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Atypical Signaling Defects Prevent IL-2 Gene Expression in *lpr/lpr* CD4–CD8– Cells

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Abstract

T cells with CD4–CD8– (double negative, DN) phenotype in MRL-*lpr/lpr* mouse serve as a model to establish the correlation between the extremely low IL-2 gene expression and the specific signaling inactivation. The extent of non-responsiveness in *lpr* DN cells was distinctive in several unusual defects. First, the poor IL-2 production in *lpr* DN cells could not be restored by supplement of signals known to augment IL-2 response in normal T cells. Second, the activations of both mitogen-activated protein (MAP) kinase and c-Jun N-terminal kinase (JNK) were attenuated in *lpr* DN cells upon direct activation by TPA/A23187. Third, IL-2 mRNA was degraded much faster in *lpr* DN cells than that in normal T cells. Fourth, of the four major transcriptional elements on IL-2 promoter, only AP-1 and nuclear factor of activated T cells (NFAT)-binding activities were suppressed in *lpr* DN T cells. Altogether, these results suggest that an extremely low level of IL-2 production in *lpr* DN T cells was due to both the increased instability of mRNA and the reduced activation of IL-2 gene promoter, the latter defect could be attributed to the inactivation of AP-1 and NF-AT as well as the poor activation of the upstream MAP kinase and JNK.

Key Words
Fas
lpr/lpr
SLE
IL-2 gene expression
JNK
MAP kinase
Signaling defect

MRL mice homozygous for the *lpr* (lymphoproliferative) mutation (MRL-*lpr/lpr*) develop spontaneous systemic lupus erythematosus (SLE)-like syndromes [11]. Defects in *lpr* mice are due to inactivation of *Fas* gene caused by insertion of retroviral transposon into the second intron [1, 45, 48]. Fas (APO-1), a 45-kD membrane protein, triggers programmed cell death when cross-linked by anti-Fas antibody or Fas ligand [35, 49]. Among the signaling components downstream of the Fas-initiated signal, the activation of acidic sphinogomyelinase which leads to the hydrolysis of sphingomyelin and generation of ceramide is well characterized [9, 16].

Lymphadenopathy in MRL-*lpr/lpr* mice consists primarily of T cells with unusual phenotypes (CD4–CD8–, CD2–, IL-2R–, TCR-αβ+, CD3+, B220+) [32, 47]. *Lpr* DN population is distinct from other T cells in that little IL-2 could be produced in response to mitogenic stimulation or TCR/CD3 engagement [3, 24]. The defect in IL-2 gene activation is not due to absence of TCR signalling, as evidenced by the increase of intracellular calcium and the generation of inositol phosphate [5]. Nonresponsiveness of *lpr* DN T cells has been correlated with the absence of CD2 [6]. *Lpr* DN cells also differ from normal T lymphocytes by the constitutive tyrosine phosphorylation of CD3ζ, increased level of c-myb, and overexpression of

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p59^{fyn} [25, 33, 39], suggesting that these T cells are previously activated. In addition, *lpr* DN T cells express a high level of CD28 [14]. Such selective abnormality in *lpr* DN T-cell signaling provides an opportunity to probe a link between T-cell stimulation and IL-2 gene expression. Results from this study reveal unusual features in *lpr* DN cells not previously reported in other T cells, and indicate that nonresponsiveness of *lpr* CD4–CD8– cells was a result of multiple defects in kinase activity, transcriptional activation, and mRNA stability.

Methods

Mice and Reagents. MRL-*lpr/lpr* and MRL-+/+ mice and their breeders were obtained from Jackson Labs (Bar Harbor, Me., USA). 14- to 18-week-old mice of both strains were used in the present study. Most chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). C_2-ceramide was obtained from Biomol (Plymouth Meeting, Pa., USA). Anti-mouse CD28 37.51 was a generous gift of Dr. Nan-Shih Liao (Institute of Molecular Biology, Academia Sinica). The following antibodies were purchased from Santa Cruz Biotech. (Santa Cruz, Calif., USA): anti-ERK2 antibody (C-14), anti-RelA(p65) antibody (A), anti-c-Rel antibody (N), anti-p50 antibody (NLS), anti-c-Jun (D) and anti-IκB-α antibody (C-15). Anti-NFAT$_p$ antibody was obtained from Upstate Biotech. (Lake Placid, N.Y., USA). Anti-JNK1 polyclonal antibody Ab101 [31] was a kind gift of Dr. Tse-Hua Tan (Baylor College of Medicine, Houston, Tex., USA).

T-Cell Isolation. Splenic and lymph node T lymphocytes from MRL-+/+ and MRL-*lpr/lpr* mice were isolated by panning twice on plates precoated with goat anti-mouse Ig antibody (Sigma). DN T cells were further purified by incubating with anti-CD4 (RL-172.4) and anti-CD8 (3.155), and followed by 1/20 volume of rabbit complement (Cedarlane, Ont., Canada). The final DN population was >98% CD4–, CD8–, and surface Ig– as analyzed on FACScan (Becton Dickinson, Palo Alto, Calif., USA). IL-2 was quantitated by the proliferation of IL-2-dependent cell line HT-2 (ATCC CRL 1841) [26] and using recombinant IL-2 as standard.

Oligonucleotides. All oligonucleotide probes were synthesized and purified as previously described [10, 27]. The sequences of the oligonucleotides are: metallothionein AP-1, 5′ gatcCGTGACTCAGCGCG; AP-1 of −180 bp murine IL-2 promoter, 5′ tcgaTAAATCCATTCAGTCAGTGTATG; NFAT of murine IL-2 promoter, 5′ gatcAAAGAGGAAAATTTGTTTCATACAG; Oct site, 5′ gatcGTGCTCATGAATATGCAAATCAATTGG; NFκB of IL-2 promoter, tcgaACCAAGAGGGATTTCACCTAAATCC; NFκB of Ig κ chain, 5′ tcgaGAGGGGACTTTCCGAGGGGACTTTCCGAGA [22].

