Molecular basis of a multiple lymphokine deficiency in a patient with severe combined immunodeficiency

Emanuela Castigli*, Rajendra Pahwa†, Robert A. Good‡, Raif S. Geha*, and Talal A. Chatila*

*Division of Allergy and Immunology, Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115; †Department of Pediatrics, North Shore University Hospital, Manhasset, NY 11030; and ‡Department of Pediatrics, All Children's Hospital, University of South Florida, St. Petersburg, FL 33701

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ABSTRACT We have previously reported that the T lymphocytes of a child with severe combined immunodeficiency are defective in the transcription of several lymphokine genes that include *IL2*, *IL3*, *IL4*, and *IL5*, which encode interleukins 2, 3, 4, and 5 (IL-2, -3, -4, and -5). To determine whether the defect in the patient's T lymphocytes involved a trans-acting factor common to the affected lymphokine genes, we examined the ability of nuclear factors from the patient's T lymphocytes to bind response elements present in the regulatory region of *IL2*. Nuclear factor NF-kB, activation protein 1 (AP-1), OCT-1, and NF-IL-2B binding activity were normal. In contrast, the binding of the nuclear factor of activated T cells (NF-AT) to its response element in the *IL2* enhancer and to an NF-AT-like response element present in the *IL4* enhancer was abnormal. To ascertain whether the abnormal NF-AT binding activity was related to an impaired function, we transfected patient and control T lymphocytes with constructs containing the reporter gene encoding chloramphenicol acetyl transferase (CAT) under the control of the entire *IL2* regulatory region or of multimers of individual enhancer sequences. CAT expression directed by the *IL2* regulatory region or by a multimer of the NF-AT-binding site was markedly lower in the patient relative to controls. In contrast, CAT gene expression directed by a multimer of the OCT-1 proximal (OCT-1p)-binding site was equivalent in patient and controls. These results indicate that an abnormality of/or influencing NF-AT may underlie the multiple lymphokine deficiency in this patient.

Induction of lymphokine gene expression is mediated by trans-acting factors that interact with specific DNA elements present in the regulatory region of these genes. The regulation of interleukin 2 (IL-2) gene (designated *IL2*) expression is one of the best characterized among the T cell-specific lymphokine genes. *IL2* transcription requires the binding of common regulatory factors, such as OCT-1, activation protein 1 (AP-1), and nuclear factor NF-kB, which are involved in the regulation of the expression of several genes in lymphoid as well as nonlymphoid cells (reviewed in ref. 1) (Fig. 1). In addition, *IL2* expression requires specific regulatory factors like NF-AT, the nuclear factor of activated T cells (1). Expression of NF-AT, like the expression of *IL2*, is T cell-restricted (2) and tightly linked to T-cell activation (3, 8–10).

In a previous report we described a child with severe combined immunodeficiency with a normal number of circulating T cells and poor T-lymphocyte proliferation to mitogens, which was corrected *in vitro* and *in vivo* by recombinant IL-2 (rIL-2) (11). Further studies showed that the patient's T lymphocytes were defective in the expression of IL-2, IL-3, IL-4, and IL-5 mRNAs as a result of decreased transcription of *IL2*, *IL3*, *IL4*, and *IL5*. The expression by T lymphocytes of other cytokines such as granulocyte/
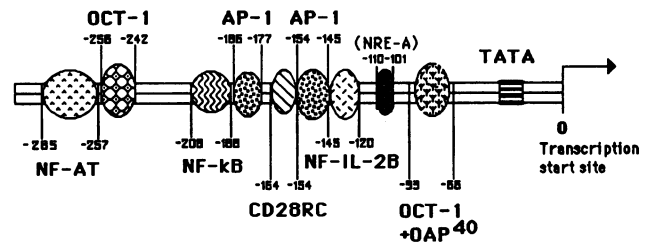

Fig. 1. Transacting factors binding their response elements in the enhancer of *IL2*. The numbers represent the 5' position relative to the transcription start site (0). Nuclear factors are NF-AT, OCT-1, NF-kB, AP-1, NF-IL-2B (1–4), and OCT-1 + OCT-1 associated protein (OAP⁴⁰) (5). Recently two more trans-acting factors involved in *IL2* regulation have been described. CD28 stimulation induces expression of a CD28 response complex (CD28RC), which possesses enhancer activity (6). The second one suppresses *IL2* expression and binds a sequence called "negative response element A" (NRE-A) (7).

macrophage colony-stimulating factor and interleukin 6, both of which are not T lymphocyte-restricted, was not affected (12). A defect in a T lymphocyte-specific trans-acting protein required for the transcription of *IL2* and other T cell-restricted lymphokine genes could account for impaired lymphokine expression.

