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DEREGULATED C-FOS AUGMENTS THE EXPRESSION OF IL-2 GENE IN T CELLS STIMULATED WITH ANTI-CD3 ANTIBODY

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INDEXING WORDS

c-fos; transgenic mice; IL-2; AP-1; IL2-κB; NFAT

SYNOPSIS

The expression of IL-2 gene in T cells stimulated with immobilized anti-CD3 antibody is controled by several transcription factors like as AP-1, IL-2κB and NFAT. We studied an effect of deregulated c-fos, a component of AP-1, in the expression of IL-2 gene in T cells by using splenic T cells from transgenic mice carrying the exogenous c-fos gene under the control of the H2-K^b promoter (H2-c-fos). The IL-2 production of H2-c-fos T cells stimulated with anti-CD3 antibody was enhanced and prolonged. Levels of AP-1 and IL-2κB transcription factors in the nuclear extract from the H2-c-fos T cells were also augmented. These results suggest that c-Fos is one of the major regulatory factors for the expression of IL-2 gene in T cells activated with anti-CD3 antibody.

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INTRODUCTION

The activation of T cells through the T cell receptor/CD3 complex induces the expression of IL-2 gene that is crucial for subsequent T cell proliferation and differentiation.[4),14),22),23)] The IL-2 promoter contains several highly conserved regions like as AP-1, IL-2κB and NFAT binding sequences.[4),8)] Those factors regulate the level of IL-2 expression in the T cells.[9),10)] However, the role of each transcription factor in the IL-2 production is not known.

We have generated transgenic mice carrying the mouse c-fos gene under the control of the H2-K^b gene promoter (H2-c-fos).[24)] Splenic T cells from H2-c-fos mice constitutively produce a large amount of c-fos protein (c-Fos) that is a component of AP-1.[5)] In order to examine an effect of deregulated c-Fos on the expression of IL-2 gene, splenic T cells from H2-c-fos mice were stimulated with immobilized anti-CD3 antibody. IL-2 production of the T cells was augmented and prolonged. Levels of AP-1 and IL-2κB in nuclear extract from T cells were also enhanced. We discuss a role of c-Fos in the expression of IL-2 gene in T cells.

MATERIALS AND METHODS

Mice:

C57BL/6CrSlc mice were purchased from Japan SLC Co. Ltd. (Hamamatsu, Japan). Transgenic mice carrying the mouse c-fos gene under the control of the H-$2K^b$ gene promoter have been described elsewhere.[24)]

Preparation of splenic T cells:

Splenic T cells were isolated from spleen cells by a nylon wool

column.[24] Briefly, $5.0x10^7$ of spleen cells in 1ml of RPMI1640 medium (GIBCO) with 5% fetal calf serum (FCS) (FILTRON) were incubated in a nylon column at 37°C for 45 min. Then, the nylon wool column was washed with RPMI1640 medium plus 5% FCS and viable cells in the efferent were collected. The purity of $CD3^+$ T cells was more than 95% in viable cells. The purified T cells were resuspended in RPMI1640 supplemented with $5x10^{-5}$M 2-mercaptoethanol, 100μg/ml kanamycin and 10% FCS.

Anti-CD3 stimulation of splenic T cells:

Monoclonal anti-CD3 (145-2C11) antibody[15] (10μg/ml) was coated on culture plates (Corning, 60 or 100 mm Tissue Culture Dish) at 37°C for 60 min. The plates were washed 3 times with sterile PBS. Splenic T cells were cultured on the coated plate at 37°C for 4 days.