Quantitation of IL-2 mRNA. 2 μg of total RNA was used for cDNA synthesis using oligo-dT as primer [26]. One tenth of the cDNA synthesized was then amplified in the presence of each serially diluted IL-2 MIMIC (Clontech, Palo Alto, Calif., USA) according to the manufacturer's protocol. RNA stability was determined by adding cyclosporin A (100 μg/ml) in T-cell culture preactivated with TPA/A23187 for 7 h, and then the RNA was isolated at different times for quantitation of IL-2 mRNA. The half-life of mRNA was calculated by second-order polynomial curve fit on CA-Cricket Graph III (Computer Associates, Islandia, N.Y., USA).

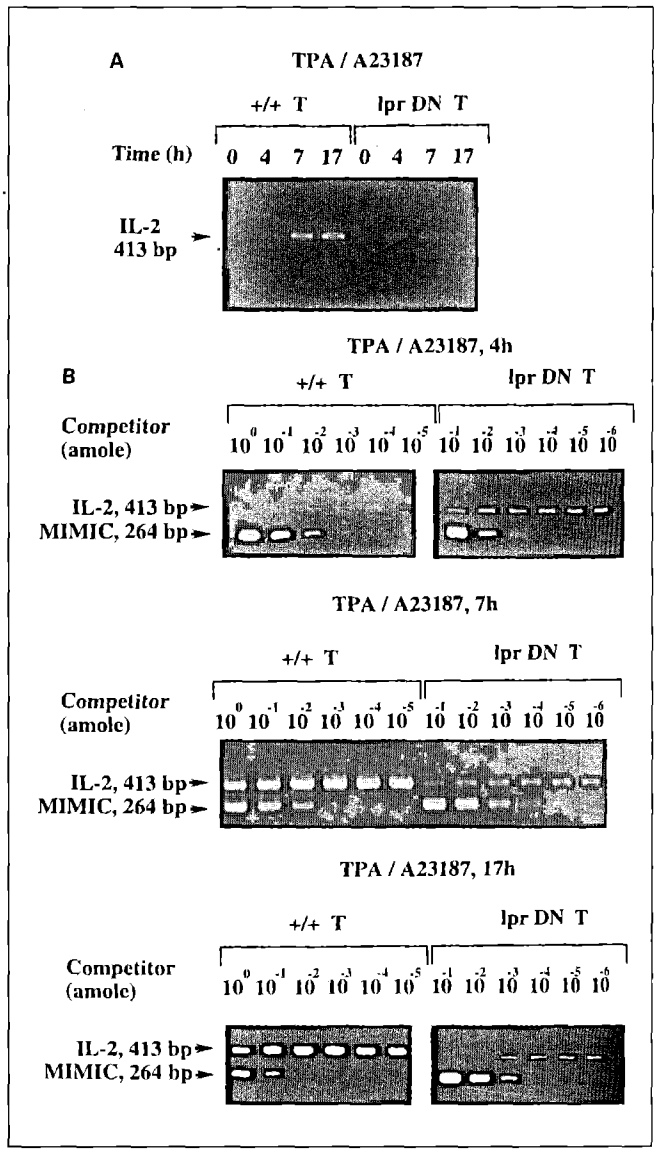

Fig. 1. A IL-2 mRNA in activated *lpr* DN T cells was much lower than +/+ T cells. Total T lymphocytes or DN lymphocytes were isolated from 14- to 18-week-old mice as described in Methods. Total RNA were isolated 4, 7 and 17 h after activation. The amount of IL-2 cDNA was determined by IL-2 MIMIC (Clontech), where intensity of MIMIC product equaled the IL-2 product. **B** Further PCR reaction with twofold dilution of MIMIC indicated that IL-2 mRNA (in amol, 10^{-18} mol) was: 4 h, 2×10^{-3} for +/+ T cells and 8×10^{-3} for *lpr* DN cells; 7 h, 2×10^{-1} for +/+ and 10^{-4} for *lpr* DN; 17 h, 8×10^{-1} for +/+ and 10^{-4} for *lpr* DN.

Others. Nuclear extract preparation and electrophoretic mobility shift assay (EMSA) were performed as previously described [27, 28]. The supershifting was performed by preincubating nuclear extract with 1 μg of antibody for 10 min at 4 °C prior to probe addition. The preparation of total cell extract and the assays for MAP kinase and JNK have been previously described [19, 20].

