Functionally Distinct NF-κB Binding Sites in the Immunoglobulin κ and IL-2 Receptor α Chain Genes

SHARON L. CROSS, NANCY F. HALDEN, MICHAEL J. LENARDO, WARREN J. LEONARD

The interleukin-2 receptor alpha (IL-2Rα) chain gene contains a sequence similar to the immunoglobulin (Ig) kappa (κ) enhancer NF-κB binding site. This site, which is bound by the nuclear protein, NF-κB, is critical for Ig κ gene expression. The major T cell nuclear factor that binds to the IL-2Rα site in vitro appears indistinguishable from NF-κB. NF-κB binds to IL-2Rα and κ sequences with similar affinities; however, only the κ site potently activates transcription from heterologous promoters. Thus, high-affinity NF-κB binding in vitro cannot be equated with transcriptional activation in vivo. Mutation of the NF-κB binding site in the context of an IL-2Rα promoter construct markedly diminished promoter activity in human T cell lymphotropic virus type I (HTLV-I)–transformed MT-2 cells but not in phorbol myristate acetate–stimulated Jurkat T cells.

REGULATION OF EXPRESSION OF high-affinity interleukin-2 (IL-2) receptors is a critical control mechanism in the T cell immune response (*1*). These receptors contain β chains (also denoted as IL-2Rβ or p70), which are expressed on resting and activated T cells (*2*), and α chains (also denoted as IL-2Rα, p55, or Tac antigen), which are expressed only after activation (*3*). This induction is significantly regulated at the level of transcription initiation (*4*).

The IL-2Rα promoter is capable of directing chloramphenicol acetyltransferase (CAT) activity in phorbol myristate acetate (PMA)–induced Jurkat T cells and in T cells transformed by human T cell lymphotropic virus type I (HTLV-I) (*5, 6*). However, constructs with 5′ end points of −265 and −267 are inactive in PMA-induced Jurkat cells, but regain activity when cotransfected with a construct expressing the transactivator protein of HTLV-I (variably referred to as $p40^x$ or *tat*-I or *tax*-derived proteins) (*6*). These studies indicate the importance of the −265 region in IL-2Rα expression and show that the *tat*-I gene product alters normal IL-2Rα regulation.

The IL-2Rα −265 region, the κB site in the Ig κ chain gene enhancer, and sites in the human immunodeficiency virus type 1 (HIV-1) enhancer (*7–10*) contain a similar sequence motif (Fig. 1). After conversion to an active DNA binding form (*11*), NF-κB binds to a consensus sequence in the HIV-1 and κ enhancers and activates transcription of these genes (*7, 12, 13*). In the IL-2Rα gene, investigators disagree as to the role of NF-κB in activating transcription in PMA-induced Jurkat cells (*8–10*). We show that the IL-2Rα and κ NF-κB binding sites differ in their ability to activate transcription from heterologous promoters and that NF-κB binding in vitro cannot be equated with transcriptional activation in vivo. In contrast, previous studies have indicated a correlation between in vitro binding and transcriptional activation in cells producing NF-κB (*7, 13, 14*).

In deoxyribonuclease I (DNase I) protection assays, the −265 region (−273 to −250, see Fig. 1A) was completely protected by nuclear proteins from the HTLV-I–transformed cells MT-2 and HUT-102B2 or from PMA-induced Jurkat cells, but was minimally protected by factors in unstimulated Jurkat extracts (arrowhead, Fig. 2A). This footprint was flanked by a more weakly protected region (−249 to −240, Fig. 2B). The IL-2Rα −273 to −250 region contains two overlapping 8- of 10-bp matches (GGGAaTcTCC, −267 to −258; GGGAgaTTCC, −257 to −266) to the murine κ gene NF-κB binding site (Fig. 1B). In competition assays, an oligonucleotide spanning both HIV-1 NF-κB sites, but not one in which these sites were mutated (*7*), abolished protection of the IL-2Rα −273 to −250 region (Fig. 2B). The flanking footprint (−249 to −240) was not diminished. These experiments suggested that NF-κB and an additional factor bound to the IL-2Rα −273 to −240 region.

