Role of Egr-2 in Up-regulation of Fas Ligand in Normal T Cells and Aberrant Double-negative lpr and gld T Cells*

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We previously identified a Fas ligand regulatory element (FLRE) in the Fas ligand (fasL) promoter that binds Egr family proteins and demonstrated that Egr-3 (PILOT) but not Egr-1 (NGFI-A, Krox-24, Tis-8, and Zif-268) induces transcription of fasL. The aberrant CD4^-CD8^- T cells from lpr/lpr and gld/gld mice, which have mutations in the genes encoding Fas and FasL, respectively, have an activated phenotype and constitutively express high levels of fasL mRNA, prompting us to ask what role if any the FLRE and Egr family proteins have in this aberrant expression of fasL. Unstimulated MRL-lpr/lpr and C3H-gld/gld CD4^-CD8^- T cells constitutively contained high levels of two proteins that bound to the FLRE. Supershift analysis revealed these proteins to be Egr-1 and Egr-2 (Krox-20); Egr-3 was not detected. Activation of normal lymph node cells resulted in increased expression of Egr-1, -2, and -3. As with egr-3, expression of egr-2 was blocked by cyclosporin A. Although overexpressed Egr-1 was ineffective, overexpressed Egr-2 was as potent as Egr-3 in inducing expression of fasL. Unstimulated MRL-lpr/lpr and C3H-gld/gld CD4^-CD8^- T cells constitutively expressed high levels of fasL mRNA in HeLa cells. FasL-dependent reporter constructs in MRL-lpr/lpr and C3H-gld/gld CD4^-CD8^- T cells were constitutively active, and this activity was largely prevented by mutation of the critical Egr family binding element. Thus, Egr-2, in addition to Egr-3, regulates FasL expression in activated normal T cells, and Egr-2 is likely to play a direct role in aberrant fasL up-regulation in lpr/lpr and gld/gld CD4^-CD8^- T cells.

Apoptotic death of T cells at the conclusion of an immune response is critical for homeostasis of the immune system. T cell receptor (TCR)-mediated activation of T cells induces the transcription and synthesis of the cell surface molecule Fas and its ligand, FasL (1). Although activated T cells are initially resistant to death signals mediated by Fas, prolonged activation increases their sensitivity, presumably owing to down-regulation of such cellular inhibitors of the Fas/FADD/caspase 8 cascade as c-FLIPS (2) and IAPs (3, 4). The murine lpr and gld mutations result in the loss of function of Fas (5) and FasL (6–8), respectively. As a result, these mice lack a functional Fas death pathway, preventing deletion of activated T cells and leading to the accumulation of large numbers of abnormal CD4^-CD8^-, B220^+, TCR^- lymph node cells (referred to as double-negative (DN) T cells). Humans with defects in Fas-mediated killing have an lpr-like syndrome, with accumulation of DN TCR^-CD4^-CD8^- T lymphocytes and autoimmune phenomena (9–11). MRL-lpr/lpr (lpr) and C3H-gld/gld (gld) mice have many features characteristic of activated T cells, and their presence is associated with the development of autoimmunity. As a presumed consequence of being chronically activated, lpr and gld lymph node cells constitutively express high levels of fasL mRNA (12, 13).

Control of fasL transcription in different settings has been attributed to several transcription factors. For example, fasL can be induced by DNA-damaging agents such as UV irradiation or topoisomerase inhibitors (14). In this case, mutant reporter constructs have indicated that control of fasL transcription can be attributed directly to NF-kB and AP-1-binding sites located more than 1 kilobase upstream of the coding region. Overexpression of a dominant negative form of the NF-kB inhibitor I-kB blocked the induction of the fasL-dependent reporter by these treatments. Some correlative evidence has suggested that NF-kB also has a role in TCR-induced fasL expression. For example, NF-kB activity, which is increased by proteasome-mediated degradation of its cytoplasmic inhibitor I-kB (15), and fasL expression are both blocked by proteasome inhibitors (16). Furthermore, induction of both NF-kB transcriptional activity and fasL transcription by TCR signals is sensitive to antioxidants, and pro-oxidants such as hydrogen peroxide can induce both (17, 18). Both activation- and peroxide-induced apoptosis were inhibited by overexpression of dominant negative I-kB (18). However, the NF-kB site that apparently takes part in stress-induced fasL up-regulation lies outside of the region that confers TCR inducibility of fasL transcription, and a potential NF-kB site that does lie in this region (19) is dispensable for TCR-mediated fasL up-regulation (20–22).

