Cyclin G2 Is Up-regulated during Growth Inhibition and B Cell Antigen Receptor-mediated Cell Cycle Arrest*

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Human cyclin G2 together with its closest homolog cyclin G1 defines a novel family of cyclins (Horne, M. C., Goolsby, G. L., Donaldson, K. L., Tran, D., Neubauer, M., and Wahl, A. F. (1996) J. Biol. Chem. 271, 6050–6061). Cyclin G2 is highly expressed in the immune system where immunologic tolerance subjects self-reactive lymphocytes to negative selection and clonal deletion via apoptosis. Here we investigated the effect of growth inhibitory signals on cyclin G2 mRNA abundance in different maturation stage-specific murine B cell lines. Upon treatment of wild-type and p53 null B cell lines with the negative growth factor, transforming growth factor β1, or the growth inhibitory corticosteroid dexamethasone, cyclin G2 mRNA levels were increased in a time-dependent manner 5–14-fold over control cell levels. Unstimulated immature B cell lines (WEHI-231 and CH31) and unstimulated IgM B cell receptor (BCR)-stimulated mature B cell lines (BAL-17 and CH12) rapidly proliferate and express low levels of cyclin G2 mRNA. In contrast, BCR-stimulated immature B cell lines undergo growth arrest and coincidentally exhibit an ~10-fold increase in cyclin G2 transcripts and a decrease in cyclin D2 message. Costimulation of WEHI-231 and CH31 cells with calcium ionophores and protein kinase C agonists partially mimics anti-IgM stimulation and elicits a strong up-regulation of cyclin G2 mRNA and down-regulation of cyclin D2 mRNA. Signaling mutants of WEHI-231 that are deficient in the phosphonositide signaling pathway and consequently resistant to the BCR stimulus-induced growth arrest did not display a significant increase in cyclin G2 or decrease in cyclin D2 mRNAs when challenged with anti-IgM antibodies. The two polyclonal activators lipopolysaccharide and soluble gp39, which inhibit the growth arrest response of immature B cells, suppressed cyclin G2 mRNA expression induced by BCR stimulation. These results suggest that in murine B cells responding to growth inhibitory stimuli cyclin G2 may be a key negative regulator of cell cycle progression.

Proliferation signals promote the coordinated progression of a cell through the cell division cycle. In eukaryotes this process is controlled by the sequential formation, activation, and inhibition of cyclin-cyclin-dependent kinase (CDK)1 complexes (1). Active cyclin-CDK complexes phosphorylate specific targets such as the tumor suppressor RB, various transcription factors, DNA polymerase α, and cytoskeletal proteins (2) and thus trigger progression through the cell cycle. The levels of many cyclins oscillate during the cell cycle and act as rate-limiting positive regulators of CDK activity. Mammalian cyclins are classified into different types based on their structural similarity, functional period in the cell division cycle, and regulated expression (1, 3, 4). 12 different cyclins in mammalian cells (cyclins A–I, some with multiple subtypes) have been identified (1, 5–7) either functionally or through an ~110-amino acid homologous region essential for cyclin-CDK complex formation (8–10) referred to as the cyclin box (3, 11). Cyclin-CDK activity is also subject to regulation by CDK inhibitors (CDKIs) such as p16INK4a and p16INK4b, p21WAF1/CIP1, and p27KIP1 which, in response to negative stimuli, bind cyclin-CDK complexes and block cell cycle progression (5, 12). In addition to participation in cellular proliferation, CDKs and cyclin-CDK pairs may participate in processes not directly related to cell cycle regulation as evidenced by Pho80-Pho85 cyclin-CDK participation in yeast phosphate metabolism (13, 14), the involvement of p35CDK5 in promoting neurite outgrowth (15–17), the association of the cyclin H/CDK7 pair in the TFIIH transcription factor complex (18, 19), and the cyclin C/CDK8 and Srb10/11 cyclin-CDK regulation of RNA polymerase II (20, 21).

We studied the effects of stimulatory and inhibitory signals on cell cycle components expressed in B lymphocytes representative of two different maturation stages of development. A robust immune system has to deliver specific and effective immune responses to foreign antigens and yet be immunologically tolerant of self-antigens. Such tolerance is achieved because T and B cells pass through stages in their development when ligation of their antigen receptors by self-antigens results in negative regulatory signals that induce either unresponsiveness and functional inactivation (clonal anergy) or their physical elimination (clonal deletion) (22–24). During clonal deletion, activated autoimmune cells are eliminated from the repertoire of potentially reactive immune cells by the induction of growth arrest and apoptosis (25, 26). This process is contextual and dependent on such factors as the type of antigen, strength of the antigen-induced signal, the ontogeny of the cell, the microenvironment of the immune cell, and the presence of positive costimulatory signals (27–30). Bone marrow immature

1 The abbreviations used are: CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; ORF, open reading frame; PCD, programmed cell death; BCR, B cell receptor; LPS, lipopolysaccharide; Ca2+, cytoplasmic calcium; DAG, diacylglycerol; PdBu, phorbol 12, 13-dibutyrate; gp, glycoprotein; TGF-β, transforming growth factor β1; PKC, protein kinase C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
IgM−IgD− B cells are particularly prone to undergo growth arrest and eventual PCD upon stimulation of their antigen receptor (24, 31, 32), as are self-reactive B cells in the germinal centers of the spleen (30, 33, 34). In contrast IgM stimulation of non-self-reactive peripheral IgM+ mature B cells in the presence of appropriate T cell help results in their entry into an active cell cycle, cellular proliferation, and clonal expansion (29, 30, 35, 36). Thus, a developmental switch in the differentiation pathway of B cells coupled to a sensing of the microenvironment through appropriate costimulatory signals has a profound effect on cell cycle regulation in response to antigen receptor stimulation of B lymphocytes.