Purified bovine NF-κB (*15*) could bind to this region (Fig. 3A). The κ enhancer oligonucleotide competed for this binding, but a similar oligonucleotide with a mutated NF-κB site did not (Fig. 3A). Competition titration assays with the IL-2Rα oligonucleotide (Fig. 1C), which has IL-2Rα coordinates −269 to −257 (Fig. 3B), or the κ site indicated that NF-κB bound both sites with similar affinities. Similar results were obtained when nuclear extracts from MT-2 T cells were used instead of purified NF-κB.

When nuclear proteins were ultraviolet (UV) cross-linked (*16*) to an IL-2Rα oligo-

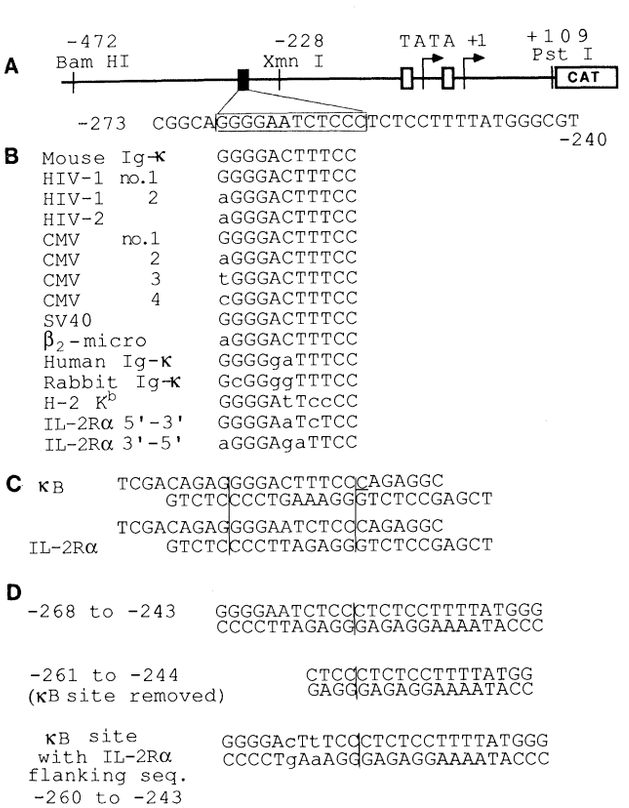

Fig. 1. (**A**) Schematic of the IL-2Rα gene, indicating the two major transcription initiation sites and TATA boxes (open boxes), and the NF-κB binding site (filled box). (**B**) NF-κB binding site similarities. Lowercase letters indicate mismatches to the murine Ig-κ site. IL-2Rα −268 to −258 and −256 to −266 represent overlapping regions of homology, only one of which could bind NF-κB at a given time. (**C**) Sequence of κB and IL-2Rα NF-κB sites with κ flanking sequences. The IL-2Rα oligonucleotide replaced the κ NF-κB binding site with IL-2Rα −267 to −257. Total identity extends from −269 to −257. One base 3′ of the site (underlined in Fig. 4A) was changed in constructs to preserve the two overlapping 8 of 10 bp matches (B). (**D**) Sequence of IL-2Rα −268 to −243 oligonucleotide and derivatives. Lowercase letters indicate mismatches to the IL-2Rα sequence.

S. L. Cross, N. F. Halden, W. J. Leonard, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
M. J. Lenardo, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

nucleotide (−273 to −250) and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE), two bands of approximately 50 kD were detected in extracts from S194 murine myeloma cells, which constitutively produce NF-κB (*12*), and from HUT-102B2 and MT-2 T cells (Fig. 3C). Similar factors were PMA-inducible in both Jurkat T cells and HeLa cells. This cellular distribution paralleled NF-κB binding activity detected by mobility shift assays, and the molecular sizes were consistent with those reported for human NF-κB [51 kD (*17*)] and murine NF-κB prepared from S194 cells (*18*). Thus, the major T cell factor binding to these IL-2Rα sequences in vitro appears indistinguishable from NF-κB. Further, HTLV-I–transformed cells express NF-κB binding activity in the absence of PMA induction, a characteristic previously observed only in certain B cell lines (*11, 12*). This binding activity may be due to the *tat*-I expression in these cells (*9, 10, 19*).