IL-2 assay:

Activity of IL-2 in supernatant from splenic T cell culture was measured by ^{3}H-thymidine uptake with CTLL-2 indicator cells according to the method described by Gillis et al.[7] Briefly, $5x10^5$ of CTLL-2 cells were cultured with serial dilutioned culture supernatant for 24 hours. The cultures were pulsed with 18.5 KBq of ^{3}H-thymidine (Amersham) for the final 4 hours. The cells were harvested onto glass filters. ^{3}H-thymidine uptake was measured by a liquid scintillation counter. IL-2 units were calculated by using the Probiot analysis as described by Gillis et al.[7]

RNase protection assays:

Levels of IL-2 and β2-microglobulin mRNA were determined by RNase protection assay, as described.[16] Briefly, total RNA from splenic T cells was extracted by the acid-guanidine method, as described.[3] Total RNA (10μg) was hybridized overnight with the ^{32}P(Amersham)-labeled RNA probes at 55°C in 80% formamide hybridization buffer, followed by

the presence of 0.5μg of poly dI-dC (Pharmacia). The resulting DNA-protein complexes were analyzed by electrophoresis at room temperature on 6% polyacrylamide gels.

The following oligonucleotides were used as probes in the electrophoretic mobility shift assays: the murine AP-1 binding site: 5'gggTTCTCCTCTGACTCAGGGTAA; the murine IL-2κB binding site: 5'gatcACCAAGAGGGATTTCACCTAAATCC; the murine NFAT binding site: 5'gatcGCCCAAAGAGGAAAATTTGTTTCATACAG

RESULTS

IL-2 production of H2-c-fos T cells stimulated with anti-CD3 antibody:

In order to examine the effect of deregulated c-Fos on IL-2 production of T cells, splenic T cells from H2-c-fos mice were stimulated with immobilized anti-CD3 antibody for 4 days. The activity of IL-2 in the culture supernatant was assayed by ^{3}H-thymidine uptake of indicator line, CTLL-2. As shown in Fig.1, IL-2 activity from H2-c-fos T cells was 4 times higher than that from control T cells. The high IL-2 activity from the H2-c-fos T cells was maintained 3 days longer than that from the control T cells.

To confirm the augmented IL-2 production by the H2-c-fos T cells, levels of IL-2 mRNA in total RNA from the T cells were analyzed by RNase protection assay. The level of IL-2 mRNA from the H2-c-fos T cells was 5 times more than that from the control T cells (Fig. 2). The high amount of IL-2 mRNA from the H2-c-fos T cells was also maintained 2 days longer than that from the control T cells.

Levels of transcription factors in nuclear extracts from H2-c-fos T cells stimulated with anti-CD3 antibody:

The expression of IL-2 gene in T cells is regulated by three different

digestion with RNaseA and RNaseT1 (Sigma) at 37°C for 1 hour. The protected fragments were separated on the 6% polyaclylamide gel. The dried gel was exposed to Kodak X-Omat AR films for 3 days at -70°C using intensifying screens (Dupont).

The following DNAs were used as templates to generate the anti-sense RNA probe by T7 (for IL-2) or SP6 (for β_2-microglobulin) RNA polymerase (Promega). IL-2: the 600bp PstI fragment of the mouse IL-2 cDNA[13] was cloned to the pGEM1 (pGEM-IL-2). This plasmid was linearized with HindIII. β_2-microglobulin: the 950bp HindIII-EcoRI fragment including the 238bp mouse β_2-microglobulin gene[19] was cloned to the pGEM4Z (pGEM-β_2-microglobulin). This plasmid was linearized with HindIII.

Preparation of nuclear extract:

Cultured cells were resuspended with hypotonic buffer (10mM HEPES, 1.5mM $MgCl_2$, 10mM KCl, 0.5mM EDTA, 0.5mM dithiothreitol, 0.5mM PMSF, 1μM leupeptin, and 1μM aprotinin ; pH 7.9) and sheared with 24 Gauge needle to disrupt cell membrane. After centifugation, nuclei were resuspended with extraction buffer (20mM HEPES, 10% glycerol, 420mM NaCl, 1.5mM $MgCl_2$, 0.2mM EDTA, 0.5mM dithiothreitol, 0.5mM PMSF, 1μM leupeptin, and 1μM aprotinin ; pH 7.9) and incubated on ice for 60 min to lyse nuclear membrane. Following centrifugation, the supernatant was rapidly frozen and stored at -70°C. Protein amount was estimated by the Micro BCA protein assay (Pierce Chemical).

