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An NF-κB-like element plays an essential role in interleukin-1-mediated costimulation of the mouse interleukin-2 promoter*

Interleukin-1 (IL-1) costimulation is required for efficient IL-2 synthesis and IL-2 receptor (IL-2R) expression of T cells. The molecular events leading to these effects are largely unknown. We utilized an IL-1-responsive and an IL-1-non-responsive subclone of the mouse thymoma cell line EL4 to investigate how IL-1 activates IL-2 gene expression. We correlated IL-2 promoter activity with the activity of the endogenous IL-2 gene, thereby showing the biological significance of our results. Our experiments provide new functional data showing that a major target of IL-1 mediated costimulation is the κB-like site, T cell element distal TCEd (GGGATTTCAC), of the IL-2 promoter. Thus, deletion or mutation of TCEd within a complete IL-2 promoter abrogated IL-1 costimulation in the IL-1 responsive EL4 subclone. Therefore. The TCEd element is functionally essential for the effect of IL-1. We also identified a nuclear factor (NF), IL-1 NF, that binds to the TCEd site after IL-1 stimulation. This factor was only present in the IL-1-reponsive EL4 subclone and not in the IL-1-non-responsive subclone after IL-1 stimulation and did not appear after phytohemagglutinin (PHA)-treatment. Binding of IL-1 NF to the TCEd site was competed by a typical κB oligonucleotide, suggesting that it is similar to NF-κB in its DNA-binding properties. However, the TCEd element was only activated by costimulation with PHA and IL-1 whereas a typical κB element was already activated by IL-1 alone. These data suggest that the biological function of the TCEd element of the IL-2 promoter differs from that of a canonical κB element. Our data provide new evidence that IL-1 acts on the IL-2 promoter by activating the TCEd element via the transcription factor IL-1 NF. Furthermore, activation of this element requires two signals, delivered by IL-1 and PHA, in this way reflecting the activation requirement for the endogenous IL-2 gene.

1 Introduction

Proliferation of T lymphocytes after activation requires both secretion of interleukin-2 (IL-2) and expression of the high-affinity IL-2 receptor (IL-2R). IL-1 plays a key role in induction of these processes. In several experimental systems IL-1 has been shown to synergize with mitogenic lectins or anti-T cell receptor (TCR) antibodies to induce expression of IL-2 and IL-2Rα chain genes [1–3]. Recent data suggest that a requirement for IL-1 may reflect the developmental status of the T cell [4]. Thus, we have shown that in mouse $CD4^-CD8^-$ thymocytes IL-1 induces high-affinity IL-2R expression in synergy with IL-2 [5]. In immature $TCR^-CD4^-CD8^-$ thymocytes IL-1 provides a necessary signal for the induction of IL-2 gene expression [4, 6]. In peripheral TCR^+ thymocytes IL-1 can be replaced by the phorbol ester PMA plus the calcium ionophore A 23187 [6]. Whether the IL-1 signal plays an enhancing or obligatory role in T cell activation, however, is controversial and the mode of IL-1 action still remains unclear (for review see [7]).

IL-1 can regulate the expression of the IL-2 gene on the transcriptional level [8, 9]. Activation of the mouse IL-2 gene is mediated through a transcriptional enhancer region located between the positions − 293 and − 7 relative to the transcriptional start site [10]. This region is highly conserved between the IL-2 genes of mouse and man and contains multiple regulatory sequence elements (for review see [11]). Numerous ubiquitous and lymphocyte-specific trans-acting factors have been found to bind to the IL-2 enhancer [10–12]. Thus, the inducible ubiquitous factors AP-1 and NF-κB as well as the lymphoid-specific factors Oct-2 and NFAT-1 have been implicated to be involved in the control of the IL-2 enhancer activity. In particular, NFAT-1 has been proposed to play a prominent role in the control of T lymphocyte-specific transcription of the IL-2 gene [13]. Most of these factors were identified after stimulation with a combination of different Tcell mitogens. However, much less is known about the nuclear events following physiological activation by IL-1 costimulation. Previous data described that the transcription factor AP-1 was induced by IL-1 and PMA as a costimulus [14]. In addition costimulation with IL-1 was shown to activate the NF-κB motif in the SV40 [15] HIV-1 [16], IL-6 [17, 18] and the mouse factor B enhancers [19]. However, it has not been shown whether the κB-like element can confer IL-1 responsiveness to the IL-2 promoter.