In order to evaluate the relative ability of the IL-2Rα and κ NF-κB sites to activate transcription, both sites were inserted into κ gene flanking sequences (Fig. 1C) and cloned upstream to the truncated c-*fos* promoter in pFCΔ56 (*20*) and the herpesvirus thymidine kinase (*tk*) promoter in pBLCAT2 (*21*). Both oligonucleotides bound NF-κB with similar affinities in vitro (Fig. 3B). In transiently transfected S194 cells, the κB oligonucleotide in normal (Fig. 4A) or reverse orientations increased CAT activity 50- to 100-fold. In contrast, the oligonucleotide with the IL-2Rα NF-κB binding site in the context of κ flanking sequences directed minimal activity in S194 cells (Fig. 4A) and in lipopolysaccharide-stimulated 38B9 pre–B cells, which express NF-κB (*13*). Neither construct was active in HeLa cells (Fig. 4B). In MT-2 (Fig. 4D) and PMA-induced Jurkat (Fig. 4C) T cells, the κ NF-κB binding site was the more potent activator. Thus, the IL-2Rα NF-κB binding site alone, in contrast to the κ site, minimally activated heterologous promoters in cells expressing NF-κB.

A larger IL-2Rα oligonucleotide (−268 to −243), more closely corresponding to the footprinted sequences (Fig. 2), boosted activity of a *tk* promoter–CAT construct 3- to 4-fold in PMA-induced Jurkat cells and 8- to 12-fold in MT-2 cells (Table 1). Little activity was seen in S194 cells (which produce high levels of NF-κB) or in HeLa cells, suggesting that the activity mediated by these sequences may be restricted to T cells.

An oligonucleotide (Fig. 1D, −261 to −244), which lacked most of the NF-κB binding site and did not compete for NF-κB binding, mediated activity similar to that of the −268 to −243 construct in PMA-induced Jurkat cells and approximately two-fold less than the −268 to −243 construct in MT-2 cells (Table 1, lines 2 versus 1). The −261 to −244 construct did not augment *tk*-CAT activity in the non–T cell lines S194 (Table 1) or HeLa, suggesting that sequences 3′ of the IL-2Rα NF-κB binding site may act as a T cell–specific positive regulatory element. A CAT construct in which the κ NF-κB binding site was placed adjacent to the IL-2Rα flanking sequences (Fig. 1D) was more active than either the −268 to −243 IL-2Rα or κB oligonucleotides in PMA-induced Jurkat cells (Table 1, line 3 versus lines 1 and 4), perhaps because of the additive activities of the κ binding site and IL-2Rα downstream sequences (lines 4 and 2). In S194 myeloma cells, however, the κB/IL-2Rα hybrid construct was significantly less active than the κB site alone (lines 3 versus 4), suggesting an inhibitory effect of the 3′ flanking sequences in these myeloma cells.

In addition to examining the function of the IL-2Rα NF-κB binding site and 3′ flanking sequences on heterologous promoters, we investigated the role of the NF-κB binding site in the context of the IL-2Rα promoter (−472 to +110, see Fig. 1A). A construct in which the NF-κB binding site was replaced with 12 bp derived from an Xba I linker (Δ−268/−257 construct) exhibited drastically decreased CAT activity in unstimulated (Fig. 5) and PMA-induced MT-2 cells. However, in PMA-induced Jurkat cells, this construct retained normal ac-