Electrophoretic mobility shift assays:

Double-strnaded radiolabeled oligonucleotide probes were prepared in the presence of [α^{32}p]dCTP (Amersham) using the Klenow fragment of DNA polymerase I (Takara) and purified with ethanol precipitation. For each binding reaction, 50,000 cpm of labeled oligonucleotide was incubated for 20 min at room temperature with 5μg of nuclear extract in

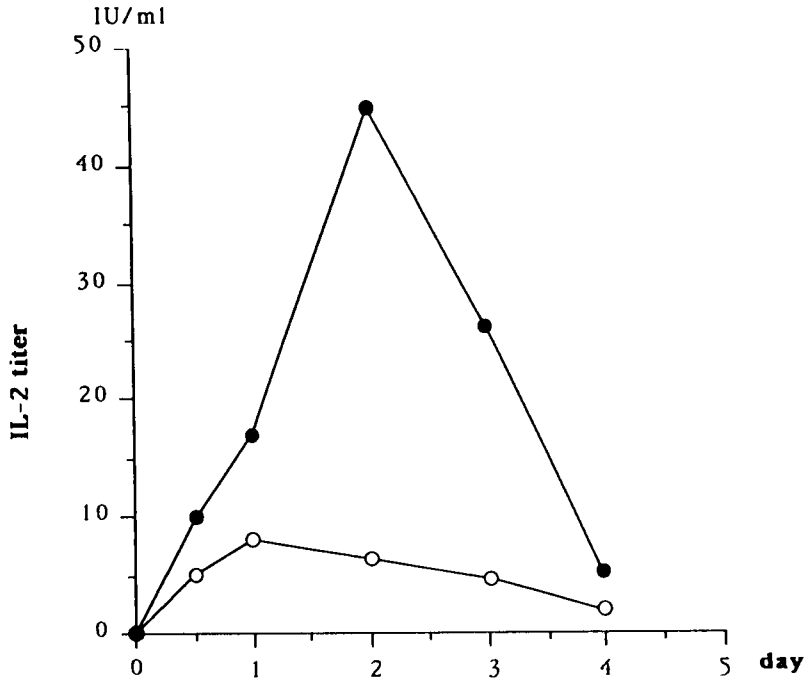

Fig. 1. Levels of IL-2 in culture supernatants from T cells stimulated with immobilized anti-CD3 antibody. Splenic T cells from control and H2-c-fos mice were cultured in the presence of immobilized anti-CD3 antibody. Activities of IL-2 in culture supernatants from control T cells (open circle) and H2-c-fos T cells (closed circle) were assayed by ^{3}H-thymidine uptake of indicator line, CTLL-2.

transcription factors, AP-1, IL-2κB and NFAT.[4),8),10),22)] Since the production of IL-2 from the H2-c-fos T cells was enhanced and prolonged, levels of these three DNA binding factors in nuclear extracts from the T cells were analyzed by electrophoretic mobility assay.

The level of AP-1 in nuclear extract from the H2-c-fos T cells was detectable even at pre-stage, increased to maximal level at 4 hours after the stimulation (Fig. 3). The high level was maintained until 24 hours after the stimulation. That from the control T cells became detectable at

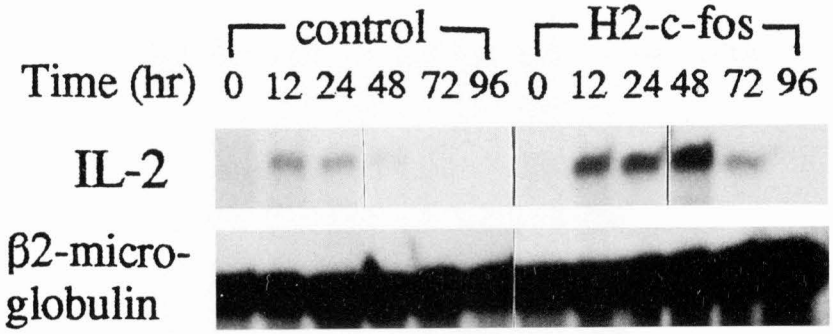

Fig. 2. The expression of IL-2 mRNA from T cells stimulated with anti-CD3 antibody. Splenic T cells from control and H2-c-fos mice were cultured in the presence of immobilized anti-CD3 antibody. Levels of IL-2 mRNA in total RNA from the T cells were measured by the RNase protection assay. Levels of β_2-microglobulin mRNA were the amount control of total RNA examined.