There are a number of lines of evidence to suggest that NF-AT plays a critical role in fasL up-regulation. First, fasL induction by TCR-mediated stimulation is sensitive to cyclosporin A (CsA) (23, 24). Furthermore, anti-TCR-induced up-regulation of fasL in vivo is impaired in NF-ATp knockout mice (25). Footprinting analysis of the fasL promoter with the use of recombinant proteins identified two potential NF-AT-binding sites (22). The distal site, located at −275 relative to the coding region, was found to contribute to fasL reporter activity in Jurkat cells (22, 26). In contrast, we found that mutation or deletion of the NF-AT site at −275 did not have a substantial effect on fasL up-regulation and described a fasL regulatory element (FLRE) located from −214 to −207 in the fasL promoter (20). The FLRE conferred the majority of TCR-inducible fasL promoter activity in a murine T cell hybridoma and in human T cell blasts and proved to be a binding site for Egr-1 and Egr-3, members of the Egr (early growth response) family

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1 The abbreviations used are: TCR, T cell receptor; DN, double-negative; CsA, cyclosporin A; FLRE, fasL regulatory element; bp, base pair(s); RT, reverse transcriptase; PCR, polymerase chain reaction; MOPS, 3-(N-morpholino)propanesulfonic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PMA, phorbol 12-myristate 13-acetate.

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Role of Egr-2 in Up-regulation of Fas Ligand

3223

of transcription factors. Overexpression of Egr-3 but not Egr-1 was sufficient to induce an FLRE-dependent reporter construct as well as an increase in cellular fasL mRNA. TCR-mediated induction of Egr-3 but not Egr-1 was sensitive to inhibition by CsA, suggesting that Egr-3 may be a transcriptional target of NF-AT. These data indicated that Egr-3 rather than the more abundant Egr-1 is likely to be a direct mediator of fasL transcription in activated T cells.

Transcription of Egr family genes is up-regulated after TCR activation (27, 28). These Zn2+ finger-containing proteins have similar DNA-binding domains and similar DNA sequence preference (29). Other domains of the proteins differ, and the expression of Egr family genes can be differentially regulated, suggesting that individual members fulfill nonredundant biological roles (30). In this report we examine MRL-lpr/lpr and C3H-gld/gld DN T cells for the presence of FLRE-binding proteins that might contribute to the constitutive activation of the fasL promoter. The results indicate that Egr-2, like Egr-3, can directly mediate TCR-induced transcription of fasL, and it is this member of the Egr family that appears to be responsible for the aberrant up-regulation of this gene in lpr and gld mice.

MATERIALS AND METHODS

Cell Lines, Mice, and Reagents—2B4.A.1 is a murine T cell hybridoma specific for peptide 81–104 of pigeon cytochrome c presented by I-Ek (31) and was maintained in RPMI 1640 (Biofluids, Rockville, MD) supplemented with 4 mm glutamine, 50 μg/ml mercaptoethanol, 100 units/ml penicillin, 150 μg/ml gentamicin, and 10% heat-inactivated fetal calf serum (complete medium). HeLa (human cervical carcinoma) cells were cultured in Dulbecco’s modified Eagle’s medium (Biofluids) with the foregoing supplements. C3H (10 weeks of age) and C3H-gld/gld MRL-lpr/lpr mice (4–5 months of age) were obtained from Jackson Laboratories (Bar Harbor, ME). In some experiments, interbred (C57BL/6 x DBA/2) F1 hybrid mice (6–8 months of age) were obtained from Berkeley Antibody (Berkeley, CA). Protein A-purified antisera against Egr-1, Egr-2, and an unknown protein (anti-Egr-2) were obtained from Sandoz. Protein A-purified antisera against Egr-1, Egr-3, WT-1, and an unknown protein (anti-Egr-2) were obtained from Santa Cruz Technologies (Santa Cruz, CA). An antisera against Egr-2 was obtained from Berkeley Antibody (Berkeley, CA).