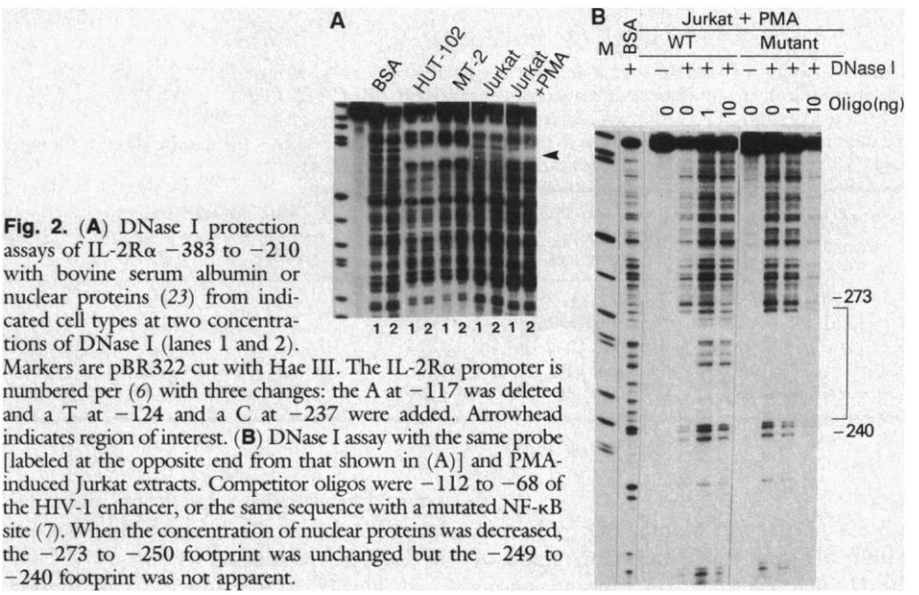

Fig. 2. (**A**) DNase I protection assays of IL-2Rα −383 to −210 with bovine serum albumin or nuclear proteins (*23*) from indicated cell types at two concentrations of DNase I (lanes 1 and 2). Markers are pBR322 cut with Hae III. The IL-2Rα promoter is numbered per (*6*) with three changes: the A at −117 was deleted and a T at −124 and a C at −237 were added. Arrowhead indicates region of interest. (**B**) DNase I assay with the same probe [labeled at the opposite end from that shown in (A)] and PMA-induced Jurkat extracts. Competitor oligos were −112 to −68 of the HIV-1 enhancer, or the same sequence with a mutated NF-κB site (*7*). When the concentration of nuclear proteins was decreased, the −273 to −250 footprint was unchanged but the −249 to −240 footprint was not apparent.

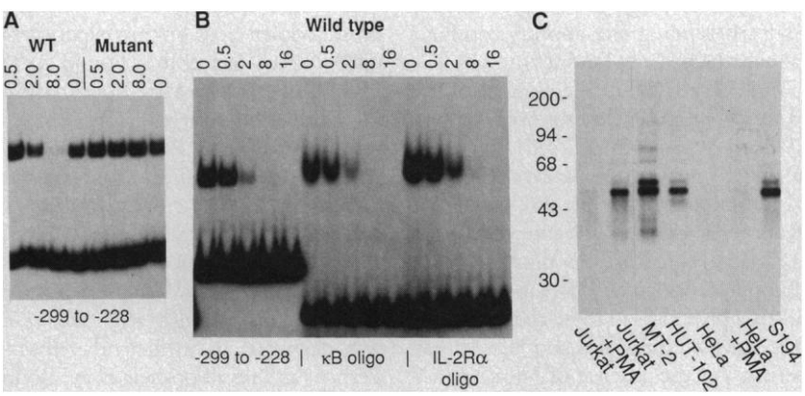

Fig. 3. Binding of purified NF-κB and T cell nuclear factors to IL-2Rα sequences. (**A**) Gel mobility shift assays (*15*) with purified NF-κB and IL-2Rα −299 to −228, and wild-type (WT) or mutant κB sequence (*7*) as competitors. (**B**) Similar assays with purified NF-κB and IL-2Rα −299 to −228, a κ enhancer NF-κB binding site, or the IL-2Rα oligonucleotide of Fig. 1C. Binding reactions were competed with the IL-2Rα oligonucleotide. (**C**) Ultraviolet cross-linking of nuclear protein from the indicated cells. IL-2Rα −273 to −250 was labeled with bromodeoxyuridine (BrdU)-triphosphate and ^{32}P-labeled deoxycytidine 5′-triphosphate (dCTP) by specific priming, ultraviolet cross-linked to nuclear proteins, and analyzed by SDS-PAGE. Similar experiments with a mutant oligonucleotide in which the GGG (−267 to −265) were replaced by CTC indicated that the 50-kD bands were specific.