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Abbreviations: **EMSA:** Electrophoretic mobility shift assay **IL-1 NF:** IL-1 induced nuclear factor **TCEd:** T cell element distal **CAT:** Chloramphenicol acetyltransferase

Key words: Interleukin-1 / Interleukin-2 promoter / Costimulation / NF-κB / T cell activation

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To investigate further the molecular events following IL-1-induced IL-2 secretion, we utilized two variants of the murine T cell lymphoma EL4, EL4 5D3 and EL4 D6/76, which differ in their IL-1 responsiveness [20]. EL4 5D3 responded to IL-1 by augmentation of PMA-induced IL-2 secretion and IL-2R expression. EL4 D6/76, however, did not respond to IL-1 although it expressed an IL-1R biochemically indistinguishable from that of EL4 5D3 cells. This defect in IL-1 responsiveness correlated with the inability to internalize receptor-bound IL-1 [20]. In addition, we have found that IL-1 increased the transcription of the IL-2 gene in the IL-1-responsive subclone. Therefore, we used these two subclones to examine the mouse IL-2 promoter for sequence motifs which are involved in the activation by IL-1.

In this report we identify the κB-like element of the IL-2 promoter as an IL-1-responsive site. Moreover, we show that the κB-like T cell element distal (TCEd) of the IL-2 promoter [21, 22] is essential for IL-1 costimulation and differs in its biological properties from a consensus κB element. In the IL-1-responsive subclone EL4 5D3 IL-1 costimulation leads to induction of a nuclear factor (IL-1 nuclear factor, IL-1 NF) that specifically binds to the TCEd element. Biological significance of this element was shown by correlation of IL-2 promoter activity with the activation of the endogenous IL-2 gene.

2 Materials and methods

2.1 Cells, cell culture and IL-2 measurement

Selection of EL4 subclones EL4 5D3 and EL4 D6/76 was described by von Hoegen et al. [20]. Cells were cultured in RPMI 1640 containing 10% FCS and 50 μg/ml gentamycin at 37 °C in 5% CO_2. Secreted IL-2 was measured in a proliferation assay using an IL-2 dependent cell line [20].

2.2 Recombinant cytokines

Human (hu) rIL-1α was kindly provided by A. Stern and P. Lomedico, Hoffmann-LaRoche, Nutley, NJ. The specific activity was 5×10^6 U/mg protein as determined by the lymphocyte activation factor assay. It was used at a concentration of 10 U/ml.

2.3 Plasmids

Plasmid pIL2-293 contains the − 293 to − 7 bp region of the murine IL-2 promoter linked to the CAT gene in the plasmid pBLCAT 2 [23]. pIL2-293 was derived from the plasmid pIL-CAT2/1+ by digestion with BglII/BamHI to eliminate the tk promoter. The construction of 4 × NFAT (previously named 4 × Pu-bd, 21), 5 × TCEd and 5 × TCEd + κBc.-CATconstructs was described previously [10, 22, 24]. The TCEd mutant TCEdM and the deletion mutant TCEdΔ were constructed by "thermal cyclic fusion PCR" as described [25] and their integrity was confirmed by sequencing [26]. 7 × NF-κB harbours seven copies of the κB-binding sequence 5′-GGGGAATTTCC-3′ of the MHC class II invariant gene in front of the tk promoter of pBLCAT2 (constructed and kindly given to us by U. Pessara, Boehringer Mannheim GmbH, Penzberg, FRG).