4 hours, slightly increased to the maximal level at 12 hours after the stimulation. The maximal level from the H2-c-fos T cells was 4 times higher than that from the control T cells.

Electrophoretic mobility assay for IL-2κB has demonstrated two specific bands.[9),17)] The upper band composed of heterodimer of p65 and p50 proteins, authentic NF-κB.[2),6)] The lower band contained the p50 homodimer proteins,[2),6)] and the transcriptional activity for the

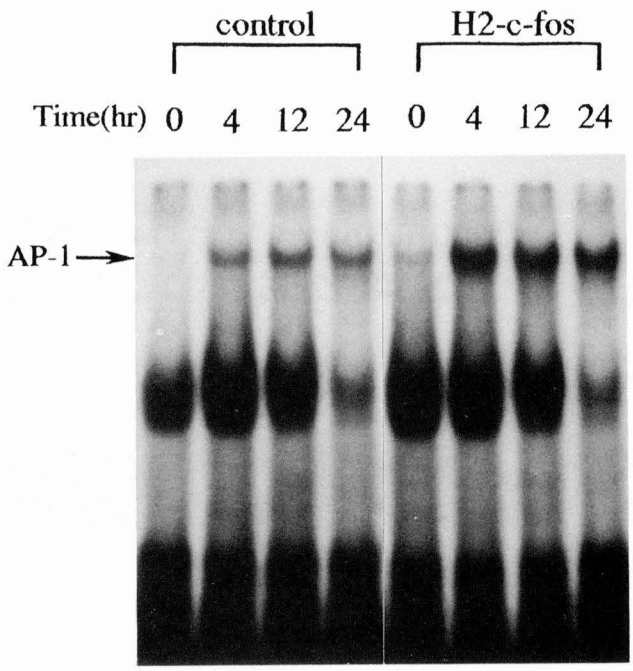

Fig. 3. Electrophoretic mobility assay for the AP-1 binding protein. Splenic T cells from control and H2-c-fos mice were cultured in the presence of immobilized anti-CD3 antibody. Nuclear extracts from the T cells were incubated with ^{32}P-labeled oligonucleotide which contains the AP-1 consensus sequence and analyzed by electro-phoresis on 6% polyacrylamide gels.

expression of IL-2 gene was unknown. As shown in Fig. 4, two bands were detected in the IL-2κB binding assay. The upper band from the H2-c-fos T cells but not from the control T cells was detectable even at pre-stage. The amount of the upper band from the H2-c-fos T cells increased to the highest level at 12 hours after the stimulation, although that from the control T cells increased until 24 hours after the stimulation. The peak level from the H2-c-fos T cells was 10 times