To address the question of whether a regulatory factor could be defective in the patient's T lymphocytes, we used electrophoretic mobility shift assays (EMSA) to examine the binding activities present in nuclear extracts from patient T lymphocytes and specific for response elements (binding sites) present in the *IL2* and *IL4* enhancer. We also examined the ability of trans-acting factors/response elements to direct the transcription of a reporter gene, chloramphenicol acetyltransferase (CAT), in patient and control T lymphocytes.

We demonstrate here that the patient's T lymphocytes have an abnormality in the binding activity of NF-AT. This abnormality was determined to be functionally important because it is accompanied by defective NF-AT–enhancer activity.

MATERIALS AND METHODS

T-Cell Lines. T-cell lines were prepared from the peripheral blood mononuclear cells of the patient or healthy volunteers by stimulation with phytohemagglutinin P (Difco) (12).

Nuclear Extracts. T lymphocytes were incubated with medium in the absence or presence of 20 ng of phorbol 12-myristate 13-acetate (PMA) (Sigma) per ml and 0.5 μM ionomycin (Calbiochem) for 2, 4, and 8 hr. Nuclear extracts

Abbreviations: IL-2, -3, -4, and -5, interleukins 2, 3, 4, and 5; rIL-2, recombinant IL-2; NF-AT, nuclear factor of activated T cells; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate; AP-1, activation protein 1; OCT-1p, OCT-1 proximal; TCGF, T-cell growth factor.

were prepared as described by Ohlsson *et al.* (13), with minor modifications in the composition of buffers A and C and in the procedure for preparation of the nuclei. Briefly 5–10 × 10^8 T lymphocytes were washed with phosphate-buffered saline (PBS), resuspended in buffer A containing 2 μg of protease inhibitors (leupeptin, antipain, and pepstatin; Sigma) per ml and 0.03% Nonidet P-40 (Sigma), and kept on ice for 5 min. Nuclei were then pelleted at 200 × *g* and resuspended in buffer C containing 2 μg of protease inhibitors per ml. All other steps were performed exactly as described in ref. 9.

Oligonucleotides. Double-stranded oligonucleotides containing the NF-AT-binding site or NF-IL-2B site from the *IL2* enhancer (Fig. 1) (2), the NF-AT-like site from the *IL4* enhancer (14), and a mutant NF-AT response element were gifts of G. R. Crabtree (Stanford University School of Medicine, Stanford, CA). The Oct-1 proximal (OCT-1p) site (of the *IL2* enhancer) (Fig. 1) (2) and the AP-1 site, derived from the human metallothionein II_A gene promoter (15), were synthesized on an Applied Biosystems DNA synthesizer 391. The NF-kB site, derived from the k light chain gene enhancer (16), was purchased from GIBCO/BRL.

EMSA. Double-stranded oligonucleotides were end-labeled with the T4 polynucleotide kinase (New England Biolabs)/[γ-^{32}P]ATP (New England Nuclear) method. Binding reactions were carried out in 16–20 μl (final volume) of binding buffer (17) containing 2.5–5 μg of nuclear proteins, 2–4 μg of poly(dI-dC) (Pharmacia LKB), and 0.05–0.1 ng of ^{32}P-end-labeled double-stranded oligonucleotide. For competition experiments, 100-fold molar excess of unlabeled double-stranded oligonucleotides were added together with the labeled ones. The binding mixtures were incubated for 60 min at room temperature when NF-AT and NF-IL-2B were tested and for 30 min at 4°C when OCT-1p, NF-kB, or AP-1 were analyzed. DNA–protein complexes were then resolved on a 5% polyacrylamide gel in 0.5× TBE (90 mM Tris/90 mM boric acid/2 mM EDTA, pH 8) at 4°C followed by autoradiography.

Plasmid Constructs. p-IL-215ΔCX, p22-6ΔXho, and pg42CAT constructs respectively contained the −319 to +52 sequence of *IL2*, the minimal *IL2* promoter spanning −70 to +52, and the γ-fibrinogen promoter, placed upstream from the CAT gene. NF-AT-CAT(×3) and OCT-1p CAT(×4) constructs contained three and four copies, respectively, of the NF-AT or OCT-1p sites from the enhancer of *IL2*, all of them placed upstream of the γ-fibrinogen promoter (2). All of these plasmids were provided by Gerald R. Crabtree. AP-1-CAT(×3), containing three copies of the AP-1 site from the human metallothionein II_A gene promoter (15), and NF-kB-CAT(×4), containing four copies of the sequence for the NF-kB site from the k light chain enhancer (16), were constructed by ligating multimerized AP-1 and NF-kB oligonucleotides to the CAT-promoter plasmid (Promega). The plasmids were purified by equilibrium centrifugation in CsCl/ethidium bromide gradient and were sequenced by standard procedures (14).