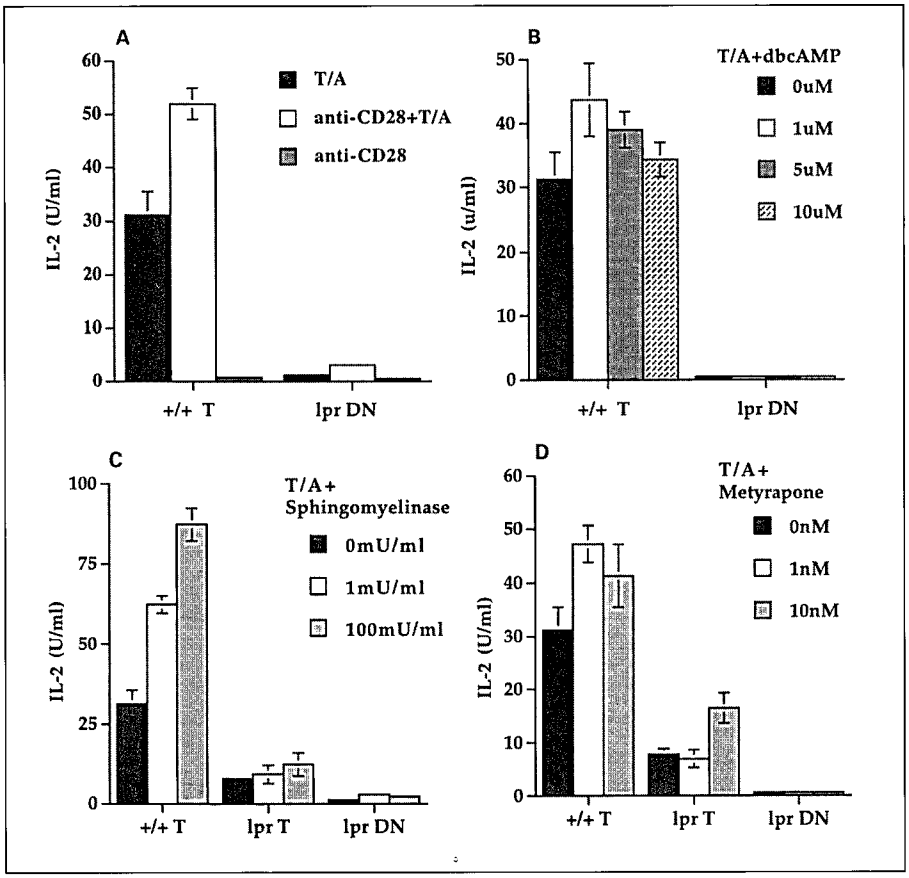

Fig. 2. Failure to restore IL-2 secretion in *lpr* DN T cells by CD28, cAMP, sphingomyelinase, and metyrapone. +/+ T cells, *lpr* total T cells, or *lpr* DN T cells were stimulated with TPA/A23187 in the presence of (**A**) immobilized anti-CD28 (coated at 2 μg/ml), or indicated concentration of (**B**) dibutyryl cAMP (dbc), (**C**) sphingomyelinase, and (**D**) metyrapone. Supernatant were collected 24 h later, and IL-2 was quantitated by the proliferation of HT-2 cells. Mean of triplicate with SE is shown.

Results

IL-2 mRNA Was at least 2,000 Times Lower in lpr *DN T cells than That in Normal T Cells.* T cells from 14- to 18-week-old MRL-+/+ and MRL-*lpr/lpr* mice and DN T cells from *lpr* mice of the same age were purified. Because *lpr* T cells differ from normal T cells in the expression of surface accessory molecules, T cells were directly activated with TPA/A23187. Little IL-2 secretion of *lpr* DN T cells was detected in all combinations of TPA/A23187 (TPA in 5–50 ng/ml and A23187 in 15–150 ng/ml). A competitive RT-PCR method was used to quantitate IL-2 mRNA in normal T cells and *lpr* DN T cells. Consistent with the suggestion that *lpr* DN T cells are previously activated [25, 29, 33], IL-2 mRNA was significantly higher in *lpr* DN T cells than that in normal T cells 4 h after activation (fig. 1A). Competitive PCR indicated that IL-2 mRNA was 2×10^{-3} amol in +/+ T cells and was 8×10^{-3} amol in *lpr* DN population (fig. 1B). However, the level of IL-2 mRNA in normal T cells rapidly exceeded that in *lpr* DN T cells (fig. 1A). Seven hours after activation, IL-2 mRNA was 0.2 and 10^{-4} amol in +/+ T cells and in *lpr* DN T cells, respectively (fig. 1B). The amount of IL-2 mRNA in activated *lpr* DN T cells was only 1/2,000 of that in normal T cells. In T cells activated 17 h after activation, the content of IL-2 mRNA was 0.8 amol in +/+ T cells and 10^{-4} amol in *lpr* DN cells. Therefore, the poor IL-2 production in *lpr* DN cells was attributed to an extremely low level of IL-2 gene expression.

IL-2 Secretion Was Not Rescued by Supplementing a Second Signal in lpr *DN Cells.* The poor stimulation of IL-2 production in *lpr* DN T cells by TPA/A23187 clearly indicates an intracellular signal defect. We have thus tested if this can be rescued by some reagents that stimulate IL-2 secretion or mimic Fas signal. First, *lpr* DN cells are distinct from their normal counterpart by expression of CD28 in the resting stage [14]. Immobilized anti-CD28 (2 μg/ml) enhanced the IL-2 generation by nearly 70% in +/+ T cells (fig. 2A). In contrast, anti-CD28 had only a weak induction of IL-2 in *lpr* DN T cells, that the amount of IL-2 stimulated by TPA/A23187/CD28 in *lpr* DN cells was less than 10% of that in +/+ T cells.