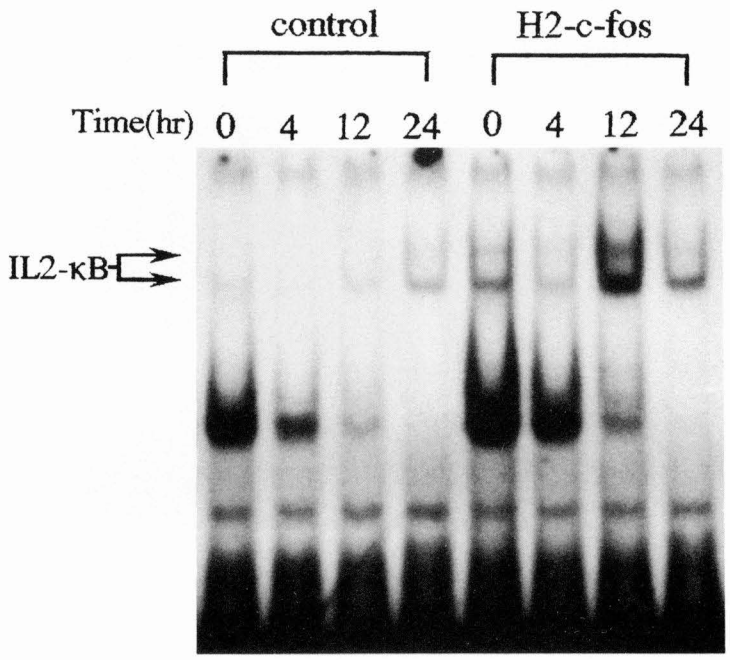

Fig. 4. Electrophoretic mobility assay for the IL-2κB binding protein. Splenic T cells from control and H2-c-fos mice were cultured in the presence of immobilized anti-CD3 antibody. Nuclear extracts from the T cells were incubated with ^{32}P-labeled oligonucleotide which contains the IL-2κB consensus sequence and analyzed by electrophoresis on 6% polyacrylamide gels.

higher than that from the control T cells.

The level of NFAT in nuclear extract from the H2-c-fos T cells was undetectable at pre-stage (Fig. 5). The level was detectable at 4 hours and was maintained until 24 hours after the stimulation. That from the control T cells gradually increased until 24 hours after the stimulation. The peak level from the H2-c-fos T cells was 2 times higher than that from the control T cells.

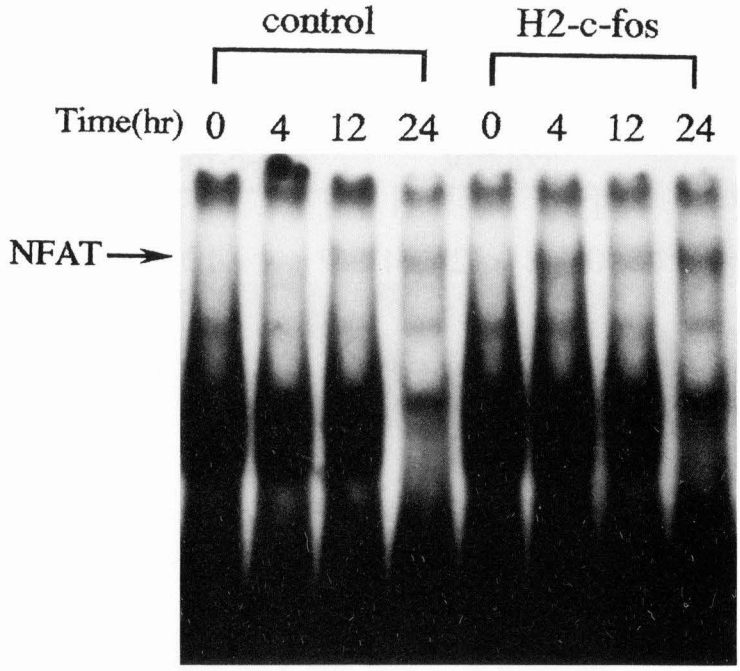

Fig. 5. Electrophoretic mobility assay for the NFAT binding protein. Splenic T cells from control and H2-c-fos mice were cultured in the presence of immobilized anti-CD3 antibody. Nuclear extracts from the T cells were incubated with ^{32}P-labeled oligonucleotide which contains the NFAT consensus sequence and analyzed by electro-phoresis on 6% polyacrylamide gels.

DISCUSSION

The expression of c-fos gene is transiently induced in T cells after the stimulation of anti-CD3 antibody.[10] When the exogenous c-fos gene is constitutively expressed in T cells from H2-c-fos mice,[24] the expression of IL-2 gene was augmented in the T cells activated with anti-CD3 antibody (Fig. 2). Since c-fos protein (c-Fos) expresses its transcriptional activity after the composition of AP-1 with jun gene products (Jun), the expression of jun gene was examined in T cells from H2-c-fos and control mice. The jun gene is a gene family and all of the jun family gene

products (c-Jun, JunB and JunD) can dimerize with c-Fos to compose AP-1.[5] JunB and junD but not c-jun mRNAs were detected in total RNA from T cells even at resting state (data not shown). Thus, the activity of AP-1 was detected in nuclear extracts from H2-c-fos T cells but not control T cells at resting state (Fig. 3). These results indicate that the activity of AP-1 is controled by the expression of c-fos gene in T cells. However, the presence of AP-1 is not enough to induce the expression of IL-2 gene in T cells (Fig. 2). This suggests that different transcription factors besides the AP-1 is required for the expression of IL-2 gene in T cells.