Transfection and CAT Assay. DEAE-dextran-mediated transfection procedure was used to introduce plasmid DNA in the T lymphocytes. Patient and control T-cell lines were stimulated with phytohemagglutinin P at 10 μg/ml in the presence of irradiated allogeneic peripheral blood mononuclear cells, partially purified T-cell growth factor (TCGF) (12), and 10 units of rIL-2 per ml. The cells were harvested 4–5 days later, centrifuged over Ficoll, washed, resuspended at 20 × 10^6 cells per ml in 1 ml of a buffer (137 mM NaCl/5 mM KCl/0.4 mM Na_2HPO_4/25 mM Tris·HCl/0.1 mg of $MgCl_2$ per ml/0.1 mg of $CaCl_2$ per ml, pH 7.4) containing 20 μg of plasmid DNA and 0.25 mg of DEAE-dextran (Sigma), and incubated for 15 min at room temperature. The cells were then resuspended in 10 ml of RPMI 1640 medium containing 10% (vol/vol) of fetal calf serum (FCS) and incubated for 30 min at 37°C. Thereafter, the cells were pelleted, resuspended

in 20 ml of RPMI 1640 medium containing 10% FCS, 2.5% TCGF, and rIL-2 at 10 units/ml, and equally divided in two flasks. Approximately 40 hr after transfection, one of the aliquots was stimulated with 20 ng of PMA per ml and 0.5 μM ionomycin. Eight hours later, the cells were spun down, washed with PBS at 4°C, and resuspended in 150 μl of 0.25 M Tris·HCl (pH 8). Cell extracts and the CAT assay were performed by following the Promega *Protocol and Applications Guide*. CAT activity was equalized for the protein content (cpm/μg). In some experiments, the T lymphocytes were cotransfected with pCMV-human growth hormone (hGH) internal control plasmid (pCMV-GH) (18) to evaluate the transfection efficiency. The hGH values were used to normalize the CAT specific activity.

Protein Assay. Protein concentrations were determined with bicinchoninic acid (BCA) protein assay reagent (Pierce).

RESULTS

We used EMSA to analyze the specific binding of nuclear proteins from patient and control T lymphocytes to functionally important sequences present in the *IL2* enhancer. EMSA performed with an oligonucleotide containing the OCT-1p-binding site of the *IL2* enhancer (2) revealed a specific retarded band when using nuclear extracts from control T lymphocytes either unstimulated or stimulated for 2, 4, and 8 hr with PMA and ionomycin (Fig. 2A, lanes 6–10). The same shift pattern was obtained with nuclear extracts from patient T lymphocytes (Fig. 2A, lanes 2–5). When nuclear extracts from stimulated T lymphocytes were used for the binding to oligonucleotides containing AP-1-, NF-kB-, or NF-IL2B-binding sites, we detected similar migration patterns in both patient and control (Fig. 2B, lanes 3–6 and 8–13; Fig. 2C, lanes 3–5 and 7–10; and Fig. 2D, lanes 3–7 and 9–13). This binding activity was either not present or weakly expressed in nuclear extracts from unstimulated patient and control T lymphocytes (Fig. 2B, lanes 2 and 7; Fig. 2C, lanes 2 and 6; and Fig. 2D, lanes 2 and 8). In contrast, using an oligonucleotide containing the NF-AT response element, we detected an abnormality in the shift pattern obtained with nuclear extracts from the patient T lymphocytes. Fig. 3A shows that stimulation with PMA and ionomycin for 2 hr induced an NF-AT binding activity in the nuclear extracts from the patient T lymphocytes that was comparable to the control (Fig. 3A, compare lane 3 with lane 9). However at 4 and 8 hr poststimulation, the patient DNA–NF-AT complex exhibited faster mobility as compared with the control (Fig. 3A, compare lanes 10 and 11 with lanes 4 and 5).

To evaluate whether such abnormal binding activity was common to NF-AT-like elements present in the enhancer of other lymphokine genes, we tested the ability of nuclear extracts from activated patient and control T lymphocytes to bind to an NF-AT-like sequence present in the enhancer of *IL4*. This sequence, which spans between −122 and −150 upstream from the transcription initiation site (14), has 68% homology with the NF-AT sequence in the *IL2* enhancer. Cross-competition experiments with oligonucleotides containing the *IL2* or the *IL4* NF-AT-like response element demonstrated the specificity of the *IL4* sequence for NF-AT (data not shown). Interestingly, EMSA performed with this sequence and nuclear extract from the patient T lymphocytes showed an abnormal shift pattern similar to that obtained with the NF-AT response element of the *IL2* enhancer (compare lanes 10 and 11 of Fig. 3A with lanes 10 and 11 of Fig. 3B).