Because the cyclin G gene was identified as a transcriptional target of the tumor suppressor p53, a key cell cycle checkpoint control protein (37–39), and its expression is induced following DNA damage, cyclin G is hypothesized to play a role in cell cycle checkpoint control (40, 41). Although the function of cyclin G has not yet been determined, cDNAs encoding a longer form of human and murine cyclin G, referred to as cyclin G1, and a novel human homolog, cyclin G2, were recently cloned and characterized (6). The mRNAs for human cyclins G1 and G2 are strongly expressed in tissues rich in terminally differentiated cells (cardiac and skeletal muscle for cyclin G1 and cerebellum for cyclin G2) and tissues populated with cells subjected to PCD (spleen and thymus). Murine cyclin G1 mRNA is expressed independently of p53 in a number of tissues of p53 null mice (e.g., brain, heart muscle, and stomach) and can be up-regulated in a p53 null murine B cell line by TGF-β treatment (6). While cyclin G1 mRNA is constitutively expressed and encodes a protein with no prototypic “destruction box” involved in ubiquitin-dependent degradation (42), or protein destabilizing PEST sequences (43), human cyclin G2 mRNA oscillates through the cell cycle, peaks in S-phase, and encodes a protein containing a carboxyl-terminal PEST sequence (6). The closest homolog of this family, cyclin I, is also strongly expressed in differentiated tissues and has been localized by in situ hybridization to terminally differentiated neurons of the hippocampus and cerebellum (7).

We cloned the cDNA encoding the murine form of cyclin G2 and investigated its expression pattern in rodent tissues and various murine cell lines. Our results indicate that cyclin G2 transcripts are present at high levels in murine B cells treated with agents causing growth inhibition or growth arrest but not in cells receiving a positive costimulus that promotes cell cycle progression. In contrast, we found that transcripts for cyclin D2, the D-type G1-phase cyclin associated with proliferation in B cells (44), are down-regulated during G1-phase growth arrest. The up-regulation of cyclin G2 mRNA during cell cycle arrest and its expression in terminally differentiated tissues suggest that this cyclin, and perhaps the related cyclins G1 and I, may function in specific contexts as negative coordinators of cell cycle progression.

EXPERIMENTAL PROCEDURES

Reagents—Phorbol 12,13-dibutyrate (PdBu), ionomycin (calcium salt), and propidium iodide were obtained from Calbiochem. Lipopolysaccharide Escherichia coli serotype 0111:B4 (LPS), porcine transforming growth factor β1 (TGF-β), dexamethasone and 5-bromo-2′-deoxyuridine (BrDu) were purchased from Sigma. Fluorescein isothiocyanate-conjugated goat anti-BrDu antibodies were obtained from Jackson-Dickinson (Mountain View, CA) and μ mouse-specific F(ab′)2 goat anti-IgM antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). COS cell supernatants containing soluble gp39 was the generous gift of Dr. Diane Hollenbaugh (Bristol-Myers Squibb) (45).

Cell Lines and Culture—The murine B cell lines WEHI-231 (46) and BAL-17 (47), CH12, CH31 (48), and the WEHI-231 mutants W88.1, W305.1, and W306.1 (49) were kindly provided by Dr. A. DeFranco (University of California, San Francisco). Cells were grown in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol at 37°C in 5% CO2. During experiments, the cultures were maintained in the exponential phase of growth (1–6 × 106). Isolation of Library cDNA Clones—Cyclin G2 cDNA probes were synthesized by polymerase chain reaction using the Genius® system digoxigenin-labeled dNTP mix from human G2 cDNA clones (Boehringer Mannheim). The resulting polymerase chain reaction fragments were purified by agarose gel electrophoresis using the GeneClean II® DNA purification kit (Bio 101, La Jolla, CA) and used to screen a λ Zap II murine thymus cDNA library (Stratagene, La Jolla, CA). Cross-species screening of the murine cDNA library with human cyclin G2 cDNA probes was done at low stringency with the hybridization buffer containing 30% formamide and filters hybridized and washed at 37°C. Development of the filters with alkaline phosphatase-conjugated digoxigenin antibodies and the Lumi-Phos® 530 reagent (Boehringer Mannheim) was done according to manufacturer’s protocol. The isolated phagemid DNA was amplified, extracted, and purified for sequence analysis following the manufacturer’s recommended methods and standard techniques (50).

Nucleotide Sequence Determination and Analysis—DNA sequences were determined using the Sequenase® (version 2.0) system following procedures recommended by the manufacturer (United States Biochemical Corp.). cDNA fragments present in the Lambda ZapII phagemid (Stratagene) cloning vectors were sequenced with either a vector-specific oligonucleotide or oligonucleotides homologous to the cloned fragment’s internal sequences. [α-32P]dATP or [α-32P]dATP (at 800 Ci/mmol) was used to radioactively label the DNA fragments. Nucleotide sequences were read from scanned gels with the aid of BioImage® sequence analysis software. The computer-aided editing and alignment of DNA sequences was accomplished using Genetics Computer Group (GCG) (Madison, WI) sequence analysis software. Additional nucleotide and cDNA-derived peptide sequence comparisons were performed using the BLAST program. Final alignments were performed using the GCG Pileup and Prettyn programs.

Counterflow Centrifugal Elutration and Analysis of Cell Cycle Position—Murine lymphocytes were separated into progressive stages of the cell cycle by centrifugal elutration, and the cell cycle position of elutriated fractions was determined as described previously (6). Unelutriated cell populations stimulated with different reagents were examined using dual-parameter flow cytometric analysis of total DNA content and newly incorporated BrDu. Briefly, following a 20–30-min pulse of a culture with 10 μM BrDu, ~2.0 × 106 cells were sedimented by centrifugation, washed in phosphate-buffered saline and fixed with ice-cold 70% ethanol, and stored at 4°C until treatment analysis could be performed. The permeabilization and staining with propidium iodide and fluorescein isothiocyanate-conjugated anti-BrDu antibodies were done following protocols supplied by Becton-Dickinson. Flow cytometry was performed utilizing either a FACScan and Lysis II software (Becton-Dickinson Instruments, San Jose, CA) or Coulter EPICS Profile II Analyzer with Multigraph and MultiCycle software (Coulter Electronics, Miami, FL).