Plasmids—The 14-mer plasmid, with a 14-bp FLRE-containing element (from –220 to –205, relative to the initiating codon) appended to a fasta minimal promoter (from –137 to –1), the “m14-mer” plasmid, in which a 4-bp substitution in the FLRE (–214 to –211: GTGG -> CACC) was made, the 350-mer, the 225-mer, and the 212-mer plasmids, all driving the luciferase reporter gene of the vector pGL3 (Promega), were described (20). The expression plasmids encoding NFGF-A (Egr-1), Egr-2, and Egr-3 were described (32).

Transient Transfection Assays—In cotransfection experiments, a Gene Pulser (BioRad) was used to electroporate 2B4.A.1 cells (2 × 10^5) in 0.2 ml of complete medium in 4-mm cuvettes, with 7.5 μg of expression plasmid and 2.5 μg of reporter plasmid, by using 960 microfarads at 220 V. The plasmid pCB6 (33) was used as a control for the expression plasmids. Each transfection was cultured in 1-ml volumes in complete medium and, the luciferase activity in 20% of each was measured. Error bars represent the standard deviation of the fold inductions (Egr-expressing plasmid relative to empty plasmid) of duplicate samples. MRL-lpr/lpr lymph node cells (4 × 10^5) were electroporated with 10 μg of fastaL-dependent luciferase-encoding reporter plasmid by using 960 microfarads at 250 V. Each transfection was cultured in 4 ml of complete medium, and the luciferase activity in 20% of it was measured. Error bars represent the standard deviation of duplicate transfections. HeLa cells were transfected by the calcium phosphate method (34). In reporter assays, triplicate 200-μl cultures were transfected with 150 ng of luciferase reporter plasmid and 150 ng of expression plasmid, and the amount of luciferase activity in 40% of each culture was measured. In RT-PCR assays, 4-ml cultures were transfected with 7.5 μg of expression plasmid, and 20% of the resulting RNA was used for synthesis of cDNA.

Gel Shift Assays—Whole cell extracts were prepared by resuspending phosphate-buffered saline-washed cells in 10 ml HEPES, pH 7.9, 400 mm KCl, 10% glycerol, 1 mm dithiothreitol, and 1 mm phenylmethylsulfonyl fluoride and subjecting them to three rounds of freezing on dry ice followed by rapid thawing in a 37 °C water bath. Extracts were obtained from the supernatants after 5 min of centrifugation at 14,000 × g at 4 °C. Binding reactions were carried out at 22 °C for 30 min by combining 3-μl extracts with 12 μl of binding buffer (10 mM HEPES, pH 7.9, 10% glycerol, 1 mm dithiothreitol, and 1 mm phenylmethylsulfonyl fluoride) containing 1 μg of poly(dI-dC) and 0.02 pmol of 32P-ended double-stranded oligonucleotide probe. In antibody supershift assays, 1–2 μl (1–2 μg) of total antiserum or phosphate-buffered saline was added to the binding reaction. Complexes and unbound probe were separated on 4.5% polyacrylamide gels with the use of 0.5× TBE running buffer.

Northern Blot Analysis—Total RNA (1 μg) isolated using the Trizol deparaffin (Life Technologies, Inc.) was separated by electrophoresis through a 1.5% agarose gel containing 6% formaldehyde and buffered with 2× SDS (Quality Biological, Inc., Gaithersburg, MD). After transfer to a Hybond-N membrane (Amersham Pharmacia Biotech), RNA was covalently bound by UV cross-linking. Hybridization with 32P-labeled cDNA probes was carried out at 65 °C in 0.5× sodium, 7% SDS, and 1× EDTA and buffered to pH 7.2 with phosphate (35). The cDNAs encoding the extracellular part of mouse FasL and the Pst-1 fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (36) were used as probes. Final washes were performed at 60 °C in 80 mM sodium phosphate-buffered as before, with 1% SDS and 1× EDTA. After exposure to detect FasL, the membrane was stripped by boiling in 0.1× SSC and 1% SDS and probed for GAPDH.