The protein subunits of NF-AT have not been fully characterized. However NF-AT has been recently described as a protein complex having at least two components: a nuclear component that is inducible upon T-lymphocyte activation but is not T cell-restricted and a constitutive cytoplasmic component that is T cell-restricted and that translocates to the nucleus after a rise in intracellular free Ca^{2+} concentra-
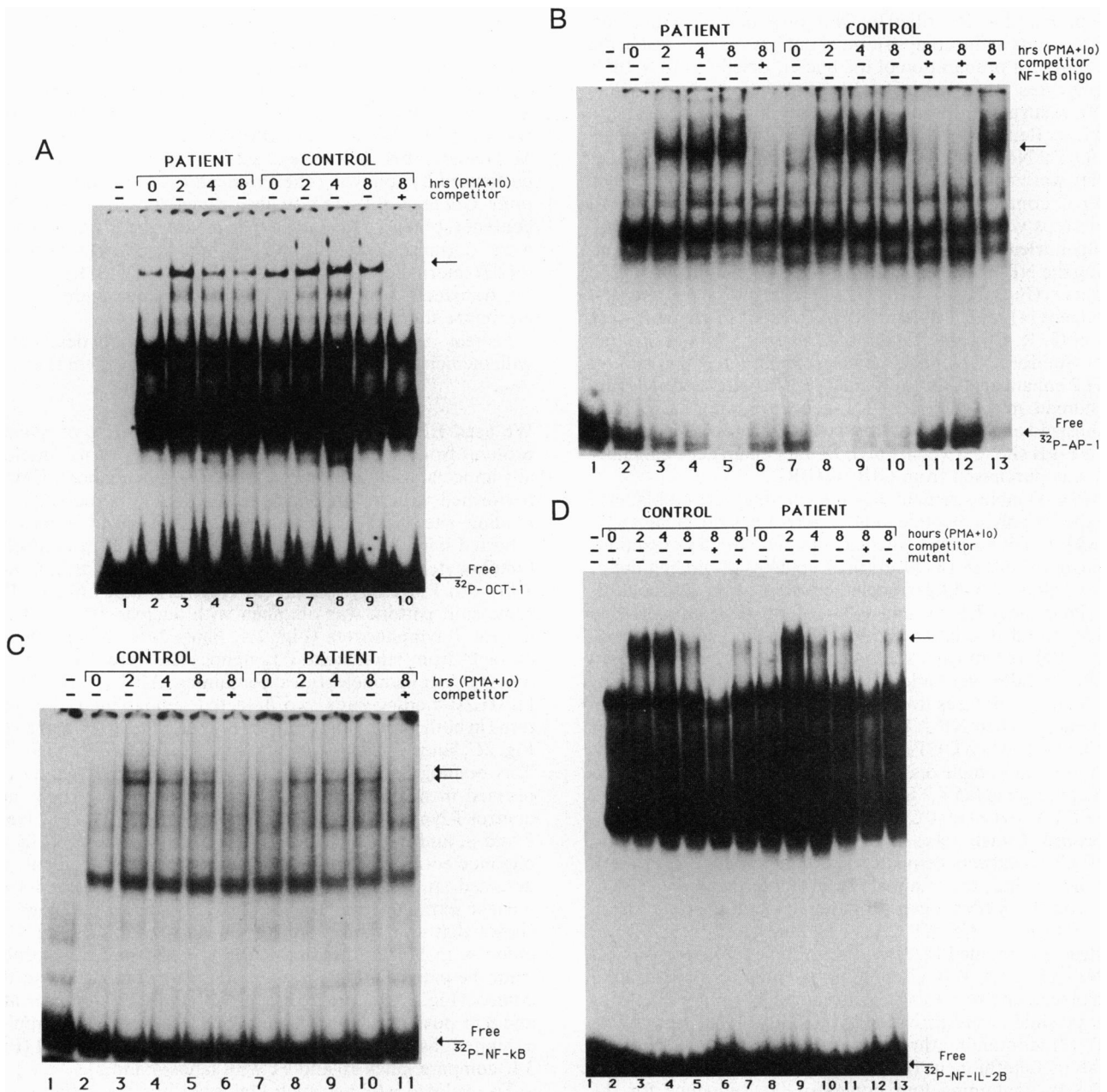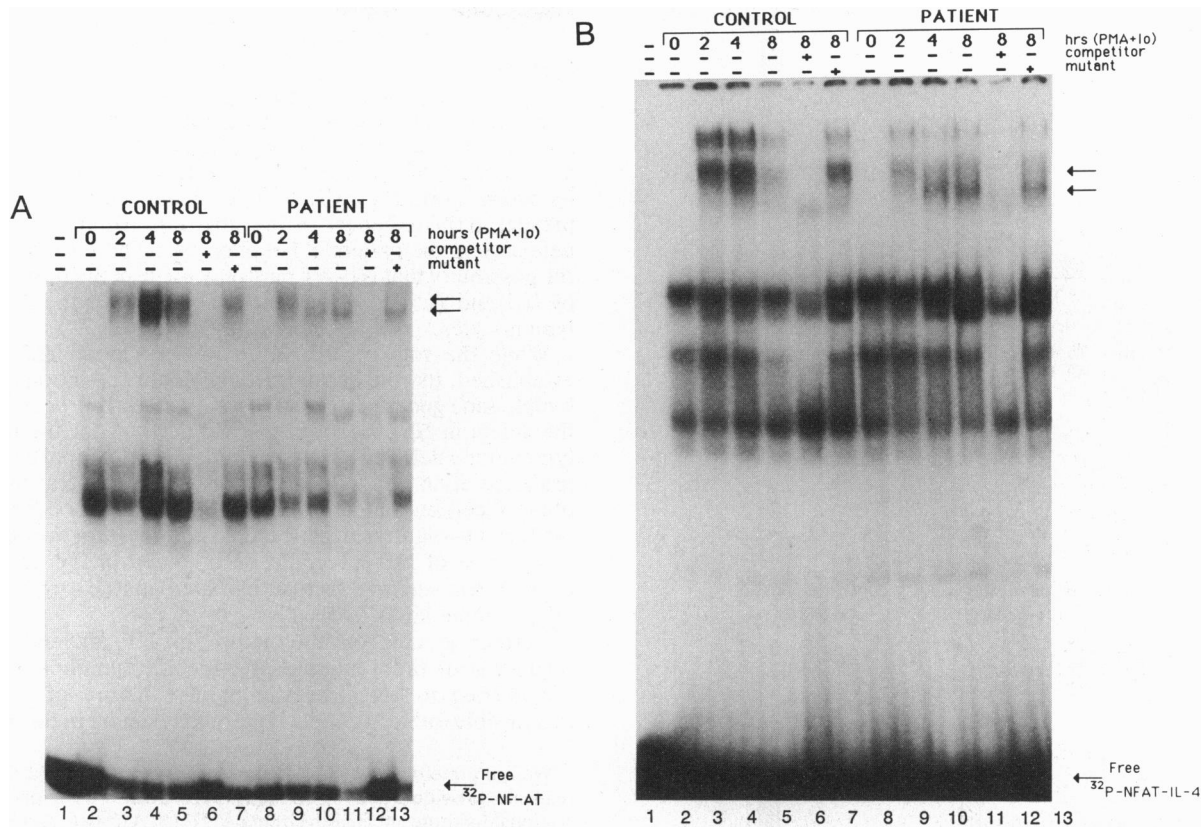
FIG. 2.   Interaction of nuclear proteins from patient and control T lymphocytes with functional response elements present in the enhancer of *IL2*. DNA–protein complexes were formed between ^{32}P-end-labeled double-stranded oligonucleotides and nuclear extracts from patient and control T lymphocytes stimulated with PMA and ionomycin (Io). ^{32}P-end-labeled OCT-1p (*A*), AP-1 (*B*), NF-kB (*C*), and NF-IL-2B (*D*) oligonucleotides were incubated in the absence (lane 1) or in the presence of nuclear extracts from patient and control T lymphocytes that were either left unstimulated (lanes 0), or stimulated for 2, 4, or 8 hr. The arrows indicate the specific bands inhibited by the addition of unlabeled oligonucleotides (competitor) and not inhibited by the addition of an unlabeled unrelated sequence (NF-kB) in *B*, lane 13, or by a mutant of NF-AT sequence in *D*, lanes 7 and 13.