Northern Blot Analysis—Total RNA was isolated from murine tissues and cells utilizing TRizol® reagent (Life Technologies, Inc.). The glyoxal denaturation of total RNA and electrophoresis in glyoxal-agarose was done following a standard protocol (50). After electrophoresis, the relative amount and quality of the RNA was controlled by short wave UV fluorescence shadowing of the ribosomal RNAs on a F-254 TLC plate. The fractionated RNAs were transferred and fixed to Magnagraph® nylon membranes (MSI, Westboro, MA) followed by removal of residual glyoxal as described (50). Membranes were routinely stained with methylene blue to control for RNA transfer efficiency as described (50). The [α-32P]dATP and [α-32P]dCTP radioactive labeling of DNA fragments was done using polymerase chain reaction generated and GeneClean II®-isolated DNA fragments as templates and reagents obtained from the Life Technologies, Inc. random priming kit. Hybridization of DNA probes to Northern blots was done according to the methods described by the manufacturer of the nylon membrane and standard protocols (50). PhosphorImaging (Molecular Dynamics) of the washed Northern blot filters was routinely obtained immediately before autoradiography. All Northern blot experiments were performed at least twice with reproducible results.

RESULTS

Cloning of Murine Cyclin G2 cDNA and Predicted Features of the Encoded Protein—Hybridization screening of a murine thy-
mic cDNA library with an internal cDNA fragment of the human cyclin G2 ORF (6) at low stringency identified 15 independent overlapping partial cDNA clones encompassing the full murine cyclin G2 ORF. One clone comprised nearly the full ORF lacking only the first two nucleotides of the translation initiation codon. In contrast to the human cDNA fragments of cyclin G2 cloned from a Jurkat lambda ZapII cDNA library (6), these murine cyclin G2 cDNA clones did not contain intron-exon junctions and represented the mature spliced form of cyclin G2. Double-stranded nucleotide analysis verified that the cDNA clones encompassed a 1035-base pair ORF with 85% nucleic acid sequence identity to the human cyclin G2 ORF. It encodes a predicted 345-amino acid protein with a molecular mass of 41 kDa which has 94.5% identity to the predicted human cyclin G2 protein (Fig. 1). Like the human homolog, murine cyclin G2 features a PEST-rich sequence near the carboxyl terminus, a motif thought to direct protein degradation (43). Murine and human cyclins G1 and G2 share a carboxyl-terminal sequence motif, previously identified in rat cyclin G (51), which is homologous to the epidermal growth factor and polyoma virus middle T antigen autophosphorylation sites (6, 51, 52). The murine cyclin G2 and murine cyclin A proteins share 47% similarity and 26% identity. Our analysis indicates that human cyclin I, a cyclin of unknown function highly expressed in brain and skeletal muscle (7), is more related to full-length human cyclins G1 and G2 (52% similarity and 30% identity for both) than to cyclin A (46% similarity and 24% identity).
Crystallography of cyclin A has defined a new structural motif consisting of two tandem repeats of a five-helix bundle referred to as the “cyclin fold” (10, 53, 54). The first repeat spans the cyclin box (α1–α5, see Fig. 1) immediately followed by the second (α1’–α5’). The amino acid identity between the cyclin G family and cyclin A is highest in the cyclin box region, yet it extends to the amino- and carboxyl-terminal regions suggesting that the G family of cyclins possess a cyclin fold structure similar to cyclin A (Fig. 1). The amino-terminal region of cyclin A contributes important residues for CDK binding and structural integrity of cyclin A and is likely to have a similar function for cyclins G1, G2, and I. Cyclin A residues Arg-211 and Asp-240 form a buried salt bridge connecting helix 1 with helix 2 and are essential for cyclin A-CDK activity (9, 10, 53, 55). The equivalent residues in the sequences of cyclins G1, G2, and I are conserved, as they are among most cyclins (3). Amino acids at position 266 (lysine) and 295 (glutamate) in cyclin A are crucial CDK contact residues (10, 53) and are maintained in cyclins G1 and G2 and human cyclin I, although the residues equivalent to the cyclin A 266 lysine in murine and human cyclin G2 have been conservatively exchanged with an arginine (Fig. 1). Alanines 235 and 264 are essential for the tight packing at the interhelical crossing points of cyclin A (10, 53) and are conserved in the amino-proximal α2 and α3 helices of the G family cyclins. Additional residues determined to contribute to the structure of cyclin A (10, 53) which are conserved in the cyclin G2 sequence are indicated by upward arrowheads in Fig. 1.

In contrast, there are nonconserved amino acid exchanges between the cyclin G family and cyclin A that might modify their structure (10, 53). Alanines 333 and 363 in the carboxyl-proximal α2 and α3 helices of cyclin A, necessary for tight packing of the helices, have been replaced by negatively charged residues in both human and mouse cyclins G1, G2, and cyclin I. Extensions of the interhelical regions in the carboxy-terminal half of cyclins G1, G2, and I suggest a distinction between the A and G family of cyclins that may allow for novel interactions of the G family with other proteins. Furthermore, crystallography of the cyclin A-CDK2-p27KIP1 complex has demonstrated the importance of the conserved cyclin A α1 and α3 helix sequences (MRAILDVD and RGKLQ; bold type indicates residues critical for p27 contact) in the interaction of p27KIP1 with cyclin A through the p27 LFG motif, a motif shared by the Kip/Cip family of CDKIs (54). A lack of sequence conservation between cyclin A and cyclins G1, G2, and cyclin I in this region (Fig. 1) suggests that the latter three do not interact with these CDK inhibitors through the p27 LFG motif.