RT-PCR—cdna was synthesized with the use of random hexamers and avian myeloblastosis virus reverse transcriptase (Invitrogen, San Diego, CA). The PCR primers, which were designed to amplify cDNA and not genomic DNA, were: human fasL, ATGTTTCAGCTCTTCCACGCTACAGA (forward) and CCAGAGAGACCTCAATAGTTGGCA (reverse), and human gapdh, AGTCGCGGTCACGCGGTTG (forward) and CACGAGAGGGGCGAGATG (reverse). 1 μl of cDNA was amplified in a 25-μl PCR reaction with the use of “Ready To Go” PCR beads (Amersham Pharmacia Biotech) with MgCl2, supplemented to 2 mM. Products derived from 40 amplification cycles of 1 min at 95 °C, 1 min at 62 °C, and 1 min at 72 °C were separated by electrophoresis through a 2% agarose gel and transferred to a HyBond-N membrane. The membrane was probed with the 32P-labeled human fasL cDNA. To control for the integrity and uniformity of the sample preparations, gapdh was amplified with 25 cycles of 1 min at 95 °C, 1 min at 55 °C, and 1 min at 72 °C, and the products were transferred and probed as before.

RESULTS

lpr and gld Lymph Node T Cells Contain Elevated Levels of the FLRE-binding Proteins Egr-1 and Egr-2—Mice of the C3H-gld/gld (gld) and MRL-lpr/lpr (lpr) background acquire massive numbers of CD4+CD8+ T cells that bear characteristics indicative of chronic activation and express constitutively elevated levels of fasL mRNA (12, 13). Because we have recently found that the fasL promoter is under the direct control of the activation-inducible and CsA-sensitive transcription factor Egr-3 (20), we asked whether T cells from lpr and gld mice contain elevated levels of FLRE-binding proteins. Extracts derived from freshly isolated lymph nodes from 5–5-month-old lpr and gld mice, which consist almost entirely of DN T cells (Ref. 37 and data not shown), were examined by the electrophoretic mobility shift assay (gel shift). Incubating extracts from either mouse with the labeled FLRE revealed a major retarded band (Fig. 1, bands I and II). The center of migration of this band was slightly faster than that of a band previously identified as Egr-1 in activated 2B4.A.1 T hybridoma cells (Fig. 1, stimulated 2B4.A.1 cells) and corresponded to the migration of Egr-1 and Egr-2 generated by transient expression in HeLa cells (data not shown). Addition of a nonspecific antisera or an antisera specific for the distantly related Egr family member WT-1, the product of the Wilms’ tumor suppressor gene locus, did not affect the gel shift patterns. In contrast, antisera specific for Egr-1 caused a reduction in the intensity of the major shifted band I, and the remaining material (band II) migrated slightly more rapidly than the mean of bands I and II. The anti-Egr-1 antibodies also caused the appearance of supershifted antibody-antigen-DNA complexes of slower mobility. Antibodies against Egr-2 also reduced the intensity of the principal band, in this case the remaining material (band I) migrated more rapidly than the mean of bands I and II.
slowly than the combined bands I and II. Anti-Egr-2 caused the appearance of a supershifted complex of mobility different from that of the Egr-1 supershifted complex. Simultaneous addition of both Egr-1 and Egr-2-specific antisera caused the complete disappearance of the major retarded species. Only a very small amount of material migrated more rapidly than band II, and, unlike activated 2B4.11 cells (Fig. 1 and Ref. 20), antisera against Egr-3 had little effect on this band III in lpr and gld cell extracts. Finally, as with 2B4.11 cells, band IV is constitutively present and is not eliminated by the unlabeled FLRE oligonucleotide (20). Therefore, unstimulated DN T cells from both lpr and gld mice have little if any Egr-3 but, unlike normal unactivated T cells (20), constitutively express Egr-1 and Egr-2, as evidenced by binding to the FLRE.