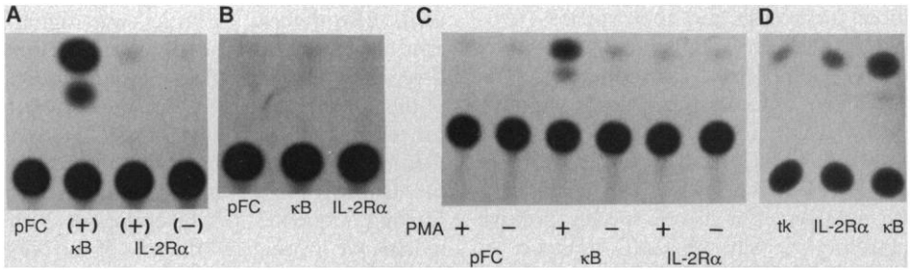

Fig. 4. IL-2Rα and κ NF-κB binding site oligonucleotides (Fig. 1C) were cloned into the Sal I sites of pBLCAT2 and pFCΔ56 (pFC), and constructs transfected with DEAE dextran (*6*) into S194, HeLa, Jurkat, and MT-2 cells [(**A**) to (**D**), respectively]. In (A) to (C), the pFCΔ56 constructs are shown; in (D), because of high background transcription by the c-*fos* promoter in MT-2 cells, *tk*-CAT constructs were used (*tk* = pBLCAT2 with no insert). In Jurkat and S194 cells, results similar to those in (A) and (C) were obtained when *tk* constructs were used. In (A), (+) and (−) indicate orientation of the inserted oligonucleotide. In (B) and (C), constructs were in the (+) orientation. In (D), the IL-2Rα oligonucleotide was in the (+) orientation and the κB oligonucleotide was in the (−) orientation. In (C), + indicates that cells were stimulated with PMA for 24 hours; − indicates no stimulation. Absolute percent conversion: S194 (A): pFC, 0.2; κB(+), 30.5; IL-2Rα(+), 0.7; IL-2Rα(−), 0.4. HeLa (B): pFC, 0.3; κB(+), 0.3; IL-2Rα(+), 0.2. Jurkat (C): pFC +PMA, 0.7; pFC −PMA, 0.6; κB(+) +PMA, 9.1; κB(+) −PMA, 1.1; IL-2Rα(+) +PMA, 0.9; IL-2Rα(+) −PMA, 0.8. MT-2 (D): pBLCAT2, 9.8; IL-2Rα(+), 15.4; κB(−), 38.1.

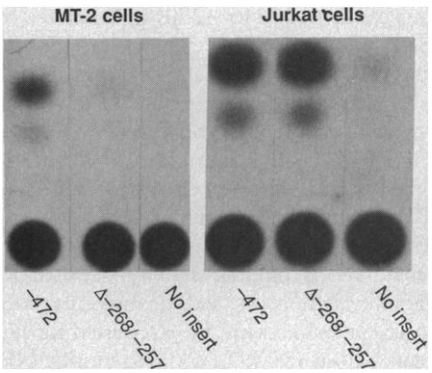

Fig. 5. CAT assay for the Δ−268/−257 construct in MT-2 and induced Jurkat T cells. Relative activities: −472 to +110, MT-2 1.0, Jurkat 1.0; Δ−268/−257, MT-2 0.1, Jurkat 1.0; no promoter MT-2 0.1, Jurkat 0.1. Δ−268/−257 was constructed by joining appropriate 3′ and 5′ deletions at their common Xba I linkers after filling in the Xba I site, replacing GGGGAATCTCCC with CTCTAGCTAGAG.

Table 1. Ability of IL-2Rα and κ sequences to transcriptionally activate the *tk* promoter. Average activities (at least two independent transfections) relative to pBLCAT2. Oligonucleotides were analyzed in both orientations for all but two constructs; orientation did not significantly affect activity except for the oligonucleotide of line 2, where activity is even greater in the (−) orientation in Jurkat (not shown). Lines 1 to 3 correspond to sequences in Fig. 1D; lines 4 and 5 correspond to sequences in Fig. 1C.

	IL-2Rα coordinates	NF-κB site/ flanking sequences	MT-2	Jurkat		S194
				−PMA	+PMA	
1.	−268 to −243	IL-2Rα / IL-2Rα	9.8	1.4	4.2	1.4
2.	−261 to −244	− / IL-2Rα	5.0	1.9	3.6	1.1
3.	−260 to −243	κB / IL-2Rα	8.7	2.1	8.2	5.6
4.		κB / κB	3.6	1.5	4.5	19.5
5.	−269 to −256	IL-2Rα / κB	1.7	1.1	1.4	1.3

tivity.