Cyclin G2 mRNA Levels in Wild Type and p53 Null Mouse Tissues and Cell Cycle Position-dependent Cyclin G2 Expression—To determine the size and distribution of cyclin G2 mRNA in murine tissues and cell lines, Northern blot analyses were performed. A single cyclin G2 mRNA band of ~2.8 kilobases was differentially expressed in murine tissues compared with cyclin G1. Abundant cyclin G2 transcripts were found in total brain, neocortex, spleen, thymus, and intestine (Fig. 2A). In contrast to cyclin G1, cyclin G2 mRNA was only weakly expressed in skeletal muscle and heart. Notably cyclin G2 transcripts are most abundant in tissues rich in either terminally differentiated cells or cells subject to growth inhibitory signals and PCD. These results were obtained twice in independent Northern blot analyses and are in agreement with our observations for human cyclin G2 (6). Functional p53 expression positively correlated with increased cyclin G1 transcripts in proliferating murine B lymphocyte cell lines and in kidney but not in other terminally differentiated tissue (such as skeletal muscle, stomach, and brain) (6). The relationship of p53 to

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**Fig. 2.** Northern analysis of the cyclin G2 transcript in wild-type and p53 null murine tissues and through the cell cycle in two murine B cell lines. Northern blot analyses were performed with ~20 μg of total RNA from various murine tissues and examined for expression of cyclin G2 relative to other cyclins, p53, and GAPDH (as a control for relative amount and quality of mRNA). A. fractionated RNAs from the indicated tissues blotted onto nylon membrane and probed for cyclin D2, cyclin G1, cyclin G2, and GAPDH. The position of RNA standard markers are shown on the right and the respective mRNA indicated on the left. B, comparison of cyclin G2 transcript levels to cyclin G1 (CycG1) in the indicated tissues from p53 wild-type (+/+), heterozygous (+/−), and null (−/−) mice. C, analysis of cyclin G2 expression at progressive stages of the cell cycle in the p53 wild-type WEHI-231 immature B cell line and p53 null BAL-17 mature B cell line. Shown are fluorescence-activated cell sorting profile of cellular DNA content from an unfractionated population (Tot Pop) and the elutriated cell fractions used for corresponding Northern blots (below). The RNAs from the respective cell cycle-positioned cells hybridized with the indicated cDNAs are shown with the corresponding elutriation fraction numbers above each lane.
cyclin G2 mRNA expression in cell lines or various developing or terminally differentiated tissues was not known. Therefore, cyclin G2 mRNA expression in various tissues from p53 null, heterozygous, and wild-type p53 mice was compared. No positive correlation between wild-type p53 and cyclin G2 mRNA expression was seen in the tissues examined (Fig. 2B). Cyclin G1 mRNA levels are increased in stomach and skeletal muscle and slightly in total brain tissue of p53 null mice (6). Now we report a notable increase in cyclin G2 mRNA in brain tissue of p53-deficient mice (Fig. 2B). As our previous investigation indicated cyclin G1 expression is inverse to p53 expression in some stages of embryonic development (6), a comparative Northern blot analysis of cyclin G2 was performed on murine embryonic mRNAs. Unlike the on-off-on expression pattern seen for cyclin G1 transcripts, cyclin G2 mRNA is very abundant throughout all stages of development and does not parallel p53 transcript levels (6) and data not shown). Taken together, cyclin G2 and cyclin G1 mRNA expression appear to be independently and differentially regulated during murine embryonic development and tissue differentiation, and cyclin G2 mRNA levels do not correlate with p53 expression.

As cyclin G2 is strongly expressed in both human (6) and murine immune system tissues, we examined cyclin G2 expression in several murine leukocyte cell lines. Cyclin G2 mRNA was present at moderate levels (when normalized to GAPDH) in the B cell lines A-20, BCL-1, and WEHI-231, the myeloid leukemia cell line CI498, and thymic epithelium TE-71 cells but was not well expressed in T lymphoid cells (EL-4 and L1210) (data not shown). To further investigate the relationship of p53 to cyclin G2 mRNA expression and examine the cell cycle position-dependent expression of murine cyclin G2, Northern blot analysis was performed on fractionated cells from the p53 wild-type, IgM- immature B cell line WEHI-231, and the p53 null, IgM- mature B cell line BAL-17 (Fig. 2C). Exponentially growing cultures of these two B cell lines were fractionated by centrifugal elutriation into populations at progressive stages of the cell cycle, and the position of each fraction was verified by flow cytometry. Northern blot analysis indicated that in WEHI-231 cyclin G2 mRNA was slightly more abundant in the S-phase fractions compared with G1-phase and, in contrast to cyclin G1 expression, decreased in subsequent stages of the cell cycle. In BAL-17 cells the cyclin G2 message was at levels similar to WEHI-231 expression, in contrast to cyclin G1, and moderately increased in S-phase fractions. Expression from the cyclin G1 gene in murine lymphocytes is constitutive (following initial up-regulation in early G1 phase) and partially dependent on transcriptional activation by wild-type p53 (6, 40). In contrast, cyclin G2 transcript levels moderately oscillate through the cell cycle and do not appear to be influenced by the expression of p53.

**Modulation of Cyclin G2 Expression by the Growth Inhibitory Factors TGF-β and Dexamethasone**—To determine if the level of cyclin G2 mRNA could be modulated by positive or negative growth stimuli we tested the effect of several growth factors on cyclin G2 expression in a variety of B cell lines. In the B cell compartment TGF-β acts as a negative immunomodulatory cytokine by inhibiting interleukin-7 growth stimulation of pre-B cells, κ light chain expression, and transition of human and murine B cells from the G1- to S-phases of the cell cycle (56–59). We have previously shown that cyclin G1 mRNA is up-regulated by TGF-β in a time-dependent manner independent of the p53 status of the B cell line (6). Here, exponentially growing cultures of BAL-17 and WEHI-231 were treated with TGF-β at 1 ng/ml over 30 h, and aliquots were periodically sampled for RNA isolation. Northern blot analysis followed by PhosphorImaging of Northern blots indicated an ~14-fold increase of the cyclin G2 message in BAL-17 relative to untreated cells within 29 h of treatment (Fig. 3), a time point when the TGF-β-mediated inhibition of DNA synthesis is ~20% (58). A similar response was seen with TGF-β-treated WEHI-231 cells, the basal level of cyclin G2 mRNA expression increasing ~10-fold in cells treated for 16–29 h (Fig. 3). Glucocorticoid hormones also act as growth inhibitory factors on lymphoid cells (58, 60–62). We examined the effect of the glucocorticoid dexamethasone on the expression of cyclin G2 mRNA. Dexamethasone inhibited the growth of a number of B cell lines with kinetics similar to that observed with TGF-β; DNA synthesis was ~83% of untreated control levels after 26 h of treatment of both BAL-17 cultures with 1 μM dexamethasone and CH12 cells with 10 μM dexamethasone. Longer periods of treatment or increasing dexamethasone concentration to 10 μM resulted in a corresponding decrease in cellular proliferation, with a 35% decrease in the S-phase population of BAL-17 cells after 31 h of culture with 10 μM dexamethasone. Cyclin G2 mRNA was increased ~5-fold in dexamethasone-treated cells as compared with untreated controls (Fig. 3 and data not shown). This effect was most obvious in the mature B cell lines BAL-17 and CH12 (Fig. 3) but was also evident in similarly stimulated WEHI-231 cells (data not shown). Reproducing all of these Northern blots with p53 cDNA showed no differences in the p53 mRNA levels of stimulated cells relative to the respective un-
treated control (data not shown). Thus cyclin G2 mRNA is up-regulated, independent of p53, during the response of murine B cells to two growth inhibitory agents.