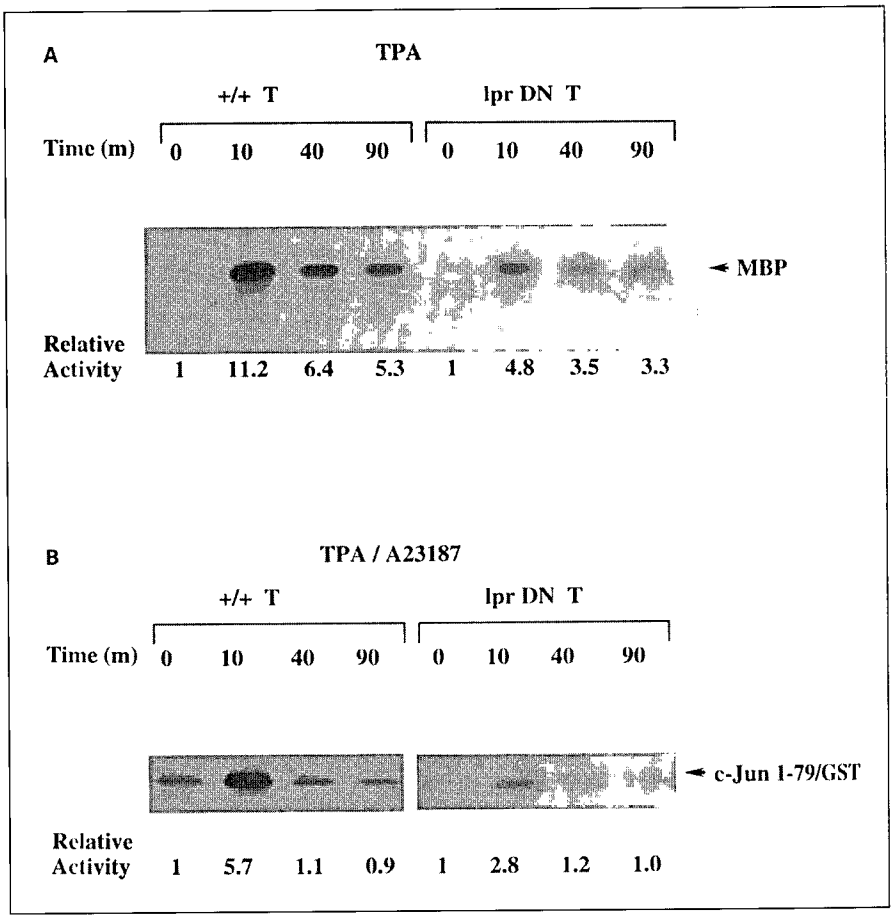

Fig. 3. Poor activation of MAP kinase and JNK in *lpr* DN T cells. +/+ T cells and *lpr* DN T cells were treated with either (**A**) TPA (10 ng/ml) or (**B**) TPA/A23187. Cell extracts were prepared at the indicated time points. **A** 200 μg of lysate was precipitated with 1 μg of anti-ERK2 C-14 antibody and protein A-Sepharose. The MAP kinase activity of the immune complexes was determined using myelin basic protein (MBP) as substrate of phosphorylation. **B** JNK assay was performed by incubating 400 μg cell extracts with anti-JNK1 Ab101 [31] and protein A-Sepharose, followed by kinase reaction using GST-c-Jun(1-79) as the substrate. JNK activity in unstimulated T cells of each mice strain was defined used as 1 relative kinase activity. Mean value of two independent measurements is shown.

Second, the nonresponsiveness of *lpr* DN T cells has been linked to the lack of CD2 expression [6]. Part of the CD2-induced signal is transmitted by intracellular cAMP [7, 17]. A low dose of cAMP was thus included in the activation of T cells by TPA/A23187 (fig. 2B). 1 μ*M* of Bt_2cAMP increased IL-2 production by 40% in +/+ T cells. The addition of Bt_2cAMP did not have any enhancing effect on the IL-2 generation in *lpr* DN T cells stimulated by TPA/A23187. Therefore, the absence of cAMP did not account for the low expression of IL-2 in *lpr* DN T cells.

Third, *lpr* T cells are known for their defect in Fas expression [1, 45, 48]. Part of signals transmitted by Fas ligation are mediated through acidic sphingomyelinase activation and ceramide generation [9, 16]. Whether the nonresponsiveness of *lpr* DN T cells may be a consequence of lacking Fas-mediated signal was thus examined by including sphingomyelinase in the stimulation mixture. The activation of +/+ T cells in the presence of 1 mU sphingomyelinase increased twofold IL-2 production (fig. 2C). Elevation of sphingomyelinase to 100 mU further enhanced IL-2 generation. Although a very weak increase in IL-2 activation was found in total *lpr* T cells, sphingomyelinase had little effect on the activation of *lpr* DN cells. Similarly, C_2-ceramide treatment did not show any additive effect in activated *lpr* DN cells, while it did enhance IL-2 production in +/+ T cells (not shown).

We had further examined the effect of metyrapone, which has been implicated in enhancing IL-2 expression and in reducing lupus manifestation [40, 44]. Metyrapone increased IL-2 production in +/+ T cells up to 60% and significantly enhanced the IL-2 generation in total *lpr* T cells (fig. 2D). However, the augmented IL-2 production in total *lpr* T cells was still lower than the level of +/+ T cells. In addition, metyrapone was ineffective to promote IL-2 production in *lpr* DN cells. Therefore, *lpr* DN T cells represent a unique type of cells where direct activation by TPA/A23187 with supplement of various signals cannot restore normal IL-2 expression.