2.4 Transfections

The two EL4 subclones were grown to a density of about 4×10^5 cells/ml. Cells (1.4×10^7) were transfected with 10 μg DNA in a final volume of 1.2 ml using the DEAE Dextran procedure [26]. Fourty-eight hours after transfection cells were adjusted to a density of 1×10^6 cells/ml and divided into four aliquots. One aliquot was used as uninduced control and the other aliquots were stimulated with IL-1 (10 U/ml), PHA (40 μg/ml) or a combination of PHA + IL-1, respectively. After 15 h of stimulation the supernatants were tested for IL-2 production [20] and the cell lysate was assayed for chloramphenicol acetyltransferase (CAT) activity as described [26]. The conversion of [^{14}C]chloramphenicol to its acetylated forms was quanitified using an automatic thin-layer chromatography linear analyzer (Berthold, Wildbad, FRG). The protein concentration of cell extracts was measured according to Bradford [27] and was used to normalize CAT activity. In addition, the plasmid pTKCAT21A [28] was used as standard for transfection efficiency in each experiment. Plasmid pBLCAT2 [23] was used as negative control and was uninducible after transfection but showed a very low basal transcriptional activity.

2.5 Electrophoretic mobility shift assays (EMSA)

The TCEd oligonucleotide 5′-gatcACCAAGAGGGATTTCACCTAAATCC-3′ was labeled with [α-^{32}P]ATP/CTP and the Klenow fragment [26] followed by purification on a 12% polyacrylamide gel. Nuclear protein extracts were prepared from 2×10^7 non-activated cells or from cells activated for 4 h, using a modified method of Dignam et al. [16]. In each binding reaction 5000 cpm (about 0.2 ng) of end-labeled probe were incubated with 5 μg protein of nuclear extracts in the presence of 3 μg poly (dI-dC) (Pharmacia LKB, Uppsala, Sweden). The binding reaction was performed for 20 min at 4 °C. Resulting complexes were analyzed by electrophoresis at room temperature on a 4% polyacrylamide gel at 200 V [26]. The gels were dried and exposed for autoradiography.

3 Results

3.1 IL-1 costimulates IL-2 promoter activity and IL-2 secretion in the IL-1-responsive subclone EL4 5D3, but not in the IL-1-non-responsive subclone EL4 D6/76

To examine molecular events of IL-1-stimulated IL-2 secretion we used two subclones of the murine T cell lymphoma EL4 that differed in their IL-1 responsiveness. Both subclones were $CD3^+$ $CD4^+$ $CD8^-$ and expressed identical numbers of surface IL-1R [20]. Subclone EL4 5D3 reacted to IL-1 with enhanced IL-2 secretion and IL-2R expression whereas the subclone EL4 D6/76 did not. The defect of EL4 D6/76 cells correlated with the inability of these cells to internalize receptor-bound IL-1 [20].

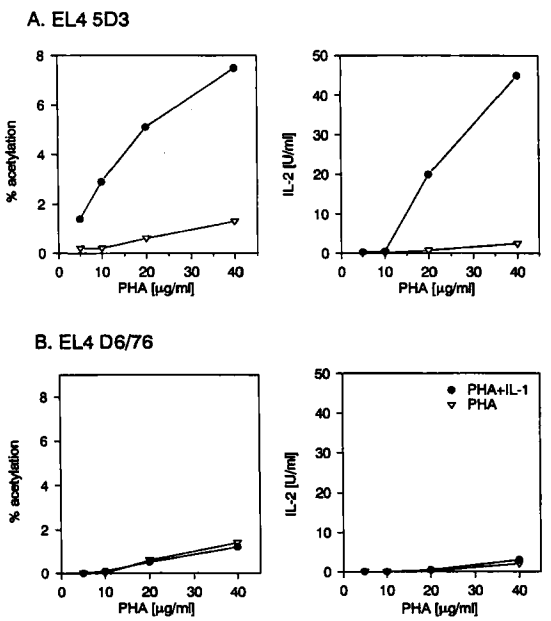

Figure 1. EL4 subclone EL4 5D3 and not subclone EL4 D6/76 reacts to IL-1 with enhanced IL-2 secretion and IL-2 promoter activity. EL4 5D3 (1A) and EL4 D6/76 (1B) cells were transfected with an IL-2 promoter-CAT construct (pIL2-293) containing a DNA fragment from position −293 to −7 relative to the transcription start site. Cells were stimulated for 15 h with increasing concentrations of PHA alone (▽), or PHA and IL-1 (●). CAT activity was measured as described [23] and the amount of IL-2 secreted into the supernatant was determined in a proliferation assay in comparison to an IL-2 standard. Unstimulated cells or cells stimulated with IL-1 alone did not produce IL-2 and the IL-2 promoter-CAT construct was not activated. One representative experiment of six performed is shown.

