Transcription Factor NF-κB Regulates Inducible Oct-2 Gene Expression in Precursor B Lymphocytes*

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Heather H. Bendall‡, David C. Scherer‡, Christine R. Edson‡, Dean W. Ballard‡§, and Eugene M. Oltz‡
From the §Department of Microbiology and Immunology and the Howard Hughes Medical Institute, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The POU transcription factors Oct-1 and Oct-2 regulate the activity of octamer-dependent promoters, including those that direct transcription from rearranged immunoglobulin genes. Unlike Oct-1, which is constitutively expressed in many cell types, Oct-2 expression is restricted primarily to B lymphocytes and can be induced in precursor B cells by stimulation with bacterial lipopolysaccharide (LPS). However, the precise factors that mediate this induction mechanism remain unknown. In the present study, we monitored Oct-2 expression in cells arrested for the activation of NF-κB, an LPS-responsive member of the Rel transcription factor family. Despite stimulation with LPS, disruption of the NF-κB signaling pathway in precursor B cells led to the loss of inducible Oct-2 DNA binding activity in vitro and the suppression of Oct-2-directed transcription in vivo. This biochemical defect correlated with a specific block to Oct-2 gene expression at the level of transcription, whereas the expression of Oct-1 was unaffected. The finding that Oct-2 is under NF-κB control highlights an important cross-talk mechanism involving two distinct transcription factor families that regulate B lymphocyte function.

The genetic program that mediates B cell development is governed by the concerted action of both ubiquitous and lymphoid-specific transcription factors. One integral component of this regulatory network is Oct-2, an inducible member of the POU family of proteins. This B cell-specific factor binds to an 8-base pair sequence, termed the octamer motif (consensus sequence ATGCAAA), which is present in the Ig heavy chain enhancer and in the promoters flanking all Ig variable region gene segments (1). However, these sites are also recognized by another POU family member, termed Oct-1, which is constitutively expressed in many cell types (2). Recent studies have shown that Oct-1 and Oct-2 associate independently with the coactivator protein OBF-1 and direct comparable levels of transcription from Ig promoters in reporter gene assays (3–5). Consistent with this evidence for functional redundancy, targeted disruption of the gene encoding Oct-2 has no significant effect on Ig gene expression in vivo (6). However, the mitogenic response of mature B lymphocytes lacking Oct-2 is severely impaired, indicating that Oct-2 plays an essential role in B cell activation that cannot be replaced by Oct-1.

Like Oct-2, members of the NF-κB/Rel family of proteins have been implicated in the control of Ig gene expression and B cell activation. These inducible transcription factors form homo- or heterodimers that are normally sequestered as latent complexes in the cytoplasm by IκB proteins (7, 8). In response to B cell activation signals, IκB is rapidly degraded and NF-κB accumulates in the nuclear compartment (7). In precursor (pre-) B and mature B cells, these nuclear NF-κB complexes contain either c-Rel or RelA as the principle transactivating subunit (9, 10). Recently, we have demonstrated that an intact NF-κB signaling pathway is required for the assembly and expression of Igκ light chain genes in pre-B cell lines (11). These findings are fully consistent with the direct role that NF-κB plays in regulating its cognate site within the κ intronic enhancer (12). However, indirect mechanisms involving the action of NF-κB-responsive transcription factors at other sites in the Igκ locus cannot be excluded.

To address this issue, we performed a biochemical survey of transcription factors that target most of the known regulatory elements within the Igκ locus (13). In the present study, we focused on Oct-2 because this particular transcription factor is induced by agents that also activate the nuclear expression of NF-κB (6, 14). We have found that disruption of the NF-κB signaling pathway in pre-B cells imposes a specific block to Oct-2 gene transcription, whereas expression of Oct-1 and OBF-1 is unaffected. The finding that Oct-2 is under NF-κB control has potential implications for the overlapping defects in B cell activation observed when either NF-κB or Oct-2 expression is disrupted in vivo (6, 15, 16). Moreover, our results provide an attractive mechanistic explanation for the constitutive expression of Oct-2 in mature B lymphocytes, which characteristically express high levels of nuclear NF-κB (14).