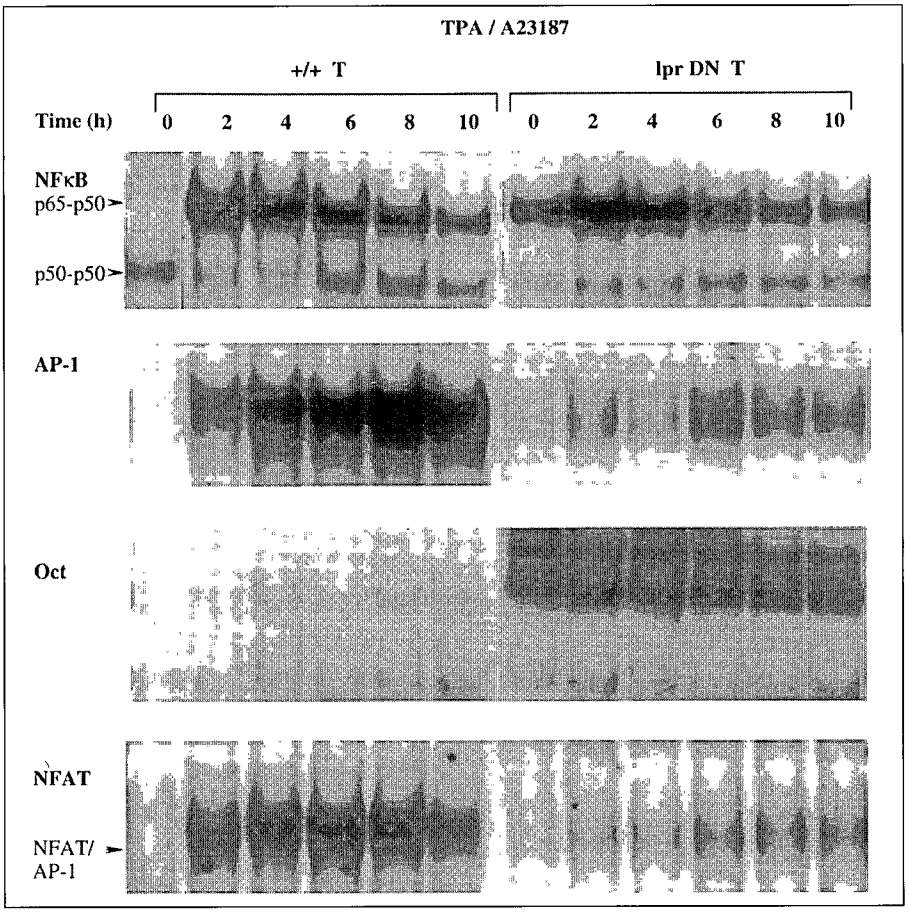

Fig. 4. Defective binding of AP-1 and NFAT in activated *lpr* DN T cells. Normal (+/+) and *lpr* DN T cells were stimulated with TPA/A23187, harvested at the indicated time for nuclear extracts preparation. 10 μg nuclear extract was incubated with radiolabeled oligonucleotide probes corresponding to binding site for NF-κB, AP-1, Oct, or NFAT at room temperature for 20 min. The binding mixtures were separated in 5% native polyacrylamide gel and autoradiography was performed. The upper NF-κB-binding complex represents p65-p50 heterodimer, and the lower complex is the p50-p50 dimer. The NFAT-binding complexes contain NFAT/AP-1 [38]. All EMSA experiments were repeated at least twice, and identical results were obtained.

Attenuated MAP Kinase and JNK Activation in lpr *DN T Cells.* Among the signaling pathways, we have examined the induction of MAP kinase and JNK, both are essential for IL-2 expression [19, 37, 42, 46]. TPA alone is an effective activator of MAP kinase in T cells [19] and was confirmed by the activation of MAP kinase (measured by the activity of the dominant form ERK2 in T cell) in +/+ T cells (fig. 3A). The induction of MAP kinase by TPA in *lpr* DN cells was at least 50% lower than that in +/+ T cells. *Lpr* DN T cells exhibited reduced JNK activation as compared to +/+ T cells. The basal JNK activity was already much lower in *lpr* DN T cells than in +/+ T cells prior to TAP/A23817 activation (fig. 3B). Both the induction ratio (expressed as relative activity in figure 3B) and the absolute activity of JNK was lower in *lpr* DN cells than in +/+ T cells. The reduced activation of MAP kinase and JNK by TPA/A23187 in *lpr* DN T cells was an interesting contrast to the tolerant T cells recently characterized [12, 29]. For the latter, MAPK/JNK were poorly activated by TCR engagement but can be fully stimulated by TPA/A23187. Therefore, *lpr* DN T cells represent the first prototype of T cells that are defective in MAPK/JNK activation even when stimulated with TPA/A23187.

IL-2 Promoter Activation in lpr *DN T Cells.* A recent study has attributed the defect of IL-2 gene expression in *lpr* DN T cells to the aberrant regulation of IL-2 promoter [10]. The IL-2 promoter activity was examined here by using oligonucleotides corresponding to NF-κB, NFAT, AP-1, and Oct [4] to quantitate the binding of the corresponding transcriptional factors by EMSA (fig. 4). The specificity of each DNA-protein complex was confirmed by competition with unlabeled specific or nonspecific oligonucleotides and by supershifting with antibodies against RelA(p65), p50, c-Rel, NFATp, and c-Jun (data not shown). The assignments of factor-DNA complex are identical to those previously documented [10, 38]. Resting +/+ T cells containing a κB-binding complex consist primarily of p50-p50 homodimer. Activation by TPA/A23187 led to appearance of p65-p50 binding complex which peaked in 4–6 h (fig. 4). This NF-κB complex (p65-