Both subclones were transfected with the plasmid pIL2-293 that contained 5′ sequences (from position −293 to −7 relative to the start site of transcription) of the murine IL-2 promoter linked to the CAT reporter gene. Fourty-eight hours after transfection the cells were stimulated for 16 h with increasing amounts of PHA alone, or PHA in combination with IL-1. CAT activity was measured in the cell lysates and secreted IL-2 was measured in the supernatants. PHA was chosen because in contrast to the PKC activator PMA it mimics activation via the T cell receptor complex [29]. In subclone EL4 5D3 the transfected IL-2 promoter construct showed synergistic stimulation with PHA and IL-1 (Fig. 1A). The same effect was observed for endogenous IL-2 expression, measured as secreted IL-2. As expected, subclone EL4 D6/76 did not react to IL-1 costimulation (Fig. 1B). Neither IL-2 secretion nor IL-2 promoter activation were found to be enhanced. These data confirm our previous results [20]. In addition, they show that IL-1 affects the activity of the IL-2 promoter and that it is able to costimulate with PHA. Therefore, the EL4 subclones are particularly suitable to study the molecular events leading to the induction of IL-2 promoter activity following IL-1 costimulation.

3.2 The κB-like TCEd, but not the NFAT-1-binding site of the IL-2 promoter was activated by IL-1 costimulation

In previous experiments IL-1 was shown to activate NF-κB elements of different genes [14–18]. However, it has not been shown whether the κB-like element within the IL-2 promoter plays a functional role for IL-1 costimulation. Therefore, our experiments focused on the κB-like site, TCEd, within the IL-2 promoter. A construct containing a pentamer of TCEd (5 × TCEd, 10) fused to the CAT gene was transfected into EL4 5D3 cells. In parallel the pIL2-293 plasmid (see Fig. 1) was used as a positive control. Since NFAT-1 binding was shown to be stimulated by PHA [13, 24] a 4 × NFAT-CAT construct [24] was used. Transfected cells were stimulated by IL-1, PHA or PHA + IL-1. The constitutively active plasmid pTKCAT21A [28] was used as control for transfection efficiency and was equally expressed in stimulated or unstimulated cells. The negative control, pBLCAT2 [23], was inactive in stimulated or unstimulated cells. All transfected cells responded to IL-1 with increased IL-2 production irrespective of the plasmid used. This shows that the plasmids did not interfere with induction of the endogenous IL-2 gene and that the induction conditions were appropriate.

Fig. 2 shows that optimal activation of the plasmid pIL2-293 was only seen with a combination of PHA and IL-1 [Stimulation index (SI) = (PHA+ IL-1)/PHA = 4.0] but not with PHA or IL-1 alone. The same synergy was seen on the level of IL-2 secretion. CAT activity of the 5 × TCEd construct was only induced by a combination of PHA and IL-1 (SI = 3.4). In contrast, the 4 × NFAT construct was activated by PHA alone, but did not show any response to IL-1 costimulation. Taken together, our experiments demonstrate for the first time that the TCEd element is an IL-1-responsive site of the IL-2 promoter and confers IL-1 inducibility to another promoter.

