylation has been temporally associated with NF-κB nuclear translocation (13, 14). IκBα is inducibly phosphorylated within 2 min after Il-1 or TNF addition (Fig. 4). SDS-PAGE analysis of anti-IκB immunoprecipitates isolated from ^{32}P-labeled, untreated Ju.1 cells shows a constitutively phosphorylated IκBα band (∼37 kD; bottom arrow in Fig. 4). In IL-1– or TNF-treated cells, the 37-kD IκBα band decreases in intensity, whereas a concomitant increase is observed in a slightly higher molecular weight band (∼40 kD) resulting from the inducible phosphorylation of the IκBα polypeptide (top arrow in Fig. 4). IL-1 pretreated Ju.1 cells have comparable levels of constitutively phosphorylated IκBα as untreated cells, and the addition of TNF to IL-1 pretreated cells results in an upshift of the phosphorylated IκBα band that is comparable to the TNF-induced phosphorylation observed in untreated Ju.1 cells. In contrast, stimulation of IL-1–pretreated cells with IL-1 fails to initiate detectable changes in either the constitutively phosphorylated or the inducibly phosphorylated IκBα band.

To determine if the inhibition of the IL-1 receptor desensitization is mediated by NF-κB, Ju.1 cells were pretreated

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[1] *Abbreviation used in this paper:* EMSA, electrophoretic mobility shift assay.
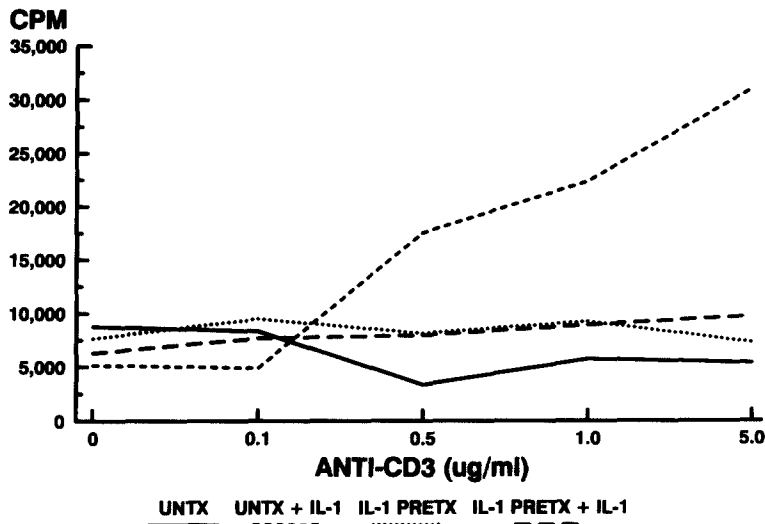
CPM

Figure 3. IL-2 responses elicited by anti-CD3 and IL-1 are inhibited by IL-1 pretreatment of Ju.1 cells. Ju.1 cells (untreated or pretreated with IL-1 overnight) were washed and then stimulated with varying concentrations of anti-CD3 in the presence or absence of IL-1 (10 U/ml). Supernatants were harvested after 24 h and IL-2 in the culture supernatants was quantitated using the IL-2–dependent cell line, HT-2 (40). Data points represent mean values from quadruplicate measurements of [³H]thymidine incorporation in the HT-2 bioassay.
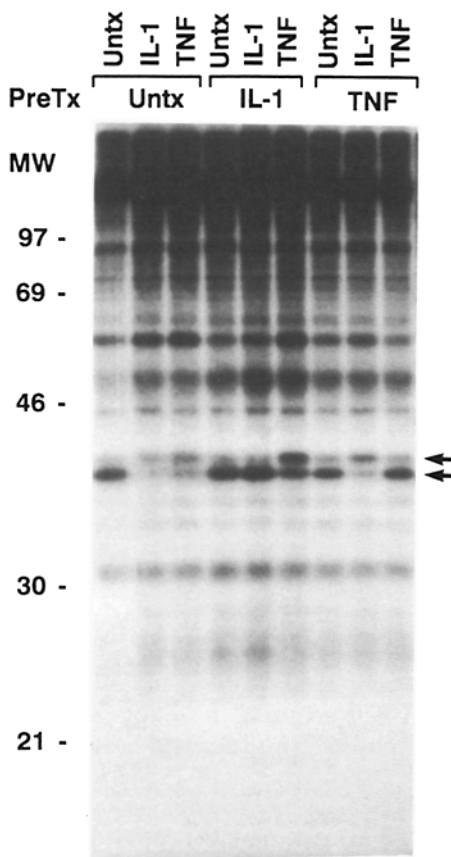