tion (19). The cytoplasmic and the nuclear components combine in the nucleus, and the resulting complex binds the DNA. To examine whether the abnormal DNA–NF-AT complex was missing one of the identified components, we used preparations containing the cytoplasmic or the nuclear component isolated from Jurkat cells (gift of G. R. Crabtree) to complement the nuclear extracts from patient T lymphocytes stimulated for at least 4 hr. The results are shown in Fig. 4. Neither the nuclear (Fig. 4, lane 6) nor the cytoplasmic (Fig. 4, lane 7) components were able to restore the normal electrophoretic mobility (Fig. 4, lanes 2 and 12) when added to the nuclear extracts from activated patient T lymphocytes. The combination of the nuclear and the cytoplasmic components from Jurkat (Fig. 4, lanes 8 and 9) gave rise to a binding

activity that exhibited faster electrophoretic mobility compared with the control DNA–NF-AT complex (Fig. 4, compare lane 8 with lane 12), in agreement with the observation of Flanagan *et al.* (19). Interestingly this migration was similar to that observed with the nuclear extracts from the patient's T cells (Fig. 4, compare lane 8 with lane 4).

To analyze whether the abnormal NF-AT binding activity was associated with an impaired enhancer function, we transiently transfected patient and control T lymphocytes with constructs in which either the regulatory region of *IL2* or multimers of the NF-AT site linked to the γ-fibrinogen promoter were inserted upstream of the CAT reporter gene. Inducible CAT activity was then determined in cell extracts from transfected T lymphocytes that were either left unstim-

FIG. 3. Interaction of nuclear proteins present in nuclear extracts from patient and control T lymphocytes with response sequences present in the enhancer of *IL2* and *IL4*. DNA–protein complexes were formed between ^{32}P-labeled NF-AT (*A*) or NFAT-IL-4 (*B*) double-stranded oligonucleotides and nuclear extracts from patient and control T lymphocytes stimulated with PMA and ionomycin (Io). The same results were obtained with nuclear extracts obtained from two separated preparations. ^{32}P-end-labeled oligonucleotides were incubated in the absence (lane 1) or in the presence of nuclear extracts from patient and control T lymphocytes which were either left unstimulated (lanes 2 and 8), or stimulated for 2 (lanes 3 and 9), 4 (lanes 4 and 10), or 8 (lanes 5–7 and 11–13) hr. Arrows indicate the induced bands inhibited by the addition of 100-fold molar excess of unlabeled oligonucleotide (lanes 6 and 12) and not inhibited by NF-AT mutant (lanes 7 and 13).

ulated or were stimulated for 8 hr with PMA and ionomycin. Induction of CAT expression via the *IL2* regulatory region was severely depressed in the stimulated patient T lymphocytes (<2 times the activity of unstimulated cells) compared with control cells (up to a 9-fold increase) (Fig. 5*A*). Most important, induction of CAT expression via NF-AT enhancer activity was significantly reduced (by up to two-thirds) in patient T lymphocytes compared with controls (Fig. 5*B*). The defective NF-AT enhancer function was specific because the enhancer function of another inducible nuclear factor (5), OCT-1p, was comparable in patient and control T lymphocytes (Fig. 5*C*). The defect in the NF-AT enhancer function was also reproduced when patient T lymphocytes were compared with T-cell lines derived from different donors (data not shown). Transfections with constructs in which multimers of AP-1 or NF-kB sites directed the transcription of CAT gene resulted in similarly high basal CAT activity in both patient and control T lymphocytes, which was not further induced upon stimulation with PMA and ionomycin (data not shown). These findings might reflect the transfection conditions; culture of transfected T lymphocytes with TCGF and rIL-2 during the 40 hr after transfection could have induced AP-1 and NF-kB via signal transduction pathways activated by the interaction of IL-2 with its receptor or of other growth factors with their receptors. In contrast, NF-AT and OCT-1p are both transcription factors that are responsive only to signals derived from the T-cell receptor.

DISCUSSION

We have demonstrated that the binding and the enhancer activity of NF-AT are selectively defective (Figs. 3*A* and 5*B*) in a patient with a multiple lymphokine deficiency.