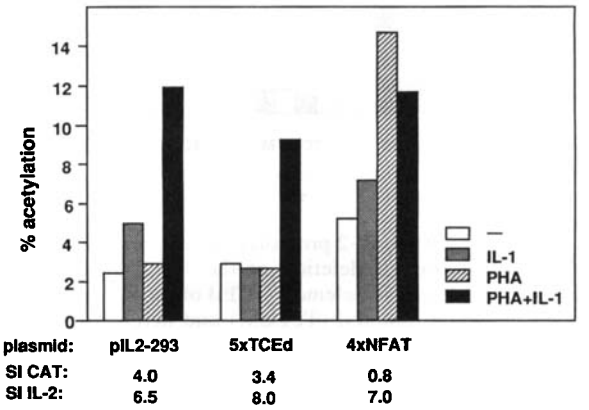

Figure 2. The NF-κB-like element TCEd of the IL-2 promoter is a site for IL-1 costimulation. Plasmid pIL2-293 contains the minimal promoter element of the murine IL-2 gene spanning the nucleotides from −293 to −7 relative to the transcription start site [10]. 5 × TCEd contains five copies of the κB-like element TCEd [10, 19] and 4 × NFAT contains four copies of the distal NFAT-1 binding site (Pu-bd) in front of the tk promoter in pBLCAT2 [21], respectively. These plasmids and the control plasmids pTKCAT21A and pBLCAT2 were transfected into EL4 5D3 cells. Cells were either left unstimulated (−) or were stimulated with PHA (40 µg/ml), IL-1 (10 U/ml) or a combination of PHA and IL-1. CAT activity of the various transfected cells is shown as % acetylation. The positive control pTKCAT21A was constitutively expressed and % acetylation determined was: 68% (−), 72% (IL-1), 75% (PHA), 73% (PHA + IL-1). The negative control pBLCAT2 was not inducible and exhibited a low basal transcription activity of less than 2% acetylation. SI for CAT activity and IL-2 secretion were calculated as (PHA + IL-1)/PHA. One representative experiment out of four performed is shown.

3.3 The TCEd element of the IL-2 promoter is essential for IL-1 action

To investigate the importance of the TCEd element for IL-1 costimulation of the whole IL-2 promoter a site specific mutant was constructed by PCR. The clustered mutation (5′GGG→TTT) of the κB-like TCEd element, TCEdM, was selected because identical base substitutions had been shown to abolish the specific binding of NF-κB to a consensus NF-κB site [30]. In addition, a deletion mutant, TCEdΔ, lacking the TCEd element (from − 207 to − 195) was constructed. EL4 5D3 cells were transfected with these constructs and stimulated with PHA, IL-1 and PHA + IL-1. CAT activities and IL-2 secreted into the supernatant were measured. As shown in Fig. 3 both the mutation (TCEdM) and the deletion (TCEdΔ) of the TCEd element completely abolished responsiveness of the IL-2 promoter to IL-1 costimulation. Therefore, the TCEd element of the IL-2 promoter is functionally essential for the action of IL-1.

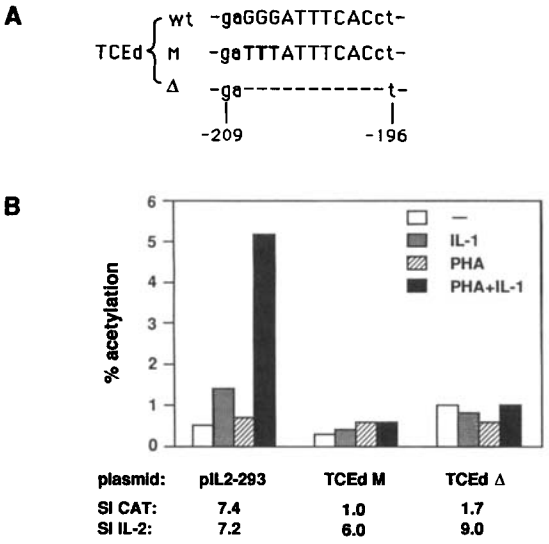

Figure 3. Induction of the IL-2 promoter by IL-1 costimulation is abrogated by mutation or deletion of the TCEd element. A) Sequences of the NF-κB-like element TCEd of the IL-2 promoter and sequences of the mutated (TCEdM) and deleted (TCEdΔ) elements are shown. B) EL4 5D3 cells were transfected with plasmids pIL2-293, TCEdM and TCEdΔ. Stimulation was done as described in Fig. 2. Controls were transfected in parallel and % acetylation obtained was 56% (−), 54% (IL-1), 58% (PHA), 62% (PHA + IL-1) for pTKCAT21A and 0.3% (−), 0.2% (IL-1), 0.3% (PHA), 0.3% (PHA + IL-1) for pBLCAT2. SI were calculated as described in Fig. 2. One representative experiment out of six performed is shown.