Thus, an NF-κB binding site is located within a functionally important region of the IL-2Rα gene (*6, 22*). The analogous locus in the murine IL-2Rα gene can also bind NF-κB. Although the role for the IL-2Rα NF-κB binding site appears complex, several conclusions can be drawn:

1) The NF-κB site in the context of the IL-2Rα promoter is critical for expression in HTLV-I–transformed MT-2 cells, since mutation of this site abrogated expression. In contrast, mutation of the site did not inhibit IL-2Rα expression in PMA-induced Jurkat cells (Fig. 5). Thus, NF-κB binding is apparently not essential for PMA induction of the IL-2Rα promoter in these cells, suggesting that other binding sites and factors may compensate for the absence of NF-κB binding in the mutated construct.

2) Although the IL-2Rα NF-κB binding site (−269 to −257) did not significantly stimulate transcription from a heterologous promoter, a larger construct (−268 to −243) did. Further, the sequence from −261 to −244 (which cannot bind NF-κB but is protected in DNase I footprinting assays) functioned as a specific positive regulatory element in the T cells studied but not in S194 or HeLa cells.

3) The IL-2Rα −269 to −257 region and κ enhancer sequences bound NF-κB with similar affinities in vitro. On the basis of ultraviolet cross-linking experiments, the major T cell factor that bound to the IL-2Rα NF-κB site in vitro appeared indistinguishable from NF-κB. This result differs from the report by Böhnlein *et al.* (*8*), who used an affinity purification technique to identify an 86-kD factor as the major species binding to this region in induced Jurkat cells. No major factors of 86 kD were observed in our assays, although we cannot exclude the possibility that such a species was degraded or inefficiently cross-linked. Perhaps as a result of their assay, Böhnlein *et al.* did not detect proteins approximating the size of NF-κB (*8*).

4) The IL-2Rα and κ gene NF-κB binding sites differed significantly in their ability to activate transcription from heterologous promoters. Only the κ site functioned as a potent enhancer in cells producing NF-κB binding activity (S194, PMA-Jurkat, and MT-2 cells). Thus, we have demonstrated that high-affinity binding of NF-κB to a DNA sequence in vitro cannot be equated with transcriptional activation in vivo. Because of the similar binding affinities observed in vitro, we hypothesize that NF-κB binds to both the κ and IL-2Rα sites in vivo, but that the 2-base difference between these sites profoundly influences the ability of the resulting protein-DNA complex to activate transcription.

Since NF-κB binding alone may not be sufficient for potent transcriptional activation, it is likely that the sequence context of NF-κB binding is critical. This hypothesis may explain the apparent paradox posed by the dramatic decrease in IL-2Rα promoter activity in MT-2 cells when the NF-κB site is mutated, although this site alone cannot significantly activate a heterologous promoter in MT-2 cells.

In conclusion, NF-κB is unlikely to be solely responsible for the exquisite cell type specificity and PMA-inducibility of expression seen for various IL-2Rα constructs described in this report. These results support the conclusion that other factors and sequence elements in addition to NF-κB will be required to explain why the κ chain generally is expressed only in B cells and the IL-2Rα generally is expressed only in T cells.

REFERENCES AND NOTES

1. W. J. Leonard, M. Sharon, S. L. Cross, in *The Year in Immunology 1988*, J. M. Cruse and R. E. Lewis, Eds. (Karger, Basel, Switzerland, 1989), vol. 5, pp. 46–58; W. J. Leonard and M. Sharon, in *Molecular Genetics of Cancer*, J. Cossman, Ed. (Elsevier, New York, in press).
2. M. Sharon, R. D. Klausner, B. R. Cullen, R. Chizzonite, W. J. Leonard, *Science* **234**, 859 (1986); M. Tsudo, R. W. Kozak, C. K. Goldman, T. A.