The faster electrophoretic mobility of the DNA–NF-AT complex indicated that the patient T lymphocytes are missing a component of the NF-AT protein complex. Complementation experiments, performed with nuclear extracts from the patient's T cells and the cytoplasmic or the nuclear compo-
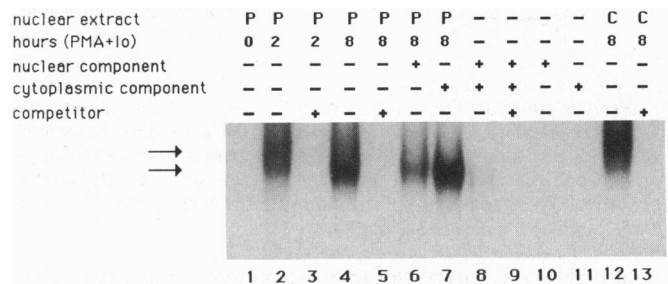
FIG. 4. Reconstitution experiments using nuclear extracts from patient T lymphocytes and nuclear and/or cytoplasmic components from Jurkat cells. DNA–protein complexes were formed between a ^{32}P-labeled double-stranded NF-AT oligonucleotide (0.1 ng) and nuclear extracts (2.5 μg or 5 μg in lanes 6 and 7) from patient T lymphocytes unstimulated (lanes 1–5) or stimulated with PMA and ionomycin (Io) for 2 hr (lanes 2 and 3) or 8 hr (lanes 4–7) to which 5 μg of preparations from Jurkat cells containing the nuclear (lane 6) or the cytoplasmic component (lane 7) were added. Labeled NF-AT oligonucleotide was also incubated with 5 μg of the nuclear (lane 10) or 5 μg of the cytoplasmic (lane 11) or the nuclear and the cytoplasmic preparations from Jurkat cells (lanes 8 and 9) and with the nuclear extract from stimulated control T lymphocytes (lanes 12 and 13). Arrows indicate the induced bands inhibited by the addition of unlabeled NF-AT oligonucleotide (lanes 3, 5, 9, and 13).
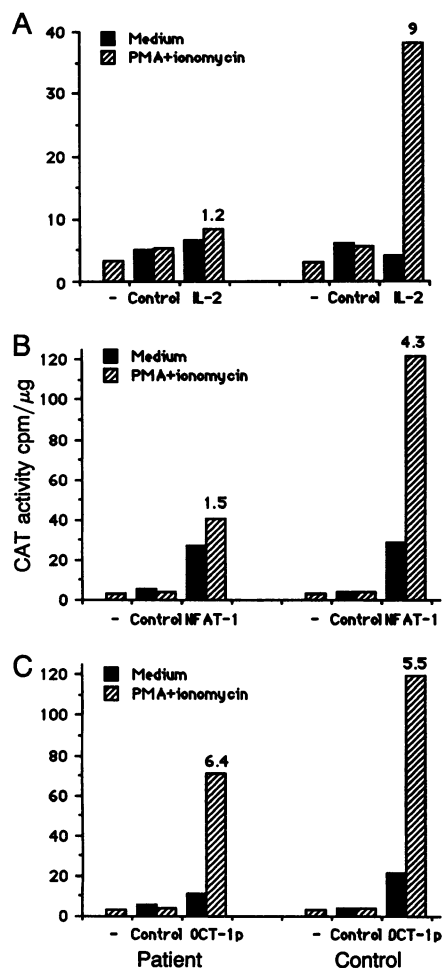
FIG. 5. Enhancer activity in PMA- and ionomycin-stimulated patient and control T lymphocytes transiently transfected with expression vectors containing the entire IL2 regulatory region (IL2 bars) or the IL-2 minimal promoter (Control bars) coupled upstream of the CAT gene (A); three copies of NF-AT site from the IL2 enhancer fused to the γ-fibrinogen promoter (NF-AT bars) or the γ-fibrinogen promoter alone (Control bars), which are in turn ligated to the CAT gene (B); or four copies of OCT-1p site from the IL2 enhancer driving the transcription of the CAT gene through γ-fibrin-ogen promoter (OCT-1p bars) or the γ-fibrinogen promoter alone (Control bars) (C). Bars labeled "−" represent the background measured with untransfected cells. CAT activities were normalized for protein concentration and transfection efficiency and are ex-pressed as cpm/μg. The numbers over the hatched bars indicate the fold increase of the CAT activity in transfected T lymphocytes (ratio of cpm/μg of stimulated cells:cpm/μg of unstimulated cells). In A and C, one representative experiment of two and in B, one repre-sentative experiment of six performed with the same plasmid and the same control T cell line are shown.

nent of NF-AT, suggested that the patient's T lymphocytes lacked a component of the NF-AT protein complex that was not present either in the nuclear or cytoplasmic components of NF-AT already identified in Jurkat T cells (Fig. 4) (19).

At present we can only speculate on the nature of the patient NF-AT defect. The defect may directly involve a component of NF-AT. Alternatively, the NF-AT defect may be the result of a distinct abnormality in the activation pathways regulating NF-AT assembly. Because of its faster mobility, the patient DNA–NF-AT complex seems to lack an-as-yet unidentified component that is important for NF-AT function as a transcriptional activator (Figs. 3 A and C and 5 B and A). The delayed appearance of the aberrant DNA–NF-AT complex suggests a susceptibility of a mutated

component of NF-AT to proteolysis. Alternatively, the miss-ing component could be a protein that must be rapidly synthesized or modified upon stimulation. A defect affecting the availability or the regulation of this component could account for the observed abnormality in NF-AT.