**Up-regulation of Cyclin G2 and Down-regulation of Cyclin D2 mRNAs Coincident with BCR-induced Growth Arrest and Apoptosis**—We next investigated whether other growth inhibitory signals also elicit an up-regulation of cyclin G2 mRNA in B lymphocytes. IgM immature B cell lines and IgM mature B cells exhibit differential responses to surface IgM (BCR) ligation (27–29). WEHI-231 and CH31 are immature B cell lines that undergo growth arrest and eventual apoptosis following BCR cross-linking (63–65) analogous to self antigen-induced clonal abortion (24, 28) and are often used as in vitro models for B cell tolerance and the elucidation of BCR-mediated signal transduction pathways (66–69). Logarithmically growing cultures of WEHI-231 and CH31 cells were stimulated with goat anti-IgM antibodies. The viability and growth of treated and control cultures was monitored by microscopy and trypan blue exclusion. Northern blot analysis for cyclin G2 and GAPDH mRNA indicated that BCR cross-linking of immature B cells induces a 5–10-fold amplification of cyclin G2 transcripts by 12 h of treatment in WEHI-231 and 14 h in CH31, with the increased level maintained through 24 h of stimulation (Fig. 4). Hybridization of the same Northern blot with either cyclin G1 or p53 cDNA probes did not show a change in their mRNA levels (data not shown). Flow cytometry DNA analysis indicated that the increase in cyclin G2 transcripts paralleled G1-phase growth arrest and, in the case of CH31, apoptosis (tabulated in Fig. 4). Probing the same Northern blots with cyclin D2 cDNA revealed that cyclin D2 mRNA levels decreased relative to untreated controls, coincident with the onset of growth arrest and the accumulation of cyclin G2 transcripts (Fig. 4). In contrast, BCR cross-linking of BAL-17 cells did not result in an increase of cyclin G2 mRNA or decreased levels of cyclin D2 mRNA (Fig. 4) and induces a proliferative response rather than growth arrest (Fig. 4). Likewise, BCR stimulation of CH12 cells does not induce growth arrest (70) and did not produce a significant elevation of cyclin G2 mRNA at either 14 or 24 h of treatment (data not shown).

Anti-IgM-stimulated WEHI-231 cells arrest in the G1-phase of the cell cycle (71, 72). Some have proposed that WEHI-231 late G1- and S-phase cell populations are insensitive to the negative signals elicited by BCR cross-linking (71), whereas others have shown that the efficiency of G1-phase growth arrest is dependent on the length of time between signal initiation and arrival of the population at the G1-phase restriction point (72). We wanted to determine whether there was a cell cycle position dependence for cyclin G2 up-regulation and cyclin D2 down-regulation in response to anti-IgM-mediated growth arrest. Purified populations of early G1-, G1/S-, and early S-phase WEHI-231 cells were isolated by centrifugal elutriation and cultured in the absence or presence of anti-IgM antibodies. Aliquots were obtained at progressive times for Northern blot analysis and flow cytometry (Fig. 5). Significant G1-phase growth arrest was achieved for each population by 25 h of treatment, and full arrest was attained by 39 h. However, comparison of the early G1-phase fraction to the S-phase fraction indicated that the early G1 fraction accumulated a small but detectable population of cells that appeared to be arrested by 6 h of treatment and a more significant population was observed by 15 h (one cell cycle) of treatment. In contrast, only a very small arrested population in the anti-IgM-treated S-phase (fraction 5) culture was observed by 18 h. Thus there was an 12–16-h delay in the initial accumulation of G1 cells following BCR cross-linking the S-phase population in comparison to the G1-phase population. Similar results were seen with
the G$_1$/S-phase population (fraction 3); a detectable accumulation in G$_1$ cells was only seen after 18 h of stimulation. Analogous studies examining the sensitivity and timing of G$_1$-phase arrest initiation of mid G$_1$-, S-, and G$_2$-phase populations from 10 to 28 h of treatment with anti-IgM had comparable results; the majority of cell cycle arrest occurs following one full cell cycle as the population cycles for a second time into G$_1$ and reaches the G$_1$ arrest point (data not shown).

The down-regulation of cyclin D2 and up-regulation of cyclin G2, like the induced G$_1$-phase arrest, is a gradual process. The decrease in cyclin D2 preceded the gradual increase in cyclin G2 message (Fig. 5). This decrease in cyclin D2 followed by a continuous decline was obvious by 3–21 h of anti-IgM stimulation of the early G$_1$-phase fraction but was not clear until 6 h through at least 15 h of IgM cross-linking of either the G$_1$/S- or early S-phase fractions. The increase in cyclin G2 mRNA, when normalized to GAPDH, was apparent by 6 h of signaling in the G$_2$/S fraction and clearly detectable by 7 h in the early G$_1$ population. There appeared to be an additional 2–3-h delay in the up-regulation of cyclin G2 mRNA in the S-phase-stimulated fraction. This occurred while the cells were proceeding through mitosis and entering G$_2$-phase but had not yet entered the S-phase (Fig. 5). A similar experiment with an enriched S/G$_2$ fraction yielded comparable results. Cyclin D2 down-regulation occurred through 7–35 h of stimulation, but the onset of detectable cyclin G2 expression was delayed until after 10–14 h of stimulation (data not shown). Although the length of stimulation necessary for cyclin G2 mRNA induction may be moderately influenced by the cell cycle stage where the signaling begins, the induction of cyclin G2 mRNA accumulation is mostly cell cycle position-independent and precedes a nearly complete G$_1$-phase arrest by 10–15 h.