The expression of the different Egr family transcription factors in DN T cells was further analyzed by Northern blot analysis. RNA from freshly isolated lpr and gld DN T cells had elevated levels of egr-1 and egr-2 when compared with unactivated normal C3H T cells (Fig. 2). In contrast, the level of egr-3 in DN T cells was, although slightly higher than that in unstimulated normal T cells, substantially lower than that in activated normal T cells. These data demonstrate that lpr and gld lymph node DN T cells have an activated phenotype with regard to Egr family protein expression, with constitutively high levels of Egr-1 and Egr-2 and relatively little Egr-3.

Egr-2 Is Among the Induced FLRE-binding Proteins in Activated 2B4.11 and Normal T Cells—The expression of Egr-2 in a T hybridoma cell line and normal T cells was examined. Egr-2, whose migration is only slightly faster than Egr-1, was not previously recognized by gel shift analysis in extracts of activated 2B4.11 T hybridoma cells, and no supershifted material was detected with a commercially available putative anti-Egr-2 polyclonal antiserum (20). Reexamination of cell extracts from activated 2B4.11 cells revealed, however, that supershifting with anti-Egr-1 and anti-Egr-3 antibodies left a faint band that migrated slightly faster than the bulk of the Egr-1 material and co-migrated with recombinant Egr-2 (Fig. 1 and data not shown). Furthermore, addition of a different anti-Egr-2 antiserum, which we have found supershifts recombinant Egr-2 (data not shown) removed this band entirely (Fig. 1), indicating that the FLRE binding activity in activated 2B4.11 cells consists of Egr-1, Egr-2, and Egr-3. To determine what FLRE-binding proteins are induced in normal T cells, C3H lymph node cells were studied. Small amounts of shifted material were detected in the region corresponding to bands I, II, and III could be seen in untreated lymph node cells (Fig. 3). All of these bands, in particular the slower species corresponding to bands I and II, were increased in cells stimulated for 3 h with PMA and ionomycin. Antisera against Egr-1 removed most, but not all, of the more slowly migrating species and caused the appearance of supershifted material, and antisera against Egr-2 removed a part of the more slowly migrating species and
caused the appearance of a correspondingly smaller amount of supershifted material. The combination of the two antisera completely removed bands I and II. As with 2B4.11 cells, an antiserum against Egr-3 specifically removed band III. Consistent with the gel shift data, Northern blot analysis demonstrated that mRNA for all three Egr family members was induced by activation (Fig. 2). Therefore, in normal lymph node T cells, activation induces the appearance of Egr-1, -2, and -3.

T-cell-mediated induction of Egr-2 expression is inhibited by CsA, which blocks activation of the NF-AT transcription factor as a consequence of inhibiting the phosphatase calcineurin (38, 39). Similarly, activation-induced activity of the FLRE reporter and induction of Egr-3, but not Egr-1, are sensitive to CsA (20). The issue of whether induced Egr-2 expression is sensitive to inhibition by CsA is controversial. In one study with peripheral blood cells (28), CsA was found to have a relatively small effect on egr-2 induction, whereas in another study on a thymocyte cell line, CsA was found to inhibit activation-induced egr-2 (40).

To determine whether Egr-2 induction in normal murine T cells is CsA-sensitive, normal lymph node cells were activated by PMA plus ionomycin in the presence or absence of CsA (Fig. 4). Egr-2 mRNA levels were substantially increased by activation, a response that was inhibited by CsA. Thus, in normal T cells Egr-2, like Egr-3 (20), is a CsA-sensitive T cell activation-inducible transcription factor.

