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Jun-B GENE EXPRESSION MEDIATED BY THE SURFACE IMMUNOGLOBULIN RECEPTOR OF PRIMARY B LYMPHOCYTES[1]

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Stimulation of primary B lymphocytes induces the nuclear expression of TPA response element binding proteins that are recognized by anti-Jun antisera. To evaluate the profile of jun gene expression, RNA was extracted from B cells and probed for c-jun. Surprisingly, c-jun mRNA was not detected either before or after stimulation with anti-Ig. Instead, stimulation through the sIg antigen receptor, or with phorbol ester containing regimens, rapidly induced expression of the related jun-B. This demonstrates a lack of coordinate regulation for jun-B and c-jun expression in these primary B cells. The role of Jun-containing TRE binding proteins in promoting B cell cycle progression remains uncertain inasmuch as Jun-B has been associated with transcriptional inhibition of the TPA response element, rather than activation as produced by c-Jun.

Primary B lymphocytes are normally arrested in the G_0 phase of the cell cycle. Crosslinking antigen specific surface immunoglobulin receptors (sIg) on B cells produces a number of early metabolic changes that eventuate in cell cycle progression and entry into S phase (reviewed in ref 1). While there is clear evidence that engagement of sIg receptors leads to translocation and functional activation of protein kinase C (PKC), little is known about the events distal to PKC activation that might be responsible for altering the resting program of gene transcription.

In non-B cell systems, phorbol ester PKC agonists have been shown to alter gene transcription through a specific DNA sequence, the TPA response element or TRE (TGAC/GTCA), that binds the protein product of the c-jun gene either alone as a homodimer or as a heterodimer with the product of the c-fos gene (2-6). A potential role for TRE binding proteins in the early phases of B cell cycle progression is suggested by our recent finding that nuclear expression of TRE binding proteins is markedly induced by B cell stimulation

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through the sIg receptor, and that the nucleoprotein complex detected by electrophoretic gel mobility shift assay is recognized by anti-Jun antisera (7).

The c-jun gene was originally identified as the cellular counterpart to the retroviral transforming oncogene, v-jun (reviewed in ref 8); subsequently, c-jun was shown to code for the major form of the transcription factor AP-1, which is identical to the Fos associated protein detected by Curran and his co-workers (8-10). More recently c-jun has been recognized as one of a family of related genes, whose members presently include jun-B and jun-D (8,11). While all three Jun related proteins bind the TRE motif, their effects on transcription differ (12,13).

Recent studies have demonstrated that c-fos gene expression is induced following stimulation of normal B cells through surface immunoglobulin (14,15). The present study was undertaken to determine whether c-jun gene expression is stimulated by B cell receptor crosslinking in a fashion analogous to that of c-fos and other early response genes, such as c-myc and egr-1 (15-19). Surprisingly, c-jun mRNA was not detected either before or after B cell stimulation. Instead, B cell stimulaton through the sIg receptor, or with phorbol ester containing regimens, markedly induced expression of the related jun-B gene.

MATERIALS AND METHODS

Cells: Purified murine B lymphocytes were prepared from BALB/c spleen cells by depletion of T cells and macrophages, as described (20). B cells were rested overnight in RPMI medium supplemented with 5% fetal calf serum (FCS) and then sedimented on a cushion of Lympholyte prior to stimulation and isolation of RNA. HeLa cells were grown to log phase in DMEM medium with FCS prior to stimulation and RNA isolation.

RNA isolation and Northern blot analysis: Total cellular RNA was extracted from B cells using the acid guanidinium isothiocyanate phenol chloroform (AGPC) procedure of Chomczynski and Sacchi (21). RNA was then separated by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde and MOPS. RNA was transferred to Duralon-UV (Stratagene, La Jolla, CA), and then covalently linked to the membrane by UV irradiation using a Stratalinker 1800 (Stratagene). Specific RNA was detected by hybridization with radiolabeled, random primed, cDNA probes followed by autoradiography as described previously (22). Where indicated, blots were re-hybridized with additional cDNA probes following two sequential washes in 0.1X SSC and 0.1% SDS at 95°C. Results were normalized by hybridization with a cDNA probe specific for glyceraldehyde-3-phosphate dehydrogenase (kindly provided by Dr. G. Frendl, Department of Medicine, Boston University Medical Center, Boston, MA).

cDNA probes: The c-jun, jun-B and jun-D probes were from EcoRI fragments of their respective murine cDNAs (obtained from the American Type Culture Collection, Rockville, MD). The c-fos probe was a PstI-SalI fragment of v-

fos, kindly provided by Dr. Richard A. Miller (Department of Pathology, University of Michigan, Ann Arbor, MI).

Reagents: Anti-Ig ($F(ab')_2$ fragments of goat anti-mouse immunoglobulin) was obtained from Jackson Immunoresearch (West Grove, PA). Monoclonal b7.6 antibody (rat anti-mouse IgM) was purified from serum free culture supernatant. Phorbol myristate acetate (PMA) was obtained from Sigma Chemical Co. (St. Louis, MO). Ionomycin was obtained from Behring Diagnostics (La Jolla, CA).