**Increased Expression of Cyclin G2 mRNA Parallels Growth Arrest and Apoptosis of Immature B Cell Lines Stimulated with Phorbol Esters Plus Calcium Ionophores**—The phosphoinositide-derived second messengers diacylglycerol (DAG) and cytosolic calcium (Ca$^{2+}$) play an important role in mediating the effects of antigen receptor stimulation (73–75). Anti-IgM-induced growth arrest and apoptosis of immature B cells can be partially mimicked by treatment of B cells with a combination of phorbol esters and calcium ionophores (72, 74); however, this pharmacological treatment does not result in growth inhibition of mature B cells (74). To examine if the increased expression of cyclin G2 mRNA and decrease in cyclin D2 mRNA can be elicited through an increase in Ca$^{2+}$, and activation of a PKC-dependent pathway, we treated logarithmically growing WEHI-231, CH31, BAL-17, and CH12 cells with 7 nM PdBu in combination with 250 nM ionomycin for a period of 24–44 h. In
Phosphoinositide Signaling Pathway Do Not Alter Cyclin G2 or Cyclin D2 mRNA Expression in WEHI-231 Cells. Cells were cultured in the presence or absence of anti-IgM antibodies with or without 1 μg/ml LPS. When stimulated with LPS alone, an increase in cyclin D2 message was found for the first 2–12 h of treatment followed by a decline to basal levels between 12 and 14 h (Fig. 8A, right panel). In addition, the decrease in basal cyclin D2 transcripts observed in anti-IgM growth-arrested WEHI-231 was inhibited through at least 10 h of costimulation with LPS, followed by a decline to basal or lower levels thereafter (Fig. 8A, left and right panel). Rather than inhibiting cyclin D2 expression, costimulation of WEHI-231 with LPS and anti-IgM antibodies strongly enhanced cyclin D2 transcript expression, as an increase in cyclin D2 mRNA was observed after 2–10 h of treatment.

The protective effect of LPS on anti-IgM-induced growth arrest also correlates with a suppression of cyclin G2 mRNA accumulation (Fig. 8A). The early rise in cyclin G2 transcripts at 6 and 10 h in anti-IgM-stimulated cells is strongly repressed by LPS and parallels the protection of WEHI-231 from BCR-induced G1 phase arrest (Fig. 8A, left panel). The rise in cyclin G2 and decline in cyclin D2 mRNA levels at 14 h coincided with loss of the protective effect of LPS (see next paragraph). WEHI-231 cultures stimulated with LPS plus BCR cross-linking antibodies do not, however, produce the same amount of cyclin G2 message as elicited by anti-IgM alone, even after 24 h of costimulation.

Following 12–14 h of stimulation B cell cultures adapt to LPS and no longer respond to its mitogenic signals (76). We tested if pretreatment of WEHI-231 with LPS for 14 h, followed by anti-IgM treatment, provides the same level of protection as obtained when LPS is applied simultaneously with anti-IgM. Cyclin G2 mRNA is increased when LPS had been added 14 h before anti-IgM for a total of 29 and 39 h as compared with simultaneous application of LPS and anti-IgM for 15 and 25 h (Fig. 8A, right panel, first 6 lanes). This increase parallels the increase in G1-phase arrested cells. Thus, due to adaptation, the protective effect of LPS against BCR-induced growth arrest of WEHI-231 is lost following 14 h of pretreatment, and this loss of protection correlates with a rise in cyclin G2 mRNA and a decline in cyclin D2 mRNA back to or below the pre-LPS stimulation basal levels.

A similar repression of cyclin G2 up-regulation was seen when the anti-μ-induced growth arrest is inhibited by a soluble form of gp39 (Fig. 8B), the murine CD40 ligand expressed on T helper cells (45, 80). In this case the reduction of cyclin G2 mRNA is more prominent at 12 h of treatment when the protective effect of soluble gp39 on growth inhibition is most pronounced. Similar to LPS, gp39 by itself strongly up-regulated cyclin D2 mRNA (Fig. 8B, left two lanes), and the inhibition of cyclin D2 mRNA down-regulation by the gp39 costimulation was more obvious earlier in the time course when the abrogation of the arrest response was most effective and fewer cells were in G1-phase arrest compared with controls (Fig. 8B). Taken together, inhibition of anti-IgM-mediated growth arrest and PCD by polyclonal activators of the B cell compartment such as LPS or gp39 correlates with the inhibition of cyclin G2 mRNA up-regulation and at least a modest up-regulation of cyclin D2 mRNA.
DISCUSSION

We present compounding evidence that expression of cyclin G2 is up-regulated in B cells during responses to negative signaling, and we present arguments for its possible role as a negative regulator of the cell cycle. The gene for cyclin G1, the closest homolog of cyclin G2 (6), is a transcriptional target of the cell cycle check point control protein p53 and may play a role in cell cycle arrest (40, 41). Our finding that cyclin G2 is expressed in both wild-type and p53-deficient murine tissues and cell lines suggests that there is no positive correlation between p53 status or proliferative state of the tissue and cyclin G2 mRNA levels. Cyclin G2 expression may be repressed by p53 as it is enhanced in the brain of p53 null mice. This increase is considerably more than we, and later others (6, 81), have observed for cyclin G1 mRNA in p53 nullizygous brain. Cyclin G1 and G2 mRNAs are abundant in terminally differentiated tissues, e.g. brain and muscle, and those rich in cells subject to PCD (e.g. spleen, thymus) (Fig. 2). We further examined cyclin G2 expression during responses to negative and positive signal transduction using immature and mature B cell lines as a model system.