Figure 4. Homologous desensitization of receptor-initiated IκB phosphorylation. Untreated, IL-1 pretreated (18 h), or TNF pretreated (18 h) Ju.1 cells were loaded with [³²P]phosphoric acid, washed, and stimulated for 10 min with IL-1 or TNF. Detergent lysates of the cells were immunoprecipitated with anti-IκB, and the immunoprecipitates analyzed by SDS PAGE.

with TNF for 16 h and then restimulated with IL-1 or TNF (Fig. 4). Analysis of anti-IκB immunoprecipitates demonstrates that TNF-pretreated Ju.1 cells exhibit an IκB phosphorylation pattern similar to that observed in untreated cells. IL-1 inducibly phosphorylates IκB in the TNF-treated cells, but TNF does not. We have not pursued experiments to identify potential mechanisms responsible for the observed TNF-mediated effects. The experiments demonstrate that TNF receptor–initiated signals, which are capable of translocating NF-κB, do not induce IL-1 receptor desensitization. Collectively, the results demonstrate that Ju.1 cells that have been pretreated with IL-1 do not respond with inducible phosphorylation of IκB by homologous receptor–initiated signals but can be stimulated by heterologous receptor–initiated signals that are capable of inducing NF-κB nuclear translocation.

Northern blot analyses of c-Jun and junB mRNA were used to evaluate IL-1 receptor desensitization effects on activation responses that are not directly associated with NF-κB. The inducible expression of c-Jun and junB mRNA by IL-1 or tetradecanoylphorbol acetate (TPA) is readily detectable in Ju.1 cells 30 min after stimulation (Fig. 5). Although the regulatory mechanisms mediating IL-1 induction of these genes are incompletely understood, their transcriptional regulation appear to be independent of NF-κB (32, 33). Northern blot analysis demonstrated that TPA induces comparable levels of both c-Jun mRNA and junB mRNA in untreated and IL-1–pretreated Ju.1 cells. In contrast, although IL-1 induces the production of c-Jun and junB mRNA in untreated Ju.1 cells, it does not induce the production of c-Jun and junB mRNA in IL-1–pretreated cells (Fig. 5). These results suggest that IL-1 receptor–distal, NF-κB–independent responses are also refractory to homologous receptor–initiated signals but not to pharmacologic stimuli in cells pretreated with IL-1.

The selective loss of IL-1 responses in IL-1–pretreated Ju.1 cells could result from the absence of unoccupied cell surface IL-1 receptors at the end of the IL-1 pretreatment period. To comparatively evaluate the number of unoccupied IL-1 receptors on untreated and IL-1–pretreated Ju.1 cells, binding
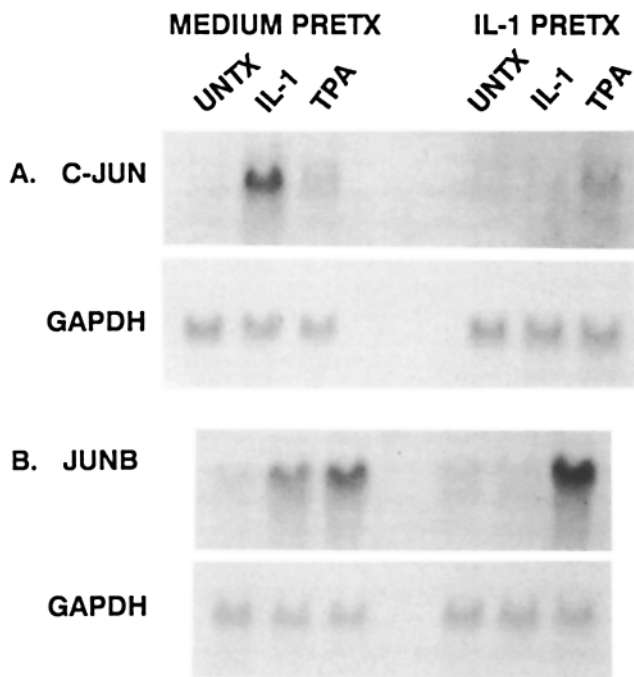
MEDIUM PRETX   IL-1 PRETX