Egr-2 Expression Is Sufficient to Induce fasL Transcription—

Egr family transcription factors contain no known dimerization domains, and their genetic target sites are asymmetrical. Therefore, they are thought to bind to DNA and activate transcription as monomers. In addition, their transcriptional activity is not thought to depend on post-translational modification (41). Accordingly, transient overexpression of Egr-3 was previously found to be sufficient for induction of a fasL promoter plasmid in the absence of any TCR-mediated activation (20). Egr-2 was likewise tested for its ability to independently induce fasL-specific transcription. 2B4.11 cells were transfected with a reporter construct encoding the luciferase gene driven by a minimal fasL promoter to which was appended the region from −220 to −205, which contains the FLRE. Enforced expression of Egr-1 had little effect on the activity of the intact reporter (Fig. 5A). As previously shown (20), the Egr-3 expression plasmid induced significantly higher activity. Overexpression of Egr-2 resulted in a response that was similar to that induced by Egr-3. Cotransfection of plasmids encoding the Egr family gene products had little effect on the activity of a reporter construct with a mutation in the FLRE that eliminated TCR-induced activity of fasL reporter constructs (20). To determine whether Egr-2 activates the FLRE in a nonlymphoid cell, the effect of its overexpression was tested in HeLa cells. As with the 2B4.11 cells, the mutated reporter was only minimally affected by cotransfected Egr-2-expressing plasmids. In contrast, although the reporter bearing the wild-type FLRE was minimally affected by cotransfected Egr-1, it was driven by Egr-3 and even more strongly by Egr-2. Thus, expression of either Egr-2 or Egr-3 is sufficient to induce the fasL promoter, and this response does not require the presence of lymphoid-specific accessory factors.

We previously showed that overexpression of Egr-3 is sufficient to induce the appearance of fasL mRNA in HeLa cells. Similar experiments were performed to determine whether the same is true for Egr-2 (Fig. 5B). Expression of Egr-1 had only a small effect on fasL mRNA levels as measured by RT-PCR. In contrast, Egr-2, just like Egr-3, induced easily detectable fasL mRNA levels as measured by RT-PCR.
Luciferase units

**Fig. 6.** An intact FLRE is required for optimal constitutive fasL-dependent reporter activity in lpr lymph node DN T cells. Lpr lymph node DN T cells were electroporated with the indicated fasL promoter-driven luciferase reporter plasmids, and luciferase activity was measured 15 h later. One representative experiment of three is shown.

mRNA. Thus, Egr-2 can activate fasL-specific transcription without other methods of cellular activation.

**Mutation of the FLRE Diminishes Expression of fasL Reporters in lpr and gld DN T Cells**—To determine whether the FLRE and by implication Egr-2 are necessary for the up-regulation of FasL in lpr and gld DN T cells, these cells were transiently transfected with reporter constructs driven by parts of the fasL promoter (Fig. 6). A reporter containing the proximal 225 bp of the fasL gene was induced to a much greater extent than one containing just the proximal 212 bp (and thus having a disrupted Egr-binding site (−214 to −207)). Moreover, a reporter driven by a larger segment of the promoter (305 bp) also was active in these cells, and this activity was largely lost by mutation of the FLRE. Similar results were obtained with gld DN T cells (data not shown). These results support the notion that the FLRE, principally by binding the constitutively present transcription factor Egr-2, plays a major role in the maintenance of fasL mRNA expression in both lpr and gld DN T cells and in an additional context confirm the role of this site in fasL regulation in T cells.

**DISCUSSION**

The importance of the FasL-triggered apoptotic death pathway for T cell homeostasis is well established. The regulation of fasL transcription is a critical control point in this phenomenon. A single Egr family binding element appears to account for the majority of the activation-induced cis-acting regulation of fasL (20). In this report we identify Egr-2, along with the previously identified Egr-3, as a direct regulator of fasL transcription in activated T cells. Like Egr-3, this factor inducibly binds to the FLRE in the fasL promoter and is synthesized de novo in a CsA-sensitive manner upon T cell activation. Ectopically expressed Egr-2, like Egr-3, was sufficient to induce fasL-specific transcription, as indicated by both fasL reporter construct activity and the measurement of cellular fasL mRNA. Egr family genes are transcriptionally up-regulated from low basal levels in response to a multitude of cellular stimuli (41). TCR induction of Egr family gene expression is differentially sensitive to CsA; transcription of Egr-2 and Egr-3 is sensitive to CsA, but Egr-1 is not. That the more abundant Egr-1 protein does not up-regulate fasL was confirmed by transient expression in T and HeLa cells, in which it was found to be ineffective at inducing fasL reporter constructs and fasL mRNA expression. Thus, by these criteria Egr-2 and Egr-3 are the likely Egr family members to mediate transcriptional activation through the FLRE and thus are likely themselves to be regulated by NF-AT.