TGF-β and Dexamethasone Up-regulate Cyclin G2 mRNA—B cells as well as most other cell types have specific TGF-β receptors (57, 82). In many cell types TGF-β stimulation induces the expression or translocation of CDKIs (e.g. p27KIP1, p15INK4A, and p21CIP1) that block cell cycle progression (82–85). TGF-β not only induces G1-phase growth arrest but also apoptosis in normal human and Burkitt B lymphoma cells and potentiates anti-Ig-induced apoptosis of murine immature B cell lines (57–59, 70, 86, 87). It is not known if a CDKI is involved in TGF-β-mediated growth inhibition of WEHI-231, but TGF-β treatment of WEHI-231 and CH31 results in the accumulation of a hypophosphorylated form of the retinoblastoma tumor suppressor protein RB (70).

TGF-β growth inhibition in various B cell lines is a slow process, with a ~20% decrease in cellular proliferation after 24 h of culture, which for WEHI-231 increases to 40% by 72 h of treatment (58). In contrast to the early transient increase in cyclin G1 mRNA for TGF-β-treated BAL-17 and WEHI-231 cells (6), cyclin G2 mRNA steadily increased in similarly treated cultures of both cell lines, reaching a 10–14-fold elevation at 29 h of culture. In the p53 null cell line BAL-17, up-regulation of cyclin G2 mRNA was detectable after 15 min, typical for an early response, but in WEHI-231 this effect was not seen before 3–8 h of culture. In both cell lines this increase did not accompany a change in p53 or, at least in the case of BAL-17, cyclin D2 mRNA levels2 and preceded a more complete inhibition of cellular proliferation. Analogous to the induction of several CDKIs by TGF-β in other systems (82, 85), TGF-β induces cyclin G2 mRNA accumulation in murine B cells. Dexamethasone inhibits the proliferative response of B cells to anti-Ig and interleukin-4 (58, 60, 61). It caused a strong increase in cyclin G2 mRNA in CH12 and BAL-17 cells coinciding

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2 M. Horne, unpublished observations.

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[FIG. 8. LPS and gp39 suppress the BCR-dependent cyclin G2 mRNA up-regulation. Cyclin G2, cyclin D2, and GAPDH mRNA detected by Northern blotting. A, WEHI-231 cells were cultured for the period shown (in hours) above each lane with either a combination of 2 μg/ml F(ab′)2 anti-IgM plus LPS (1 μg/ml), anti-IgM alone, LPS alone or no additives (top two panels). In some cases LPS treatment preceded anti-IgM addition by 14 h. B, RNA extracted from WEHI-231 cells cultured for the indicated times with either soluble gp39 (1/10 of COS cell supernatant) alone, anti-IgM alone, or costimulated with both agents in parallel and a nontreated control. The percentage of cells in the specific phases of the cell cycle at the time of sampling determined by two-parameter flow cytometry is shown below the corresponding lanes in A and B.]
with a moderate level of growth inhibition observed at 22–31 h (Fig. 3). This treatment did not change p53 or cyclin D2 mRNA levels (data not shown). Enhanced cyclin G2 mRNA expression is thus correlated with a p53 independent growth inhibitory response of B cell lines to two immunosuppressive agents known to have negative effects on cell cycle progression. It is not known if cyclin G2 mRNA levels continue to increase with extended treatment of murine B cells with either TGF-β or dexamethasone, but it is interesting that both agents enhance anti-IgM-induced growth arrest of WEHI-231 and block the ability of LPS to protect WEHI-231 from this growth arrest response (see below) (58).

**Up-regulation of Cyclin G2 mRNA during BCR-mediated Growth Arrest**—BCR stimulation of both immature and mature B cells induces protein tyrosine phosphorylation, hydrolysis of phosphoinositide, and activation of PKC (69). This stimulation strongly up-regulated cyclin G2 mRNA in WEHI-231 and CH31 cells coincident with the onset of growth arrest and apoptosis (Figs. 3, 6, and 8, and data not shown) but not in proliferating populations of unstimulated controls or in the anti-IgM-treated mature B cell lines BAL-17 and CH12. Cyclin G2 mRNA accumulates to detectable levels by 6 h in unsynchronized anti-IgM-stimulated WEHI-231 and rises by 14 h to near maximum levels that are maintained for at least 35 h (data not shown). Simultaneous application of phorbol diesters and calcium ionophores partially mimics the anti-IgM growth arrest response in WEHI-231 and CH31 (72, 74) and up-regulates cyclin G2 mRNA. In addition, the anti-IgM-resistant WEHI-231 mutants W305.1 and W306.1, which are deficient in either the phosphoinositide signaling pathway or in a downstream component responsive to Ca2+ elevation and PKC activation (49), did not up-regulate cyclin G2 mRNA in response to BCR cross-linking. Thus BCR signaling pathways involving a combination of Ca2+ elevation and PKC activation are likely to be important for the up-regulation of cyclin G2 mRNA. The transcription factors c-myc, junB, c-fos, egr-1, nur77, nup475, and pip92 are immediate early response genes that are activated by BCR stimulation and PKC activation with phorbol esters (69, 88). In addition the pre-existing transcription factors NF-xB, CREB, and Ets-1 are altered upon BCR stimulation, the first two becoming activated through PKC signaling events, and the latter inhibited by CaM kinase II phosphorylation (69, 89). While it is not yet established if a burst of transcriptional activity or the accumulation of a more stable transcript is responsible for rises in cyclin G2 mRNA, any one of the above transcription factors could be involved.