UNTX IL-1 TPA   UNTX IL-1 TPA

A. C-JUN

GAPDH

B. JUNB

GAPDH

Figure 5. IL-1 pretreatment desensitizes Ju.1 cells to IL-1–initiated production of c-jun and junB mRNA. Two Northern blots were used to characterize the inducible expression of c-jun and junB mRNA in untreated or IL-1–pretreated Ju.1 cells that had been stimulated with IL-1 or TPA (20 ng/ml) for 30 min. Northern blots were probed sequentially with c-jun or junB and GAPDH cDNAs. Autoradiographs of the two Northern blots were exposed for comparable periods of time.

analyses were performed with ^{125}I-labeled IL-1. Results in Table 1 demonstrate that untreated Ju.1 cells express ∼4,900 unoccupied receptors per cell whereas IL-1–pretreated cells express ∼3,900 unoccupied receptors per cell. The K_d calculated from the IL-1 binding analyses on untreated and IL-1–pretreated cells were comparable to each other and to previously published values for type I IL-1 receptors (34). We and others (6, 35) have demonstrated previously that as few as 10 ligated IL-1 receptors per cell are needed to elicit a biologic response from T lymphocytes (6, 35). Thus, it is unlikely that the homologous desensitization initiated by IL-1 pretreatment of the Ju.1 cells results from the absence of unligated IL-1 receptors on the surface of these cells.

Receptor desensitization should be followed by a time-dependent recovery of IL-1–mediated function once excess IL-1 is removed from the T cell culture. To evaluate the kinetics of receptor recovery, IL-1–pretreated Ju.1 cells were

Table 1. *Unligated IL-1 Receptors on Ju.1 Cells Treated 18 h with rIL-1α*

Pretreatment	$K_d(\times 10^{-10})$	Sites/cell
Medium	8.65	4,866 ± 341
IL-1	8.40	3,898 ± 273

washed free of IL-1, and cultured for varying periods of time in medium before being restimulated for 30 min with IL-1. As shown in Fig. 6, a detectable increase in IL-1–initiated NF-κB translocation can be observed after 2 h of culture, with full functional recovery by 18 h. These results demonstrate that in IL-1–pretreated cells, IL-1 receptors recover their functional activity in a time-dependent manner.

We previously demonstrated that primary murine CD4+ T lymphocytes express no detectable IL-1 receptors and undergo no detectable IL-1–mediated biological responses (Podzorski, R., manuscript submitted for publication). However, secondary CD4+ T cell populations derived from stimulating lymph node/spleen–derived T cells with anti-CD3 antibody and lymphokines for 10–14 d express detectable levels of functional type I IL-1 receptors. Preliminary experiments demonstrate that IL-1–stimulated NF-κB nuclear translocation is maximal in nuclear extracts from cells stimulated with IL-1 for 15 min, and is reduced to near background in cells stimulated for 30 min (data not shown). To determine if IL-1–pretreated CD4+ secondary T lymphocytes are refractory to homologous receptor–initiated NF-κB translocation, secondary lymphocytes were cultured overnight in either the presence or absence of IL-1, washed, and then restimulated with IL-1 or TNF. The gel shift analysis in Fig. 7 shows that TNF stimulates NF-κB translocation in untreated and in IL-1–pretreated secondary CD4+ T lymphocytes. In contrast, IL-1 stimulates NF-κB nuclear translocation from untreated but not from IL-1–pretreated CD4+ T lymphocytes. The relatively small amount of NF-κB nuclear translocation observed in extracts from the IL-1–treated CD4+ T lymphocytes (as compared to the Ju.1 cells) probably results from the presence of IL-1 receptors on only a subset of the secondary T lymphocytes. Collectively, the results presented are consistent with the hypothesis that IL-1 receptor ligation elicits intracellular signals that stimulate the T cell activation pro-
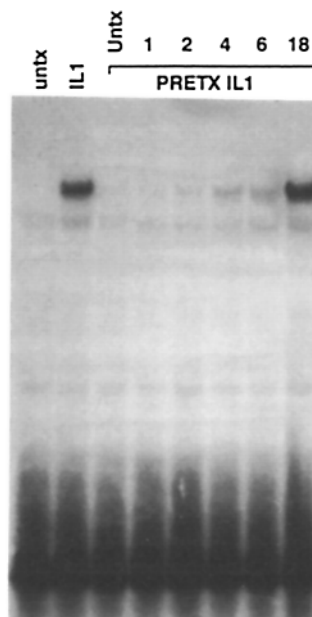