EXPERIMENTAL PROCEDURES

Cell Culture and Reporter Gene Assays—Control and IκBΔN-expressing derivatives of the pre-B cell lines 38B9 and 70Z/3 were cultured as described previously (11). Oct-2-responsive constructs contained a luciferase reporter gene driven either by the chicken lysozyme promoter (pCL) alone or by pCL in combination with wild type (pCLED) or mutant octamer sites (pCLED) (17). Transient transfection of these reporters was performed using a modified DEAE-dextran procedure as described (11). In brief, 38B9 pre-B cells (2 × 10⁶) were transfected with luciferase reporters (2 μg) and a control vector encoding the plasmid alkaline phosphatase protein (PEP.PAP, 2 μg) (11). Transfected cells were cultured in the presence or the absence of LPS (10 μg/ml) for 20–24 h. Protein extracts were assayed for luciferase activity using an enhanced luciferase assay kit (Analytical Luminescence Laboratory). Control assays for PAP activity were performed on the same extracts with a phosphatase kit (Tropix).

The abbreviations used are: pre-B cell, precursor B cell; LPS, lipopolysaccharide; GAPDH, glyceraldehyde phosphate dehydrogenase; PAP, placental alkaline phosphatase.
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Gel Retardation Analyses—Pre-B cells were cultured in the presence or the absence of LPS (10 μg/ml) for 12 h, and nuclear extracts were prepared as described previously (11). Extracts (4 μg) were incubated with radiolabeled probes corresponding to either a consensus κB site derived from the IL-2Rα promoter (18) or a consensus octamer binding site (19) under standard DNA binding conditions (11, 19). DNA-protein complexes were resolved on native 5% polyacrylamide gels and visualized by autoradiography. For supershift analyses, nuclear extracts were preincubated with either OCT-1- or OCT-2-specific antibodies (Santa Cruz Biotechnology) for 1 h at 4°C prior to the addition of radiolabeled probe.

Northern Blot Analyses—Total RNA was isolated from pre-B cells cultured in the presence or the absence of LPS for 24 h by the lithium chloride method. Isolated RNA (15 μg) was fractionated on 1% agarose formaldehyde gel, transferred to Zeta-Probe membranes (Bio-Rad), and sequentially hybridized with a 561-base pair EcoRI-HindIII fragment from the Oct-2 cDNA (14), the entire CD36 (21) and OBF-1 cDNAs (5), and a 1.1-kilobase PstI fragment derived from the rat glycerolaldehyde phosphate dehydrogenase (GAPDH) (11). Hybridization probes were radiolabeled with [α-32P]dCTP by random priming using a commercially available kit (NEN Life Science Products).

RESULTS AND DISCUSSION

In recent studies with Abelson-transformed pre-B cells, we demonstrated that an intact NF-κB signaling pathway is required for the induction of Igκ transcription by LPS (11). However, the Igκ locus contains regulatory motifs for other LPS-inducible proteins that might be under NF-κB control, including Oct-2 (13, 14). Consistent with this hypothesis, NF-κB and Oct-2 are both induced in pre-B cells by LPS (14). To determine whether NF-κB is required for Oct-2-directed transcription, we employed a mutated form of IκBα that functions as a constitutive repressor of NF-κB activity (20). Previous studies have shown that the inhibitory effects of this mutant, termed IκBAN, are restricted to its cognate signal transduction pathway (11, 20). Pre-B cells stably expressing IκBAN were transfected with luciferase reporter plasmids containing a basal promoter from the chicken lysozyme gene (pCL) linked to either wild type (pCLED) or mutated (pCLED) octamer motifs. Prior studies have demonstrated that the transcriptional activity of pCLED correlates directly with functional Oct-2 levels (17). As shown in Fig. 1, neither pCL nor pCLED was responsive to LPS when introduced into control transfectants lacking ectopic IκBAN, whereas the wild type pCLED reporter was significantly induced in these cells. Despite the presence of LPS, the Oct-2-dependent transcriptional response of pCLED was suppressed in IκBAN-expressing cells. These data suggest that an intact NF-κB signaling pathway is required for the induction of Oct-2-directed transcription in pre-B cells.