Waldmann, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9694 (1986); K. Teshigawara, H. M. Wang, K. Kato, K. A. Smith, *J. Exp. Med.* **165**, 223 (1987). J. P. Siegel, M. Sharon, P. L. Smith, W. J. Leonard, *Science* **238**, 75 (1987).
3. T. Uchiyama, S. Broder, T. A. Waldmann, *J. Immunol.* **126**, 1393 (1981).
4. W. J. Leonard, M. Krönke, N. J. Peffer, J. M. Depper, W. C. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6281 (1985).
5. W. J. Leonard *et al.*, *Science* **230**, 633 (1985).
6. S. L. Cross *et al.*, *Cell* **49**, 47 (1987).
7. G. Nabel and D. Baltimore, *Nature* **326**, 711 (1987).
8. E. Böhnlein *et al.*, *Cell* **53**, 827 (1988).
9. K. Leung and G. J. Nabel, *Nature* **333**, 776 (1988).
10. S. Ruben *et al.*, *Science* **241**, 89 (1988).
11. P. A. Baeuerle and D. Baltimore, *ibid.* **242**, 540 (1988).
12. R. Sen and D. Baltimore, *Cell* **46**, 705 (1986); *ibid.* **47**, 921 (1986); M. Lenardo, J. W. Pierce, D. Baltimore, *Science* **236**, 1573 (1987).
13. J. W. Pierce, M. Lenardo, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1482 (1988).
14. B. Nelsen, L. Hellman, R. Sen, *Mol. Cell. Biol.* **8**, 3526 (1988).
15. M. J. Lenardo, A. Kuang, A. Gifford, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8825 (1988).
16. L. A. Chodosh, R. W. Carthew, P. Sharp, *Mol. Cell. Biol.* **6**, 4723 (1986).
17. K. Kawakami, C. Scheidereit, R. G. Roeder, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4700 (1988).
18. M. J. Leonard and D. Baltimore, unpublished results.
19. D. W. Ballard *et al.*, *Science* **241**, 1652 (1988).
20. M. Z. Gilman, R. N. Wilson, R. A. Weinberg, *Mol. Cell. Biol.* **6**, 4305 (1986).
21. B. Lückow and G. Schutz, *Nucleic Acids Res.* **15**, 5490 (1987).
22. N. Suzuki *et al.*, *J. Biol. Chem.* **262**, 5079 (1987); J. W. Lowenthal, E. Böhnlein, D. W. Ballard, W. C. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4468 (1988).
23. J. D. Dignam, R. M. Lebowitz, R. G. Roeder, *Nucleic Acids Res.* **11**, 423 (1983).
24. We thank R. D. Klausner, A. Rabson, D. I. Cohen, K.-T. Jeang, and M. Sharon for valuable discussions and D. Baltimore for support and encouragement. Partially supported by the Cancer Research Institute (S.L.C.), by a National Cancer Institute Clinical Investigator Award (M.J.L.), and by an American Cancer Society and an NIH grant to D. Baltimore (M.J.L.).

18 November 1988; accepted 9 February 1989

Role of Na^+/H^+ Exchange by Interferon-γ in Enhanced Expression of JE and I-A_β Genes

VERONICA PRPIC, SHEAU-FUNG YU, FLORENCIO FIGUEIREDO, PAUL W. HOLLENBACH, GOWIND GAWDI, BRIAN HERMAN, RONALD J. UHING, DOLPH O. ADAMS

The rapid transductional sequences initiated by interferon-γ (IFN-γ) on binding to its receptor regulate functional and genomic responses in many cells but are not well defined. Induction of macrophage activation is an example of such functional and genomic changes in response to IFN-γ. Addition of IFN-γ to murine macrophages, at activating concentrations, produced rapid (within 60 seconds) alkalinization of the cytosol and a concomitant, rapid influx of $^{22}Na^+$. Amiloride inhibited the ion fluxes and the accumulation of specific messenger RNA for two genes induced by IFN-γ (the early gene JE and the β chain of the class II major histocompatibility complex gene I-A). The data indicate that IFN-γ initiates rapid exchange of Na^+ and H^+ by means of the Na^+/H^+ antiporter and that these amiloride-sensitive ion fluxes are important to some of the genomic effects of IFN-γ.