untx IL-1 Untx 1 2 4 6 18

PRETX IL1

Figure 6. Kinetics of the recovery of IL-1–inducible NF-κB from IL-1–desensitized Ju.1 cells. Ju.1 cells were pretreated for 18 h with IL-1, washed, and cultured for 1, 2, 4, 6, or 18 h. Cells were subsequently restimulated with IL-1 for 30 min and nuclear extracts from the cells were analyzed for NF-κB DNA binding activity by EMSA.
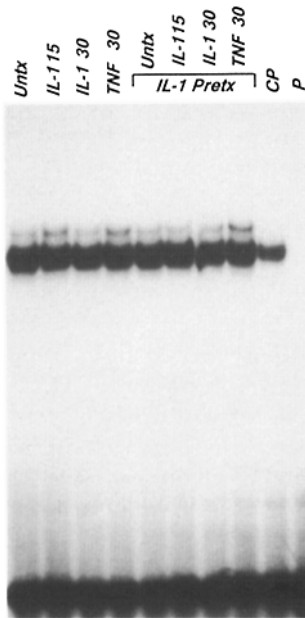
Figure 7. IL-1 pretreatment of CD4+ T lymphocytes inhibits homologous but not heterologous ligand-induced NF-κB nuclear localization. CD4+ T lymphocytes (cultured 18 h in the presence or absence of IL-1) were stimulated for the times indicated (minutes) with IL-1 or TNF, and nuclear extracts from the cells were analyzed for NF-κB DNA binding activity by EMSA. (CP) The 15-min IL-1 treated extract (lane 2) incubated with ^{32}P-labeled NF-κB probe plus unlabeled NF-κB probe. (P) ^{32}P-labeled NF-κB probe only.

gram as well as signals that uncouple the receptor from downstream signaling pathways.

Discussion

The T helper lymphocyte activation program consists of a coordinated series of gene activation and inactivation events that result in antigen receptor–stimulated T cells transiting through the competence (lymphokine and lymphokine receptor expression) and progression (lymphokine receptor mediated) phases of the cell cycle. Antigen receptor–initiated signals, which alone elicit suboptimal activation responses, usually require additional signals generated by IL-1 or other costimulatory receptors (CD28) to maximally initiate the T cell activation program. IL-1 receptor ligation rapidly induces the production of transcription factors that interact cooperatively with antigen receptor–activated transcription factors to stimulate gene expression events in the competence phase of the activation program.

Studies characterizing the IL-1 receptor–proximal events in human and murine T cell model systems have demonstrated that IL-1 initiates phosphorylation of IκBα (13) and dissociation of IκBα from NF-κB. The dissociated IκBα is rapidly degraded in the cytosol (13–15). The NF-κB complex translocates to the nucleus where it binds to target sequences and positively regulates a variety of gene enhancers, including IκBα (36) and the p105 precursor of the NF-κB p50 polypeptide (37). As a consequence of the IL-1–induced NF-κB nuclear translocation, IκBα and p105 polypeptide synthesis is induced and these proteins reappear in the cytosol 60 min before stimulation. Although IL-1 is a potent stimulus to initiate NF-κB nuclear translocation, gel shift (Fig. 1) and Western blot (data not shown) analyses demonstrate that NF-κB is largely gone from the nucleus 4 h after IL-1 has been added to the cells. Thus, the transient localization of IL-1–induced NF-κB DNA

binding activity in the nucleus of human Ju.1 and in murine LBRM 33 cells occurs at the same time that abundant levels of IκB–NF-κB complexes exist in the cytosol of IL-1–treated cells. The cytosolic NF-κB complexes in IL-1–pretreated cells were shown to be translocation competent by demonstrating that TNF induced NF-κB nuclear translocation (Fig. 2). These observations suggested that either cell surface IL-1 receptors on the IL-1–pretreated cells were not available for ligation, or that the IL-1 receptors were uncoupled from downstream signaling pathways.

The amount of IL-1α added to the Ju.1 cell cultures to elicit the desensitization response was calculated to ligate only a fraction of the cell surface receptors. Binding assays with ^{125}I-labeled IL-1 on untreated and IL-1–pretreated Ju.1 cells demonstrated that the IL-1–pretreated cells have, on average, more than 3,900 unligated receptors per cell (Table 1). In addition, the affinity of the available receptors was unaffected by the IL-1 pretreatment. Concentration–response analyses of murine CD4+ clones T_H2 (6) have confirmed a previous report (35) that as few as 10 type I IL-1 receptors need to be ligated to initiate 50% of the maximal biological responses from cells. Thus, it is clear that the availability of cell surface IL-1 receptors is not responsible for the desensitization phenomenon. Given the low level of receptor occupancy needed to elicit an IL-1 response in T cells, it is not surprising that a mechanism exists to desensitize IL-1 receptor responses after receptor ligation.