To extend these results to a more physiologically relevant gene, we next examined the inhibitory effects of IκBAN on CD36 expression. Prior studies have demonstrated that transcription of this B cell-specific gene is critically dependent on the action of Oct-2 (21). As revealed by Northern blot analyses (Fig. 2), steady-state levels of CD36 transcripts were substantially elevated in two different pre-B cell lines following LPS treatment (lanes 1, 2, 7, and 8). Similar results were obtained using cells stably transfected with wild type IκBα (lanes 3 and 4), which is rapidly inactivated by LPS treatment (11). In contrast, induction of the CD36 gene was completely blocked in the IκBAN background (lanes 5, 6, 9, and 10). These data establish that NF-κB is required not only for induction of Oct-2-directed transcription from a synthetic promoter but also from an endogenous transcription unit known to be under Oct-2 control.

In pre-B cells, elevated levels of nuclear Oct-2 DNA binding activity are detected within 8 h after LPS stimulation (14). To determine whether NF-κB is required to generate this inducible activity, pre-B cells expressing either wild type IκBα or IκBAN were stimulated with LPS for 12 h, and nuclear extracts were prepared for gel retardation studies with a consensus κB probe. As expected, LPS efficiently induced the expression of nuclear NF-κB complexes in control cells lacking ectopic IκBα and in transfectants expressing wild type IκBα (Fig. 3, top panel). Based on prior DNA-protein cross-linking studies (11), the predominant NF-κB species induced in these cells contain either c-Rel or RelA as the transactivating subunit. Parallel experiments performed with a consensus octamer motif as the radiolabeled probe revealed a similar pattern of inducible DNA binding for Oct-2, whereas the constitutive DNA binding activity of Oct-1 was unaffected (Fig. 3, bottom panel). The presence of Oct-1 and Oct-2 in the two nucleoprotein complexes observed in these experiments was confirmed by antibody supershift analyses (lanes 11 and 12 and data not shown). Unlike wild type IκBα, IκBAN exerted potent inhibitory effects on the DNA binding activities of both NF-κB and Oct-2 (lanes 6 and 10). These effects could not be attributed to clonal variation because the same pattern of repression was observed in multiple clones derived from three independent pre-B cell lines (Ref. 11 and data not shown). These findings strongly suggest that the in-
that induction of the Oct-2 gene in these pre-B cell lines is the expression of Oct-1 (Fig. 3) and OBF-1 (Fig. 4). We conclude functional NF-

report suggest that the Oct-2 promoter contains one or more mRNA stability cannot be excluded, the data presented in this evidence that this transcription factor relationship is physiologically transformed pre-B cells, thus providing further evidence with either preimmune (P.I.) serum (lane 11) or an anti-Oct-2 antisera (lane 12) before addition to DNA binding reactions. wt, wild type.

duction of Oct-2 DNA binding activity by LPS requires deployment of NF-kB to the nuclear compartment.

In contrast to NF-kB, which is activated by a post-translational mechanism (7, 8), Oct-2 is regulated primarily at the level of transcription (14). However, the transcription factors that regulate Oct-2 gene expression have not been defined. To determine whether NF-kB stimulates transcription from the Oct-2 gene, we monitored the amount of Oct-2 mRNA in control and NF-kB-arrested pre-B cells. As shown in Fig. 4, LPS induced high levels of Oct-2 messages in transfecteds lacking ectopic IxB (lanes 2 and 8) and in transfecteds expressing wild type IxB (lane 4). In contrast, the induction of Oct-2 transcripts by LPS was completely blocked in cells arrested for NF-kB expression (lanes 6 and 10). These inhibitory effects were highly specific for Oct-2, because IxB failed to perturb the expression of Oct-1 (Fig. 3) and OBF-1 (Fig. 4). We conclude that induction of the Oct-2 gene in these pre-B cell lines is contingent upon the activation of NF-kB.

In summary, we have found that NF-kB is required for transcriptional activation of the Oct-2 gene in transformed pre-B lymphocytes. Similar results were obtained with conditionally transformed pre-B cells, thus providing further evidence that this transcription factor relationship is physiologically relevant (data not shown; see Ref. 22). The finding that NF-kB and Oct-2 are functionally coupled has several important implications. First, although effects of NF-kB on Oct-2 mRNA stability cannot be excluded, the data presented in this report suggest that the Oct-2 promoter contains one or more functional NF-kB binding sites. Consistent with this, recent studies have identified kB sites within the transcriptional control elements that regulate Oct-2 gene expression. Second, a hallmark feature of mature B cells is their constitutive expres-