THE PLEOTROPIC ACTIONS OF IFN-γ include antiviral, antiproliferative, and immunoregulatory effects in a wide variety of responsive cells (*1*). One cellular target is the macrophage, which IFN-γ activates to present antigen to T lymphocytes and to kill facultative and obligate intracellular parasites and tumor cells (*2*). These regulatory effects are initiated when IFN-γ occupies a cell surface receptor, an event that leads to priming for protein kinase C activation, an oxidative burst, eicosanoid production, and the induction of genes coding for proteins such as class II major histocompatibility complex (MHC) molecules, also referred to as Ia molecules. Surface expression of Ia molecules is essential to functions of the activated macrophage and is stringently regulated (*3, 4*). The initial transductional events occurring after IFN-γ occupies its receptor have not been delineated (*5, 6*). We reported earlier that addition of IFN-γ to murine macrophages results in an enhanced efflux of $^{45}Ca^{2+}$ (*7*). Because this enhanced efflux was observed only after a lag of 5 to 10 min, we examined the possibility that it is preceded by more rapid ion fluxes. We now show that addition of IFN-γ to murine macrophages results in a rapid cytosolic alkalinization and an enhanced influx of $^{22}Na^+$. The ability of amiloride to inhibit these responses and to inhibit the accumulation of mRNA for the gene JE and the β chain of the class II MHC gene I-A (I-A_β), both of which are induced by IFN-γ, indicates the importance of Na^+/H^+ exchange in the IFN-γ–induced activation of murine macrophages.

IFN-γ at physiologic concentrations (10 to 100 units of recombinant IFN-γ per milliliter)—that is, at concentrations that induce macrophage activation for tumor cytotoxicity and induce surface expression of Ia—rapidly raised cytosolic *p*H by as much as 0.1 *p*H unit (Fig. 1A). An increase in intracellular *p*H (pH_i) was manifest within 1 min after IFN-γ was added to the macrophage cultures and continued for 10 to 15 min, after which pH_i began a slow decrease to basal levels (Fig. 1A). This alkalinization was inhibited by prior treatment of the macrophages with amiloride at concentrations that inhibit the Na^+/H^+ antiporter (*8*) (Fig. 1A). The increase in cytosolic *p*H was dose dependent and was observed with as little as 1 unit of IFN-γ per milliliter (*9*). In contrast, 50 units of interferon α/β per milliliter did not alter *p*H (*9*).

IFN-γ also induced the rapid influx of $^{22}Na^+$ into macrophages; this was readily observable within 2 min (176% ± 24% of the basal value; mean ± SEM, $n = 3$ experiments) after IFN-γ was added to the cultures (Fig. 1B). At these early times, the IFN-γ–induced influx of $^{22}Na^+$, when compared to the appropriate buffer control, was inhibited ~50% by prior exposure of the cultures to 0.05 m*M* amiloride and ~90% by prior exposure to 0.3 m*M* amiloride at the extracellular sodium concentrations used. Both the increase in IFN-γ–stimulated pH_i and $^{22}Na^+$ influx were also inhibited by amiloride analogs according to their relative efficacies for inhibition of the antiporter (*9*). As shown in Fig. 1, amiloride exhibited small inhibitory effects on basal $^{22}Na^+$ influx, as also described in other cells (*8*). The effects of IFN-γ on $^{22}Na^+$ influx were dose-dependent, and increases were observable with as little as 0.1 unit of IFN-γ per milliliter (*9*). The half-maximal dose for IFN-γ–stimulated, amiloride-inhibitable influx of sodium was ~7.5 units of IFN-γ per milliliter.

Addition of IFN-γ to macrophage cultures resulted in increased accumulation of mRNA for numerous genes, including the gene JE and the genes coding for Ia molecules such as I-A_β (*10*). The increased accumulation of mRNA for JE in macrophages is due to an increase in mRNA stability (*11*), whereas the increase in mRNA for Ia is transcriptionally regulated (*10*). We thus examined a potential role of Na^+/H^+ exchange in IFN-γ–mediated regulation of mRNA for JE and I-A_β. When amiloride

V. Prpic, S.-F. Yu, F. Figueiredo, P. W. Hollenbach, R. J. Uhing, D. O. Adams, Laboratory of Cell and Molecular Biology of Leukocytes, Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC 27710.
G. Gawdi and B. Herman, Laboratories for Cell Biology, Department of Cell Biology and Anatomy, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599.