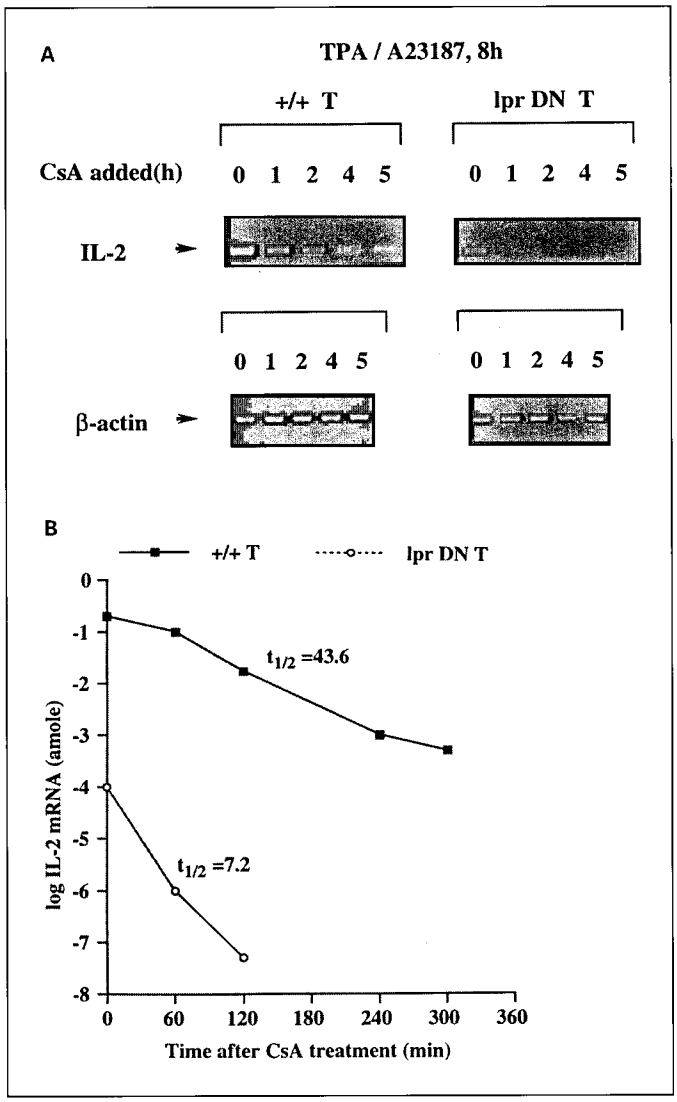

Fig. 5. Decreased stability of IL-2 mRNA in *lpr* DN T cells. T cells were activated with TPA/A23187 for 7 h, then cyclosporin A (100 μg/ml) was added in culture medium. Cells were harvested at the indicated intervals for RNA preparation. Direct PCR and competitive PCR were performed. **A** Direct amplified PCR products of IL-2 and β-actin mRNA. **B** Decay curve of IL-2 mRNA for +/+ T cells and *lpr* DN T cells. mRNA of *lpr* DN T cells with CsA treatment longer than 120 min was beyond the detecting limit of IL-2 MIMIC kit. The half-life was calculated by second-order polynomial curve fit. Average value of two measurements is shown.

p50) was present in the nucleus of the resting *lpr* DN T cells, which could be enhanced further upon activation. There existed a constitutive high level of Oct-binding protein in resting *lpr* DN T cells but not in +/+ T cells. On the contrary, the induced binding of NFAT and AP-1 was much lower in *lpr* DN cells than in +/+ T cells (fig. 4). In summary, IL-2 promoter-binding activities were different in *lpr* DN T cells in three aspects. First, NF-κB p65-p50 and Oct-binding complex were constitutively expressed in resting *lpr* DN cells but not in normal T cells. Second, the overall binding of Oct was much stronger in activated *lpr* DN T cells than that in activated normal T cells. Third, AP-1 and NFAT bindings were significantly attenuated in activated *lpr* DN T cells.

Accelerated IL-2 mRNA Degradation in lpr *DN T Cells.* We have examined if an additional mechanism controlling IL-2 expression was also affected in *lpr* DN cells. IL-2 mRNA is also known to be regulated by the rate of degradation [41, 43]. The half-life of IL-2 mRNA was determined by the rate of mRNA degradation after the addition of cyclosporin A [41] to block further RNA synthesis (fig. 5). The presence of cyclosporin A had no effect on the half-life of housekeeping gene mRNA such as β-actin (fig. 5A). The half-life of IL-2 mRNA determined for +/+ T cells was 43.6 min, which was very close to the value reported [41, 43]. Even though the low level of IL-2 mRNA in *lpr* DN T cells precluded an accurate determination, its half-life was estimated to be 7.2 min (fig. 5B), supporting that IL-2 mRNA in *lpr* DN T cells was degraded significantly faster than that in normal T cells.

Discussion

Dependence of IL-2 Gene Activation on MAPK/JNK. It has been shown that both MAP kinase and JNK are essential for both the activation of IL-2 gene and inhibition of each kinase interfering IL-2 gene activation [19, 37, 42, 46]. The poor IL-2 induction seen in *lpr* DN T cells therefore can be attributed, at least in part, to the insufficient activation of two major kinase cascades in T cells. The attenuation of MAP kinase and JNK activities in *lpr* DN T cells (fig. 3) did not lead to suppression of all transcriptional elements on IL-2 promoter (fig. 4). The selective attenuation of NFAT/AP-1 in *lpr* DN cells suggests their activation were dependent on MAP kinase and JNK cascade in T cells. JNK is able to phosphorylate and consequently activate of c-Jun [18]. It is evident that a poor stimulation of JNK results in insufficient activation of c-Jun, the major component of AP-1. The induction of c-fos, the other AP-1 element, requires the phosphorylation by MAP kinase [30]. It has also been shown that MAP kinase kinase, activator of MAP kinase, costimulates NFAT activity in Jurkat T cells [46]. Expression of MAP kinase kinase-negative mutant suppressed NFAT activation as well as IL-2 activation. The present results hence

serve as additional evidence that NFAT/AP-1 activation require both MAPK and JNK.

Multiple Defects in the Regulation of IL-2 Gene Expression. It has been shown that the activation of IL-2 promoter requires the full binding of each transcriptional element [4, 13, 38]. IL-2 promoter is inactivated by mutation of any single transcriptional site [38], or by cAMP inhibition of a single NF-κB site [8, 36]. In a similar scheme, the insufficient binding of AP-1/NFAT in *lpr* DN cells should diminish the transcriptional activation of IL-2 gene. It should be noted that unresponsive T cells have defective binding in AP-1 but not in NFAT, NF-κB, and Oct sites [15, 23]. The inactivation of AP-1/NFAT in *lpr* DN cells suggests that the degree of nonresponsiveness of these cells exceeds those reported for anergic cells before.