3.4 IL-1 NF, a specific IL-1-inducible nuclear factor, binds to the TCEd element

The results of transfection studies suggest that IL-1 inducible nuclear factors may bind to and control the activity of the TCEd element. To address this question we investigated the IL-1-responsive subclone EL4 5D3 and the IL-1-non-responsive subclone EL4 D6/76 for IL-1-inducible nuclear proteins that might bind to the TCEd element in EMSA.

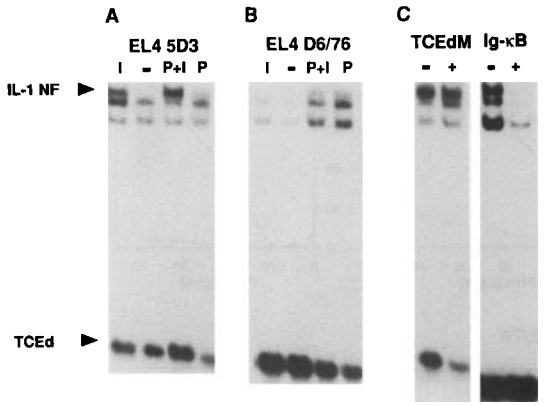

Figure 4. Binding of the IL-1-inducible factor IL-1 NF from EL4 5D3 cells to the TCEd element. EL4 5D3 cells (A) and EL4 D6/76 cells (B) were left untreated (−), or stimulated for 4 h with IL-1 (I), PHA (P), and PHA + IL-1 (P + I). Nuclear extracts were prepared as described by [13] and incubated with a labeled oligonucleotide containing the TCEd site and analyzed in electrophoretic mobility shift assays. For competition (C) the Ig-κB (5′-GGGACTTTC-C-3′) and TCEdM (5′-TTTATTTCAC-3′) oligonucleotides were used, as indicated. Arrowheads indicate the positions of the free probe and the IL-1 inducible DNA-protein complex.

Both subclones were activated with IL-1, PHA or PHA + IL-1 for 4 h and nuclear extracts were prepared and assayed in EMSA. Fig. 4A shows that in extracts of EL4 5D3 cells three individual protein factors bound to the TCEd element after stimulating the cells with PHA and IL-1. We named the most prominent factor IL-1 NF. IL-1 NF was induced by stimulation with IL-1 alone. The two faster-migrating protein complexes were already present in unstimulated cells but were up-regulated upon stimulation with PHA. IL-1 did not induce generation of IL-1 NF in EL4 D6/76 cells, correlating with their inability to react to IL-1 (Fig. 4B). In these cells, like in EL4 5D3 cells, PHA stimulation enhanced binding of the two faster migrating protein complexes. Induction of these two complexes was not sufficient for transcriptional activation of TCEd but might reflect a mode of costimulation via PHA.

Binding of IL-1 NF and the faster-migrating proteins was competetively inhibited by an excess of NF-κB consensus sequence from the Ig κ enhancer (Fig. 4C) suggesting that these proteins are either identical or similar in their DNA-binding properties to NF-κB. The mutated TCEd site, TCEdM, (see Fig. 3) was unable to compete for binding. This shows that mutation of TCEd abolishes binding of these proteins.

3.5 Activation of TCEd by IL-1 is functionally different from activation of a consensus NF-κB element

The NF-κB consensus sequence competed for binding of IL-1 NF to the TCEd element. NF-κB is an ubiquitous factor and NF-κB elements are inducible in various cell types. In contrast, the TCEd motif was shown to be a T cell-specific enhancer element [21, 22]. We wanted to investigate whether the sequence differences between the NF-κB and TCEd elements had consequences on IL-1 responsiveness of the TCEd element. Therefore, EL4 5D3 cells were transfected with the constructs 5 × TCEd,