RESULTS

RNA was prepared from primary murine splenic B lymphocytes and probed for the presence of c-jun mRNA. As shown in Fig 1a, there was essentially no hybridization of the c-jun probe to RNA obtained either from untreated B cells or from B cells stimulated through receptor crosslinking by anti-Ig for various periods of time. The probe was intact as demonstrated by the detection of c-jun expression in RNA obtained from PMA stimulated HeLa cells that was separated, probed and autoradiographed in concert with the B cell samples.

The reported induction of c-fos gene expression that follows sIg crosslinking in murine B cells (14,15) provided the means to demonstrate that the B cell RNA was intact. Blots were stripped and re-probed with a c-fos specific cDNA probe. In keeping with previous reports, we observed rapid but brief induction of c-fos in anti-Ig stimulated B cells (Fig 1c).

We have recently reported that anti-Ig mediates the induction of immunoreactive Jun and Fos containing TRE binding activity, which is dependent

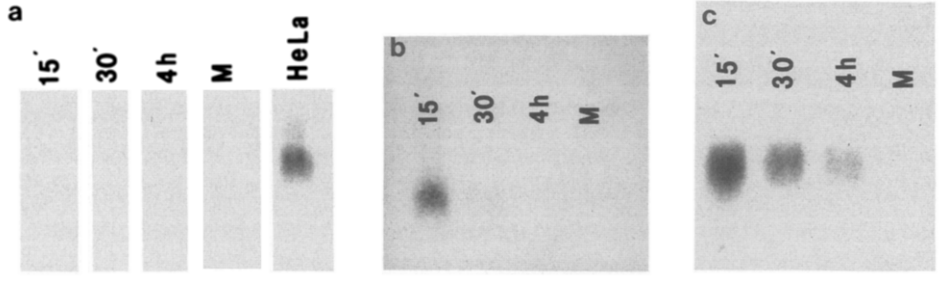

Fig 1.
Induction of jun-B and c-fos gene expression following the crosslinking of surface immunoglobulin receptors on primary B lymphocytes. B cells were cultured in medium alone (M) or in medium containing mitogenic $F(ab')_2$ fragments of goat anti-mouse immunoglobulin at 10 µg/ml for the indicated periods of time. B cells were then harvested and RNA was isolated, separated by electrophoresis through denaturing agarose gels, transferred to membrane and hybridized with radiolabeled cDNA probes specific for c-jun (a), c-fos (b), and jun-B (c), as described in Materials and Methods. RNA was isolated from phorbol ester stimulated HeLa cells (PMA at 100 ng/ml for 30 minutes) and similarly probed; results for c-jun are shown in(a)(HeLa).

on new protein synthesis (7). We therefore expected that B cell stimulation would be accompanied by increased levels of jun gene expression on the order of the enhancement associated with c-fos gene expression. Because we were unable to detect measurable levels of c-jun expression in B cells, we probed for expression of the jun related genes, jun-B and jun-D. As shown in Fig 1b, there was little hybridization of the jun-B probe to RNA obtained from untreated B cells (see also Figs 2 and 3). However, there was marked induction of jun-B gene expression following stimulation of B cells through receptor crosslinking by anti-Ig (Fig 1b). Induction of jun-B mRNA occurred very rapidly with a peak at 15-30 minutes, followed by a slow decline over a 4 hour period. The same blots were probed for jun-D, which was found to be constitutively expressed and little affected by anti-Ig treatment (data not shown).

In order to demonstrate that these results were not idiosyncratic to the anti-Ig reagent used, the effect of B cell stimulation using a mitogenic monoclonal rat anti-mouse IgM antibody (b7.6) was tested. As before, b7.6 failed to induce c-jun gene expression but, as shown in Fig 2, b7.6 did induce

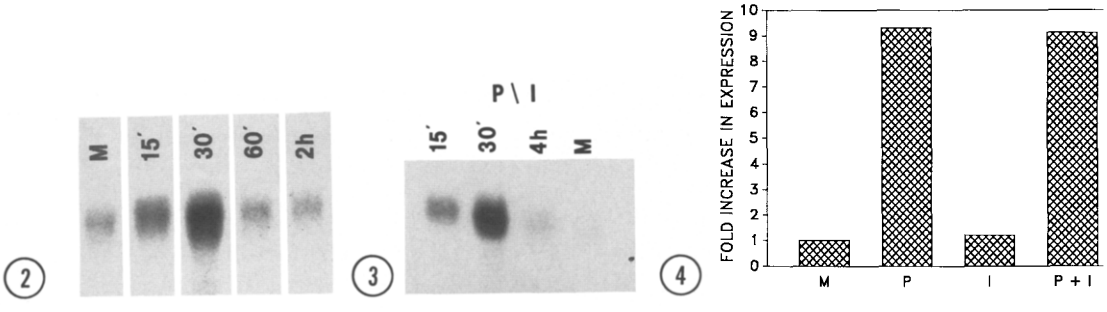

Fig 2.
Induction of jun-B gene expression following crosslinking of sIg receptors on primary B lymphocytes using a monoclonal anti-immunoglobulin antibody. B cells were cultured in medium alone (M) or in medium containing the mitogenic monoclonal rat anti-mouse IgM antibody b7.6 at 20 µg/ml for the indicated periods of time. B cells were then harvested and RNA was isolated and probed for c-jun and jun-B expression by Northern analysis as described in Materials and Methods. Results for jun-B are shown.