Little is known about the function of Egr-2 in vivo. As with the other Egr family members, egr-2 mRNA is not detected in most adult tissues by Northern analysis. An exception is the thymus, where some of the cells are undergoing antigen-driven selection (42), and activated T cells (28, 40). Egr-2 is also expressed in the course of embryogenesis (days 8–9 postcoitum in the mouse) in rhombomeres r3 and r5 of the hindbrain. Egr-2-deficient mice have disruptions of these rhombomeres (43) as well as defects in myelination of peripheral nerves (30), and the mice are nonviable. Proposed target genes of Egr-2 include HoxB-2 and HoxA-2 (44), which encode homeobox domain transcription factors (45), and EphA4, which encodes a receptor tyrosine kinase (46). Treatment of mouse embryos with retinoic acid, which activates retinoic acid receptors (RARs/RXRαs) that are themselves transcription factors, inhibits egr-2 expression in rhombomere 3 and prevents maturation of this hindbrain segment (47–49). Interestingly, in T cells and thymocytes, retinoic acid inhibits activation-induced fasL up-regulation and apoptosis (50–52). The mechanism by which retinoic acid inhibits TCR-inducible fasL promoter activity remains to be determined. Thus, some of the genetic interrelationships that underlie neural development may be recapitulated in that of the immune system.

The aberrant DN T cells found in lpr and gld mice have an activated phenotype yet are refractory to stimuli such as TCR cross-linking, interleukin-2, CD28, PMA, and phytohemagglutinin (53). The activated state presumably arises from continual contact with self-antigens because of the inability of the peripheral T cells to be deleted by the normal Fas-dependent mechanisms. DN T cells spontaneously turn over inositol phosphates (54), display elevated tyrosine-phosphorylation of TCRζ (55) and Vav (56), and express increased levels of the tyrosine kinase Fyn (57). Also increased is the DNA binding activity of the transcription factors NF-κB, AP-1, and an uncharacterized DNA binding activity, NF-ATγ, that recognized the DNA sequence of the interleukin-2 promoter NF-AT site (58). DN T cells do not proliferate upon TCR cross-linking in part because they are unable to up-regulate production of interleukin-2 (59).

To account for this, it was proposed that NFATγ functions as a transcriptional repressor that overrides the activating potential of the aforementioned constitutive transcription factors activity, although no such repressive activity was demonstrated (58). It is possible that in the context of the egr-2 and egr-3 promoters NF-ATγ functions as an activator of transcription. Attempts to analyze the promoters of these genes are currently underway.

By Northern analysis, lpr and gld DN T cells were found to contain elevated egr-2 mRNA levels relative to activated C3H normal T cells. mRNA for egr-3, and to a lesser extent egr-1, was less abundant in DN T cells than in activated normal T cells. These relative levels correlated with those observed by gel shift. On the other hand, activated 2B4.11 cells contained similar amounts of Egr-2- and Egr-3-specific FLRE binding activity. Thus, of the Egr family members capable of inducing fasL transcription, Egr-2 is selectively expressed in lpr and gld DN T cells. Differential regulation of Egr family member expression is thus another characteristic distinguishing the chronic state of activation of lpr and gld DN T cells from that of normal T cells responding acutely to antigen. Analysis of the correlations between the signaling pathways constitutively activated and the Egr family members expressed in DN T cells will further understanding of both normal and abnormal TCR signaling.

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