IL-2κB is one of the transcription factors to regulate the expression of IL-2 gene in T cells.[1),4),8),9)] The activity of IL-2κB (the upper band in Fig.4) was also detected in nuclear extract from H2-c-fos T cells at resting state (Fig. 4). Since the activity of IL-2κB was not detected in nuclear extract from control T cells at resting state, the results shown in Fig. 4 indicate that c-Fos affects the level of IL-2κB in T cells. This augmenting effect of c-Fos in resting H2-c-fos T cells was also demonstrated on the level of IL-2κB from H2-c-fos T cells stimulated with anti-CD3 antibody. However, the relation between the level of c-Fos and the activity of IL-2κB has not been reported. Since IL-2κB is consisted of heterodimer or homodimer of proteins such as p50 and p65,[2)] the expression of genes encoded those proteins may be regulated by the AP-1. Further investigations are necessary to clarify this mechanism.

The expression of IL-2 gene was not induced in the H2-c-fos T cells in the presence of AP-1 and IL-2κB at their resting state (Fig. 2), indicating that a third factor is also required for the expression of IL-2 gene in T cells. NFAT is one of the transcription factors to control the expression of IL-2 gene in T cells.[4),8)] The activity of NFAT binding protein was not detected in nuclear extract from the resting H2-c-fos T

cells (Fig. 5), suggesting that the NFAT is an essential factor for the expression of IL-2 gene in T cells. The activity of NFAT was slightly enhanced in the H2-c-fos T cells after the anti-CD3 stimulation (Fig. 5). These results partly support the notion that AP-1 is a component of NFAT.[11]

The kinetics of the activity of AP-1 but not IL-2kB and NFAT was consistent with the kinetics of the expression of IL-2 gene in the T cells stimulated with anti-CD3 antibody. These results suggest that AP-1 is the major transcription factor to regulate the expression of IL-2 gene in T cells. Promoter analysis of IL-2 gene with reporter genes has also demonstrated the major role of AP-1 among those transcription factors.[9] Indeed, no expression of IL-2 gene in anergic T cells stimulated with antigen is due to no induction of AP-1.[21] Since the activity of AP-1 is controled by the expression of c-fos gene in T cells, c-Fos may be the major regulatory factor for the expression of IL-2 gene in T cells.

In summary, the constitutive expression of c-fos gene in T cells augments the activities of AP-1 and IL-2κB followed by the enhancement of the expression of IL-2 gene in the T cells stimulated with anti-CD3 antibody. These results suggest that c-Fos is the major regulatory factor of the expression of IL-2 gene in T cells activated with anti-CD3 antibody.

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REFERENCES

1. Arima, N., Kuziel, W.A., Grdina, T.A., and Greene, W.C.: J. Immunol. 1992. 149. 83/91. IL-2 induced signal transduction involves the activation of nuclear NF-κB expression.
2. Baeuerle, P.A.: Biochi. Biophs. Acta. 1991. 1072. 63/80. The inducible transcription activator NF-κB: regulation by distinct protein subunits.
3. Chomczynski, P., and Sacchi, N.: Analytical Biochem. 1987. 162. 156/159. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction.
4. Crabtree, G.R.: Science. 1989. 243. 355/361. Contingent genetic regulatory events in T lymphocyte activation.
5. Curran, T., and Franza, B.R.: Cell. 1988. 55. 395/397. Fos and Jun : the AP-1 connection.
6. Fujita, T., Nolan, G.P., Ghosh, S., and Baltimore, D.: Genes & Dev. 1992. 6. 775/787. Independent modes of transcriptional activation by the p50 and p65 subunits of NF-κB.
7. Gillis, S., Ferm, M.M., Ou, W., and Smith, K.A.: J. Immunol. 1978. 120. 2027/2032. T cell growth factor : parameters of production and a quantitative microassay for activity.
8. Granelli-Piperno, A., and Nolan, P.: J. Immunol. 1991. 147. 2734/2739. Nuclear transcription factors that bind to elements of the IL-2 promoter.
9. Jain. J., Valge-Archer, V.E., Sinskey, A.J., and Rao, A.: J. Exp. Med. 1992. 175. 853/862. The AP-1 site at -150 bp, but not the NF-κB