Fig 3.
Induction of jun-B gene expression by the combination of a phorbol ester and a calcium ionophore. B cells were cultured in medium alone (M) or in medium containing the phorbol ester, PMA, at 100 ng/ml plus the calcium ionophore, ionomycin, at 800 ng/ml for the indicated periods of time. B cells were then harvested and RNA was isolated and probed for c-jun and jun-B expression by Northern analysis as described in Materials and Methods. Results for jun-B are shown.

Fig 4.
Induction of jun-B gene expression by phorbol ester, acting alone. B cells were cultured in medium alone (M) or in medium containing the phorbol ester, PMA, alone at 100 ng/ml (P), the calcium ionophore, ionomycin, alone at 800 ng/ml (I), or the combination of PMA plus ionomycin (P + I). B cells were harvested after 30 minutes and RNA was isolated and probed for jun-B and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression of jun-B was normalized for GAPDH expression and is reported as the fold increase above the expression present in medium control B cells.

jun-B gene expression in a fashion analogous to that described for polyclonal anti-Ig.

B cells were also stimulated by the mitogenic regimen consisting of the phorbol ester PKC agonist, PMA, and the calcium ionophore, ionomycin (23,24). In combination these pharmacologic agents are thought to mimic the alterations in PKC and intracellular Ca^{++} evoked physiologically through sIg crosslinking. RNA obtained from PMA plus ionomycin stimulated B cells expressed jun-B, as shown in Fig 3, but failed to express c-jun (data not shown). In view of the documented synergy between PMA and ionomycin in stimulating nuclear expression of TRE binding proteins (7), it was of interest to determine whether such synergy was evident at the level of jun-B expression. PMA alone markedly stimulated jun-B expression and this did not appear to be increased by inclusion of ionomycin in the mitogenic regimen at either 15 minutes (data not shown) or 30 minutes (Fig 4).

DISCUSSION

These results indicate that stimulation of primary B lymphocytes through the sIg antigen receptor, as well as via direct PKC activation, results in the very rapid and marked induction of jun-B gene expression unaccompanied by any change in the undetectable baseline level of c-jun transcripts. Preliminary run-on expriments indicate that the induction of jun-B mRNA coincides with transcriptional activation of the gene.

jun-B expression has been associated with transcriptional inhibition rather than activation. In co-transfection experiments using F9 cells it was found that not only did Jun-B fail to trans-activate a TRE-dependent reporter gene, but Jun-B inhibited trans-activation by c-Jun (12,13). Construction of chimeras between jun-B and c-jun demonstrated that this difference in transcriptional activation could not be accounted for by reduced DNA binding (12). Thus, it is remarkable that regimens that stimulate resting B cells to enter and progress through cell cycle induce jun-B, which would be expected to produce an inhibitory signal, rather than c-jun, in conjunction with c-fos. Although it might be speculated that Jun-B acts to turn off genes associated with the resting state by competition with c-Jun, the absence of detectable baseline c-jun expression casts doubt on this interpretation. Still, it has not yet been demonstrated at the protein level that the TRE binding complexes identified in B cells contain Jun-B and not c-Jun, and it remains at least formally possible that significant levels of c-Jun protein are derived from quantities of c-jun mRNA that are below the present limits of detection.

jun-B and c-jun are co-expressed in many transformed cell lines; the present results represent the first example to our knowledge of incoordinate regulation of jun-B and c-jun in primary cells. While one might attribute this to differences between primary cells and cell lines, we have recently shown in preliminary experiments that treatment of WEHI 231 B cells with anti-Ig also stimulates jun-B and not c-jun gene expression. Moreover, a lack of coordinate regulation of jun-B and c-jun has previously been observed with HeLa and NIH 3T3 cells stimulated by forskolin (12). However, c-jun transcription was stimulated in both cell lines by treatment with TPA, which failed to stimulate c-jun gene expression in primary B cells. This is similar to the effect of PMA on F9 cells (12). The basis for the incoordinate regulation of jun-B and c-jun gene expression in stimulated B cells remains to be determined; however, it may be attributable to differences within the individual promoter regions of these genes. The human jun-B promoter differs markedly from the c-jun promoter and does not appear to share with c-jun recognized sites for transcriptional regulatory DNA binding proteins (13). Alternatively, c-jun may not be functionally expressed in primary B cells due to methylation of its promoter region as recently shown for egr-1 (25).

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