Uncoupling the IL-1 receptor from intracellular signaling pathways could occur by modification of one or more components of the signaling pathway or by a direct modification of the IL-1 receptor. The most IL-1 receptor–proximal events we have measured in T cells have been associated with NF-κB nuclear translocation. In addition to inhibiting homologous ligand–induced nuclear translocation of the NF-κB complex, phosphorylation of IκBα, which is detectable within 2 min after ligation of the receptor, also is blocked by IL-1 pretreatment (Fig. 4). Although multiple intracellular events have been associated with IL-1 receptor ligation in a variety of cell types, the signal transduction pathway linking IL-1 receptors to posttranslational modifications of IκB remains unidentified. TNF receptor ligation, which also stimulates IκB phosphorylation and NF-κB nuclear translocation, does not initiate IL-1 receptor desensitization. Thus, the IL-1 receptor–initiated signal responsible for the receptor desensitization is not NF-κB dependent. The synthesis of c-jun and junB mRNA, which are IL-1 receptor–distal, NF-κB–independent events, also is inhibited by IL-1 pretreatment (Fig. 5). Together these results demonstrate that IL-1 pretreatment of Ju.1 cells may inhibit multiple IL-1 receptor–initiated signaling events and, consequently, suggest that the uncoupling event is very proximal to the IL-1 receptor. The observation that IL-1 pretreatment of Ju.1 cells inhibits the production of IL-2 elicited by the synergistic stimuli of anti-CD3 plus IL-1 clearly demonstrates the biological relevance of IL-1 receptor desensitization.

The uncoupling of IL-1–initiated signals from downstream responses could result from a direct modification of the IL-1

receptor. One report indicated that IL-1 initiates phosphory- lation of the type I IL-1 receptor in COS cells (38). How- ever, we have been unable to reproduce these experiments in LBRM 33 cells (Abraham, R.T., and D.J. McKean, un- published results). Ju.1 cells and LBRM 33 cells each express approximately 5,000 IL-1 receptors per cell. Concentrations of IL-1 that result in maximal IL-1 receptor desensitization occupy only ~20% of the cell surface receptors. Since, as discussed above, there are a large number of spare IL-1 receptors in these T cell model systems, IL-1 pretreatment would have to affect essentially all of the Ju.1 IL-1 receptors to abrogate the IL-1–induced response. Thus, it is likely that the desen- sitization event occurs at the level of a receptor-proximal trans- ducer rather than by a direct posttranslational modification of the IL-1 receptor. This postreceptor desensitization event is an obvious potential target for therapeutically antagonizing IL-1 effects in vivo.

In IL-1 receptor–positive normal secondary CD4+ T lym- phocytes and in T$_H$2 clones, IL-1 alone does not elicit the expression of lymphokines or lymphokine receptor genes. However, IL-1 does provide a potent costimulatory signal to augment antigen receptor–initiated activation responses in T lymphocytes. The IL-1 costimulatory activity is medi- ated, in part, by the enhanced production/activity of tran- scription factors, NF-κB (33) and AP-1 (39). These proteins can potentially affect the rate of transcription of many genes involved in the T lymphocyte activation program. Previous studies (1, 2, 4, 5) have indicated that the primary role for IL-1 in the T cell activation program is to upregulate genes in the competence phase. IL-1 receptor desensitization may effectively contribute to focusing the effects of IL-1 in the competence phase of the activation program where upregu- lation of lymphokine and lymphokine receptor genes is im- portant. It also is possible that transcription factors, which have positive effects on gene expression in the competence phase of the activation program, have negative effects when combined with transactivating proteins produced in later phases of the activation program.

The transient nature of the receptor desensitization also may enable cells to regain IL-1 responsiveness as the activated T cell returns to the G$_1$ phase of the cell cycle. Regaining IL-1 responsiveness after the antigen receptor has been func- tionally downregulated or physically cleared from the cell sur- face would be expected to preclude additional IL-1 augmen- tation of lymphokine gene expression. Although lymphokine gene expression in activated T cells is transient, additional rounds of cell division can occur if the cells are stimulated through lymphokine receptors in a paracrine manner. Thus, the subsequent acquisition of IL-1 responsiveness could reflect a potential uncharacterized role for IL-1 in the progression phase of the T cell activation program.

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