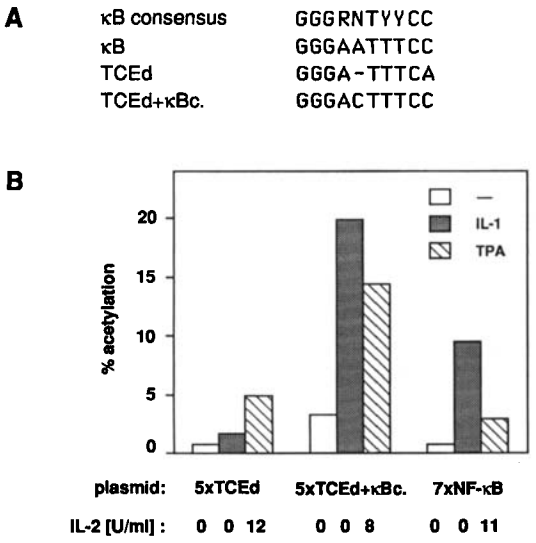

Figure 5. The κB-like site TCEd from the IL-2 promoter is functionally distinct from a consensus κB site. A) Sequences of NF-κB motifs present in the plasmids 5 × TCEd, 5 × TCEd + κBc. and 7 × NF-κB. B) EL4 5D3 cells were transfected with 5 × TCEd, 5 × TCEd + κBc., 7 × NF-κB and control plasmids. They were left unstimulated or were stimulated with PMA (2 ng/ml) or IL-1 (10 U/ml). CAT activity was measured as % acetylation. Secreted IL-2 was measured as described in Sect. 2.1. The values for the positive control pTKCAT21A and the negative control pBLCAT2 were, respectively: 62 and 0.5% (−), 58 and 0.4% (IL-1), 66 and 0.4% (PMA). One representative experiment of six performed is shown.

7 × NF-κB and with a construct containing a TCEd core which was converted to a consensus NF-κB sequence, TCEd + κBc. (κB core; see Fig. 5A). Cells were treated with IL-1 or PMA and CAT activity and IL-2 secretion were determined. PMA stimulation was used as a control for mitogen-dependent increase in CAT activity of the three constructs, as in EL4 5D3 cells PMA alone is sufficient to activate IL-2 secretion. The TCEd element was not induced by IL-1, as expected, but by PMA alone. This activation pattern correlated well with the activation of the endogenous IL-2 gene (Fig. 5). The mutation of TCEd to a TCEd + κBc. sequence had a strong effect on the IL-1-induced enhancer activity, as IL-1 was sufficient for activation. Activation with IL-1 was even stronger (6-fold induction) than activation with PMA (4.3-fold induction). The 7 × NF-κB construct exhibited the same effect. This finding was in striking contrast to the activation of the endogenous IL-2 gene. The endogenous IL-2 gene was not induced with IL-1 alone, but PMA was sufficient to activate IL-2 secretion (about 12 U/ml).

Therefore, TCEd is a T cell-specific element [22] whose activity is induced by IL-1 costimulation. It differs in its biological properties from the non-tissue-specific consensus NF-κB element.

4 Discussion

The initiation of T cell activities in immune responses requires costimulation by the cytokine IL-1. Relatively little is known about the mode of IL-1 costimulation. Utilizing IL-1-responsive and IL-1-non-responsive subclones of the murine thymoma EL4 [20] we have previously shown that IL-1 costimulates IL-2 gene expression by enhancing the activity of the IL-2 promoter [8]. In this report we further investigated IL-1 responsive elements of the IL-2 promoter. We identified the κB-like element, TCEd, as a major target of IL-1 costimulation. κB-like elements were shown to be involved in the activation of several other genes by IL-1 [15–19]. We show here that IL-1 activates IL-2 production through the κB-like element TCEd. Furthermore, we demonstrate that IL-1 costimulation of IL-2 promoter activity in the IL-1 responsive EL4 subclone was abolished by mutation or deletion of the TCEd element. Therefore, we provide functional evidence that the TCEd element is essential for the IL-1 effects on the IL-2 promoter. By comparing the non-responsive with the IL-1-responsive EL4 variant we detected an IL-1-inducible nuclear factor, IL-1 NF. DNA binding of IL-1 NF was induced by IL-1 only in the responsive subclone. According to its electrophoretic mobility, IL-1 NF differed from two other factors that were found to be upregulated by PHA stimulation. The DNA binding properties of IL-1 NF are similar or identical to NF-κB. It was shown that purified NF-κB binds to the TCEd element [22, 31]. In our experiments a κB consensus oligonucleotide competed for binding of IL-1 NF to the TCEd site. However, we present functional data that IL-1 NF is likely to be different from NF-κB. When the TCEd motif was converted to a canonical NF-κB motif (TCEd + κBc.) its functional activity was also changed. In contrast to the activation of the TCEd element IL-1 alone was sufficient for activation of the TCEd + κBc. element. This finding is in agreement with the report of Kang et al. [32] who showed that for the Ig-κB site CAT activity could be stimulated by APC alone (possibly by secreting IL-1). IL-2 κB activation, however, required both antigen and APC [32]. In addition, Briegel et al. showed that the TCEd + κBc. construct was inducible in non-T cells [22]. Nevertheless, IL-1 NF may be composed of members of the NF-κB family. This family comprises numerous structurally related factors including the c- and v-rel oncogene products and *Drosophila* dorsal gene products [33–36].