site, is likely to represent the major target of protein kinase C in the Interleukin 2 promoter.

10. Jain, J., Valge-Archer, V.E., and Rao, A.: J. Immunol. 1992. 148. 1240/1250. Analysis of the AP-1 sites in the IL-2 promoter.
11. Jain, J., McCaffrey, P.G., Valge-Archer, V.E., and Rao, A.; Nature. 1992. 356. 801/804. Nuclear factor of activated T cells contains Fos and Jun.
12. Kang, S.M., Beverly, B., Tran, A.C., Brorson, K., Schwartz, R.H., and Lenardo, M.J.: Science. 1992. 257. 1134/1138. Transactivation by AP-1 is a molecular target of T cell clonal anergy.
13. Kashima, N., Nishi-Takaoka, C., Fujita, T., Taki, S., Yamada, G., Hamuro, J., and Taniguchi, T.: Nature. 1985. 313. 402/404. Unique structure of murine interleukin-2 as deduced from cloned cDNAs.
14. Kronke, M., Leonard, W.J., Depper, J.M., and Greene, W.C.: J. Exp. Med. 1985. 161 1593/1598. Sequential expression of genes involved in human T lymphocyte growth and differentiation.
15. Leo, O., Foo, M., Sachs, D.H., Samelson, L.E., and Bluestone, J.A.: Proc. Natl. Acad. Sci. USA. 1987. 84. 1374/1378. Identification of a monoclonal antibody specific for a murine T3 polypeptide.
16. Melton, D.A., Kreig, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K., and Green, M.R.: Nucl. Acid. Res. 1984. 12. 7035/7056. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter.
17. Molitor, J.A., Walker, H.A., Doerre, D., Ballard, D.W., and Greene, W.C.: Proc. Natl. Acad. Sci. USA. 1990. 87. 10028/10032. NF-κB: A family of inducible and differentially expressed enhancer-binding proteins in human T cells.
18. Nakabeppu, y., and Nathans, D.: EMBO J. 1989. 8. 3833/3841. The basic region of Fos mediates specific DNA binding.

19. Parnes, J.R., and Seidman, J.G.: Cell. 1982. 661/669. Structure of wild type and mutant mouse β_2-microglobulin genes.

20. Ransone, L.J., Wamsley, P., Morley, K.L., and Verma, I.M.: Mol. Cell. Biol. 1990. 10. 4565/4573. Domain swapping reveals the modular nature of Fos, Jun and CREB proteins.

21. Schmid, R.M., Perkins, N.D., Duckett, C.S., Andrews, P.S., and Nabel, G.J.: Nature. 1991. 352. 733/736. Cloning of an NF-κB subunit which stimulates HIV transcription in synergy with p65.

22. Shaw, J.P., Utz, P.J., Durand, D.B., Toole, J.J., Emmel, E.A., and Crabtree, G.R.: Science. 1988. 241. 202/205. Identification of a putative regulator of early T cell activation genes.

23. Smith, K.A.: Annu. Rev. Immunol. 1984. 2. 319/333. Interleukin-2.

24. Takao, S., Sakai, N., Hatano, M., Koizumi, T., Hanioka, K., Rüther, U., and Tokuhisa, T.: Int. Immunol. 1991. 3. 369/375. IgG response is impaired in H2-c-fos transgenic mice.