In addition, enhanced degradation of IL-2 mRNA in *lpr* DN cells contributed to the poor IL-2 expression. This is distinct from naive T cells in which IL-2 gene expression is determined mainly by transcription [4]. Granulocyte-macrophage colony-stimulating factor mRNA has been shown to be stabilized by TPA in EL4 [46]. T-cell activation also leads to stabilization of other mRNA [21]. It is thus possible that the decreased half-life of IL-2 mRNA is also correlated with the aberrant T activation observed in *lpr* T cells. The generation of DN T cells is caused by the absence of Fas molecule. It has been shown that Fas transduces activation signals in normal human T lymphocytes on certain occasions [2]. How the absence of Fas molecule leads to the development into T cells of unusual unresponsiveness deserves further characterization.

Acknowledgments

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References

1 Adachi M, Watanabe-Fukunaga R, Nagata S. Aberrant transcription caused by the insertion of an early transposable element in an intron of Fas antigen gene of *lpr* mice. Proc Natl Acad Sci USA 90:1756–1760;1993.

2 Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F, Lynch DH. Fas transduces activation signals in normal human T lymphocytes. J Exp Med 178: 2231–2235;1993.

3 Altman A, Theofilopoulos AN, Weiner R, Katz DH, Dixon FJ. Analysis of T cell function in autoimmune murine strains: Defects in production and responsiveness to interleukin-2. J Exp Med 1954:791–808;1981.

4 Brorson KA, Beverly B, Kang SM, Lenardo M, Schwartz RH. Transcriptional regulation of cytokine genes in nontransformed T cells: apparent constitutive signals in run-on assays can be caused by repeat sequences. J Immunol 147: 3601–3609;1991.

5 Budd RC, Winslow G, Inokuchi S, Imoden JB. Intact antigen receptor-mediated generation of inositol phosphates and increased intracellular calcium in CD4–CD8– T lymphocytes from MRL *lpr* mice. J Immunol 145:2862–2872; 1990.

6 Budd RC, Russel JQ, van Houten N, Cooper SM, Yagita H, Wolfe J. CD2 expression correlates with proliferative capacity of $\alpha\beta^+$ or $\gamma\delta^+$ CD4–CD8– T cells in *lpr* mice. J Immunol 148: 1055–1064;1992.

7 Carrera AC, Rincon M, De Landazuri MO, Lopez-Botet M. CD2 is involved in regulating cyclic AMP levels in T cells. Eur J Immunol 18: 961–964;1988.

8 Chen D, Rothenberg EV. Interleukin-2 transcription factors as molecular targets of cAMP inhibition: delayed inhibition kinetics and combinational transcription roles. J Exp Med 179:931–942;1994.

9 Cifone MG, Maria RD, Roncaioli P, Rippo MR, Azuma M, Lanier LL, Santoni A, Testi R. Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. J Exp Med 177:1547–1552;1993.

10 Clements JL, Cooper SM, Budd RC. Abnormal regulation of the IL-2 promoter in *lpr* CD4– CD8– T lymphocytes results in constitutive expression of a novel nuclear factor of activated T cell-binding factor. J Immunol 154: 6372–6381;1995.

11 Cohen PL, Eisenberg RA. *Lpr* and *gld:* Single gene models of systemic autoimmunity and lymphoproliferative disease. Annu Rev Immunol 9:243–269;1991.

12 Fields PE, Gajewski TF, Fitch FW. Blocked Ras activation in anergic CD4+ T cells. Science 271:1276–1278;1996.

13 Garrity PA, Chen D, Rothenberg EV, Wold BJ. Interleukin-2 transcription is regulated in vitro at the level of coordinated binding of both constitutive and regulated factors. Mol Cell Biol 14:2159–2169;1994.

14 Giese T, Allison JP, Davidson WF. Functionally anergic *lpr* and *gld* B220+ T cell receptor (TCR)-α/β^+ double-negative T cells express CD28 and respond to costimulation with phorbol myristate acetate and antibodies to CD28 and the TCR. J Immunol 151:597–609;1993.

15 Go C, Miller J. Differential induction of transcriptional factors that regulate the interleukin-2 gene during anergy induction and restimulation. J Exp Med 175:1327–1336;1992.

16 Gulbins E, Bissonnette R, Mahboubi A, Martin S, Nishioka W, Brunner T, Baier G, Baier-Bitterlich G, Byrd C, Lang F, Kolesnick R, Altman A, Green D. Fas-induced apoptosis is mediated by a ceramide-initiated Ras signalling pathway. Immunity 2:341–351;1995.

17 Hahn WC, Rosenstein Y, Burakoff SJ, Bierer BE. Interaction of CD2 with its ligand lymphocyte function-associated antigen-3 induced adenosine 3′,5′-cyclic monophosphate production in T lymphocytes. J Immunol 147:14–21; 1991.

18 Hibi M, Lin A, Smeal T, Minden A, Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that bind and potentiate the c-Jun activation domain. Genes Dev 7:2135–2148;1993.

19 Hsueh YP, Lai MZ. JNK but not MAP kinase is sensitive to cAMP in T lymphocytes. J Biol Chem 270:18094–18098;1995.

20 Hsueh YP, Liang HE, Ng SY, Lai MZ. CD28 costimulation activates CREB in T lymphocytes. J Immunol 158:85–93;1997.

21 Hsueh YP, Lai MZ. Overexpression of activated transcriptional factor 1 in lymphomas and in activated lymphocytes. J Immunol 154: 5675–5683;1995.