Our findings are in agreement with the report by Novak et al. [9] correlating IL-1 costimulation with augmented levels of two TCEd binding factors. In their experiments PMA alone induced binding of these two NF-κB-like factors in EL4 cells. The binding activities especially of the slower-migrating factor which comprise IL-1 NF were further enhanced by IL-1. They also showed that IL-1 alone was able to up-regulate IL-1 NF. We also found that activation with PMA, in contrast to PHA stimulation gives only suboptimal IL-1 NF induction. Addition of IL-1, however, upregulated binding activity of IL-1 NF preferentially (data not shown). The striking negative effect of mutation or deletion of the TCEd motif on IL-1 mediated costimulation of the IL-2 promoter provides new functional data that the TCEd element is essential for IL-1 effects.

The role of AP-1 in IL-1 costimulation of the IL-2 promoter is controversial. Muegge et al. [37] showed that in the mouse thymoma LBRM-331A5 IL-1 induced transcription of c-jun, a component of AP-1. These authors further showed that deletion of a fragment from − 218 to − 176 of the IL-2 promoter abrogated the effect of IL-1. Therefore,

they concluded that a potential AP-1-binding site located around position − 180 of the IL-2 promoter was responsible for this effect. However, they also deleted the essential IL-1 responsive TCEd element, thereby abrogating the IL-1 effect. Furthermore, Muegge et al. did not show functional data that the AP-1 element at − 180 of the IL-2 promoter was the IL-1 responsive site, because they used the collagenase AP-1 sequence in their transfection experiments which differs from the AP-1 site at − 180. Therefore, there is no convincing functional data that the AP-1 element at − 180 is an IL-1-responsive element. Several other studies have shown that deletion of this element has no effect on inducibility of the IL-2 promoter [10, 12, 32]. Leonard et al. reported that antibodies against c-jun and c-fos failed to react with complexes formed at this site and neither the IL-2 AP-1 (at − 150) nor the collagenase AP-1 oligonucleotides could compete with the complexes [29]. Rothenberg et al. showed that IL-1 costimulation resulted in enhanced activity of an AP-1 like factor that binds to the proximal AP-1 site around position − 150 of the IL-2 promoter [9]. However, Muegge et al. have shown that deletion from − 169 to − 101 did not affect IL-1 responsiveness. In our system mutation of the proximal AP-1 site within the IL-2 promoter did also not affect IL-1 activity (data not shown). Taken together, these data indicate that AP-1 does not seem to play a major role in IL-1 costimulation of the IL-2 promoter.

Mizel proposed a model of IL-1 activity [7] in which IL-1 initiates IL-2 and IL-2Rα synthesis and expression. Concomitant increased expression of the IL-1R then renders the cells fully responsive to IL-1. A common transcription factor was described to be involved in the regulated expression of IL-2 and IL-2 Rα genes [38] that both contain NF-κB-like elements. The transcription factor bound to TCEd and to the IL-2Rα κB-like site but displayed properties different from NF-κB [38]. It is likely that this factor is related to or identical to IL-1 NF. Therefore, IL-1 may regulate both IL-2 and IL-2Rα expression through induction of IL-1 NF. Further studies to investigate this question are in progress.

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