This defect affected not only the NF-AT interaction with its response element present in the enhancer of IL2 but also its interaction (Fig. 3B) with a similar response element present in the enhancer of IL4, the transcription of which is defective in the patient T lymphocytes. These findings raise the possibility that NF-AT may play a role in the transcription of IL3 and IL5, which are also defective in the patient T lymphocytes.

While the role of NF-AT in the regulation IL2 is well established, its role in the transcriptional activation of other lymphokine genes remains to be identified. It is possible that the defect in NF-AT activity could account for the multiple lymphokine deficiency in this child. This is supported by the presence of an NF-AT-like response element in at least one other T cell-restricted lymphokine gene that is defective in the patient—namely, IL4. It is also possible that the defective component of NF-AT could be involved in the activity of distinct trans-acting factors involved in the regulation of other lymphokines in T cells.

Further studies on the nature of NF-AT and on the regulation of the T lymphocyte-specific lymphokine genes are needed to elucidate the precise nature of the gene responsible for the multiple lymphokine defect in this patient.

1. Ullman, K. S., Northrop, J. P., Verweij, C. L. & Crabtree, G. R. (1990) Annu. Rev. Immunol. 8, 421–452.
2. Durand, D. B., Shaw, J., Bush, M. R., Replogle, R. E., Belagaje, R. & Crabtree, G. R. (1988) Mol. Cell. Biol. 8, 1715–1724.
3. Durand, D. B., Bush, M. R., Morgan, J. G., Weiss, A. & Crabtree, G. R. (1987) J. Exp. Med. 165, 395–407.
4. Kamps, M. P., Corcoran, L., LeBowitz, J. H. & Baltimore, D. (1990) Mol. Cell. Biol. 10, 5464–5472.
5. Ullman, K. S., Flanagan, M. W., Edwards, C. A. & Crabtree, G. R. (1991) Science 254, 558–562.
6. Fraser, J. D., Irving, B. A., Crabtree, G. R. & Weiss, A. (1991) Science 251, 313–316.
7. Williams, T. M., Moolten, D., Burlein, J., Romano, J., Bhaerman, R., Godillot, A., Mellon, M., Rausher, F. J., III, & Kant, J. A. (1991) Science 254, 1791–1794.
8. Siebenlist, U., Durand, D. B., Bressler, P., Holbrook, N. J., Norris, C. A., Kamoun, K., Kant, J. A. & Crabtree, G. R. (1986) Mol. Cell. Biol. 6, 3042–3049.
9. Riegel, J. S., Richie, E. R. & Allison, J. P. (1990) J. Immunol. 144, 3611–3618.
10. Granelli-Piperno, A., Nolan, P., Inaba, K. & Steinman, R. M. (1990) J. Exp. Med. 172, 1869–1872.
11. Pahwa, R., Chatila, T., Pahwa, S., Paradise, C., Day, N. K., Geha, R., Schwartz, S. A., Slade, H., Oyaizu, N. & Good, R. A. (1989) Proc. Natl. Acad. Sci. USA 86, 5069–5073.
12. Chatila, T., Castigli, E., Pahwa, R., Pahwa, S., Chirmule, N., Oyaizu, N., Good, R. A. & Geha, R. S. (1990) Proc. Natl. Acad. Sci. USA 87, 10033–10037.
13. Ohlsson, H. & Edlund, T. (1986) Cell 45, 35–44.
14. Arai, N., Nomura, D., Villaret, D., DeWaal Malefijt, R., Seiki, M., Yoshida, M., Minoshima, S., Fukuyama, R., Maekawa, M., Kudoh, J., Shimizu, N., Yokota, K., Abe, E., Yokota, T., Takebe, Y. & Aray, K. (1989) J. Immunol. 142, 274–282.
15. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) Cell 49, 729–739.
16. Sen, R. & Baltimore, D. (1986) Cell 46, 705–716.
17. Emmel, E. A., Verweij, C. L., Durand, D. B., Higgins, K. M., Lacy, E. & Crabtree, G. R. (1989) Science 246, 1617–1620.
18. Skalnik, D. G., Strauss, E. C. & Orkin, S. H. (1991) J. Biol. Chem. 266, 16736–16744.
19. Flanagan, W. M., Corthesy, B., Bram, R. J. & Crabtree, G. R. (1991) Nature (London) 352, 803–807.