22 Jain J, Valge-Archer VE, Sinskey AJ, Rao A. 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 interleukin-2 promoter. J Exp Med 175:853–862;1992.

23 Kang SM, Beverly B, Tran AC, Brorson K, Schwartz RH, Lenardo MJ. Transactivation by AP-1 is a molecular target of T cell clonal anergy. Science 257:1134–1138;1992.
24 Katagiri K, Katagiri T, Eisenberg RA, Ting J, Cohen PL. Interleukin-2 responses of *lpr* and normal L3T4/Lyt-2 T cells induced by TPA plus A23187. J Immunol 138:149–156;1987.
25 Katagiri T, Urakawa K, Yamanishi Y, Semba K, Takahashi T, Toyoshima K, Yamamoto T, Kano K. Overexpression of a *src* family gene for tyrosine kinase $p59^{fyn}$ in CD4–CD8– T cells of mice with a lymphoproliferative disorder. Proc Natl Acad Sci USA 86:10064–10068; 1989.
26 Lai MZ, Jang YJ, Chen LK, Gefter ML. T cell receptor V(D)J junctional diversity in the response specific for λ repressor: Selection of a highly restricted junctional element. J Immunol 144:4851–4856;1990.
27 Lee MR, Chung CS, Liou ML, Wu M, Li WF, Hsueh YP, Lai MZ. Isolation and characterization of nuclear proteins that bind to T cell receptor Vβ decamer motif. J Immunol 148: 1906–1912;1992.
28 Lee MR, Liou ML, Liou ML, Yang YF, Lai MZ. cAMP analogs prevent activation-induced apoptosis of T cell hybridomas. J Immunol 151:5208–5217;1993.
29 Li W, Whaley CD, Mondino A, Mueller DL. Blocked signal transduction to the ERK and JNK protein kinases in anergic CD4+ T cells. Science 271:1272–1276;1996.
30 Marais R, Wynne J, Treisman R. The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain. Cell 73:381–393;1993.
31 Meyer CF, Wang X, Chang C, Templeton D, Tan TH. Interaction between c-Rel and the mitogen-activated protein kinase kinase kinase 1 signaling cascade in mediating B enhancer activation. J Biol Chem 271:8971–8976;1996.
32 Morse HC III, Davidson WF, Yetter RA, Murphy ED, Roths JB, Coffman RL. Abnormalities induced by the mutant gene *lpr*: expression of a unique lymphocyte subset. J Immunol 129: 2612–2618;1982.
33 Mountz JD, Steinberg AD, Klinman DM, Smith HR. Autoimmunity and increased c-*myb* transcription. Science 226:1087–1089; 1984.
34 Mueller DL, Jenkins MK. Molecular mechanism underlying functional T-cell unresponsiveness. Curr Opin Immunol 7:375–381; 1995.
35 Nagata S. Apoptosis by death factor. Cell 88: 355–365;1997.
36 Neumann M, Grieshammer T, Chuvpilo S, Kneitz B, Lohoff M, Schimpl A, Franza Jr BR, Serfling E. RelA/p65 is a molecular target for the immunosuppressive action of protein kinase A. EMBO J 14:1991–2004;1995.
37 Pastor MI, Reif K, Cantrell D. The regulation and function of $p21^{ras}$ during T-cell activation and growth. Immunol Today 16:159–165; 1995.
38 Rooney JW, Sun YL, Glimcher LH, Hoey T. Novel NFAT sites that mediate activation of the interleukin-2 promoter in response to T-cell receptor stimulation. Mol Cell Biol 15:6299–6310;1995.
39 Samelson LE, Davidson WF, Morse III HC, Klausner RD. Abnormal tyrosine phosphorylation on T-cell receptor in lymphoproliferative disorder. Nature 324:674–676;1986.
40 Seder RA, Paul WE. Acquisition of lymphokine-production phenotype by CD4 T cells. Annu Rev Immunol 12:635–673;1994.
41 Shaw J, Meerovitch K, Bleackley RC, Paetkau V. Mechanisms regulating the level of IL-2 mRNA in T lymphocytes. J Immunol 140: 2243–2248;1988.
42 Su B, Jacinto E, Hibi M, Kallunki T, Karin M, Ben-Nerian Y. JNK is involved in signal transduction during costimulation of T lymphocytes. Cell 77:727–736;1994.
43 Umlauf SW, Beverly B, Lantz O, Schwartz RH. Regulation of interleukin-2 gene expression by CD28 costimulation in mouse T-cell clones: both nuclear and cytoplasmic RNAs are regulated with complex kinetics. Mol Cell Biol 15: 3197–3205;1995.
44 van Vollenhoven RF, Engleman EG, McGuire JL. An open study of dehydroepiandrosterone in systemic lupus erythematosus. Arthritis Rheum 37:1305–1310;1994.
45 Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356:314–317;1992.
46 Whitehurst CE, Geppert TD. MEK1 and the extracellular signal-regulated kinase are required for the stimulation of IL-2 gene transcription in T cells. J Immunol 156:1020–1029;1996.
47 Wofsy D, Hardy RR, Seaman WE. The proliferating cells in autoimmune MRL/*lpr* mice lack L3T4, an antigen on 'helper' T cells that is involved in the response of class II major histocompatibility antigens. J Immunol 132:2686–2689;1984.
48 Wu J, Zhou T, He J, Mountz JD. Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. J Exp Med 178:461–468;1993.
49 Yonehara S, Ishii A, Yonehara M. A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with receptor of tumor necrosis factor. J Exp Med 169:1747–1756;1989.