A striking decline in cyclin D2 mRNA preceded the rise in cyclin G2 mRNA during the BCR-evoked growth arrest response of WEHI-231 and CH31 cells (Fig. 4). No change in cyclin D2 mRNA was obvious in the mature B cell lines or WEHI-231 anti-IgM-resistant mutants (Figs. 4–6 and data not shown). Cyclin D2 is the major D-type cyclin promoting the progression of B cells and other hematopoietic cells through the G₁ phase of the cell cycle (44, 90, 91). Mitogenic stimulation of murine primary B lymphocytes with anti-Ig antibodies induces proliferation, cyclin D2 and CDK4 synthesis, and the formation of cyclin D2/CDK4 holoenzymes capable of phosphorylating RB (44). In contrast, anti-IgM treatment of WEHI-231 considerably reduces CDK4 and CDK6 protein levels by 24 h of treatment (79), resulting in accumulation of a hypophosphorylated form of RB (70, 92). During cell cycle regulation, RB and the other related pocket proteins, p107 and p130, are dephosphorylated in early G₁-phase, promoting their binding to the E2F family of transcription factors and thereby preventing the transcription of genes important for entry into S-phase, DNA synthesis, and subsequent cell cycle events (93, 94). Cyclin D/CDK4 and cyclin D/CDK6 holoenzymes are believed to be the resident early and mid-G₁-phase active kinase complexes responsible for the hyperphosphorylation of RB and related proteins (4, 91). A decrease in cyclin D2 levels in WEHI-231 cells might result in decreased phosphorylation of RB, p107, and p130, a consequent continued binding of these pocket proteins to E2F transcription factors blockading cells at the G₁-phase restriction point and inhibiting their entry into S-phase (93, 95). As D-type cyclin proteins are reported to be very unstable proteins (4, 96), it would not be expected that high levels of cyclin D2 protein in WEHI-231 and CH31 cells could persist in the absence of de novo synthesis from cyclin D2 transcripts.

Others have investigated cyclin A protein expression, an S/G₂-phase cyclin, and the activity of cyclin A-CDK2 and G₁/S-phase cyclin E-CDK2 complexes in growth arresting WEHI-231 (92, 97). After 24 h of BCR cross-linking, when 70–75% of a WEHI-231 cell population is expected to be arrested in G₁, cyclin A-CDK2 and cyclin E-CDK2 associated in vitro kinase activity was decreased (97). 12–16 h after BCR cross-linking the expression of p27KIP1 is up-regulated, with increasing amounts of p27KIP1 forming complexes with cyclin A-CDK2 (97). The decrease in cyclin A-CDK2 and cyclin E-CDK2 kinase activity may be secondary to preceding G₁-phase cell cycle arrest. While Eshevsky et al. (97) did not examine cyclin D2/CDK4 or cyclin D2-CDK6-associated kinase activity nor the expression of CDK6 protein, they did not find an obvious decrease in CDK4 protein levels or cyclin D2 levels in WEHI-231 cells, in disagreement with results presented here and by others (79). The reason for this discrepancy is not clear, but as these authors indicated it could be due to differences in WEHI-231 clones. We examined transcript abundance compared with both ribosomal RNA and GAPDH mRNA levels, thereby correcting for loading differences or changes in total mRNA under the various conditions. As discussed above we would not expect the unstable cyclin D2 proteins to persist.

How would the dramatic increase in cyclin G2 transcripts and drop in cyclin D2 transcripts during BCR-induced growth arrest ultimately affect the cell cycle? Our observations on the lack of cell cycle dependence of WEHI-231 cells to negative signaling are similar to earlier findings (72) and demonstrate that the anti-IgM growth arrest response is a slow process, the efficiency of arrest increasing with the duration of treatment preceding entry into the G₁ phase restriction point. Several inhibitory regulators of the cell cycle may act at different targets to cause cell cycle arrest at the G₁-phase restriction point. Synchronized WEHI-231 cells show a considerable drop in cyclin D2 mRNA levels in the early G₁-phase fraction by 3 h of anti-IgM treatment and a rise of cyclin G2 levels starting by 6–7 h of treatment. If cyclin D2/CDK4 and cyclin D2-CDK6 complexes decrease when the cells are in G₁-phase, RB and related proteins would remain underphosphorylated and associated with E2F transcription factors, thereby inhibiting subsequent transcription of genes important for passing the restriction point. Those cells that passed this restriction point would then complete the rest of the cell cycle until they re-enter the G₂-phase and are challenged to cross the restriction point again. This may account in part for the passage of anti-IgM-stimulated late G₁ and S-phase WEHI-231 cells through one round of cell cycle before arrest occurs in the following G₁-phase, although a balance between the amount of residual D2 message and the onset of cyclin G2 expression may also be important.

As indicated by crystallographic analysis (10, 54) and our protein sequence alignments, cyclin G2 possesses a cyclin box domain similar to the cyclin A domain which mediates cyclin A-CDK2 interaction but lacks the amino-terminal motif impor-
tant for cyclin A-p27 interaction. While it is possible that cyclin G2 binds one or more CDKs, it appears unlikely that cyclin G2 interacts at its amino terminus with p27 or related CDKIs. As cyclin G2 protein abundance increases, it may displace other cyclins in cyclin-CDK complexes or may sequester CDKIs not bound to a CDK and may itself act as a CDK. Because cyclins G1, G2, and I contain carboxyl-terminal regions different from other cyclins, cyclin G2-CDK complexes could further inhibit CDK activity through a possible interaction with other proteins at its carboxyl terminus. It is intriguing that cyclin G1 has recently been reported to interact with two isoforms of the β regulatory subunit of protein phosphatase 2A (98). The concerted action of several inhibitory components on the cell cycle, with cyclin G2 perhaps acting as an anti-cyclin inhibiting the formation of active cyclin-CDK complexes not yet bound by CDKIs, could cause an eventual G1-phase arrest. Because rises with cyclin G2 perhaps acting as an anti-cyclin inhibiting the regulatory subunit of protein phosphatase 2A (98). The expression of cyclin G2 and characterization of its expression in various murine B lymphocytes (44). We showed that LPS costimulation significantly decreased the abundance of cyclin G2 transcripts and up-regulated cyclin D2 mRNA levels in anti-IgM-stimulated WEHI-231 cells. The degree of cyclin G2 repression and cyclin D2 augmentation correlated with the degree of cellular proliferation. We demonstrated that stimulation of WEHI-231 cultures with LPS alone significantly increased the level of cyclin D2 transcripts for ~12 h of treatment. After that period, it declined to a level observed in the untreated control. This increase in cyclin D2 paralleled an increase in the amount of S-phase cells. Thus, as observed by Tanguay and Chiles (44), high cyclin D2 expression may be a reflection of a highly proliferative B cell population.

A delay in the addition of LPS up to 6 h after the addition of anti-IgM does not result in a loss of protection, but longer delays decrease this protection so that by 14 h less than 20% of the maximal protection is achieved (76). In addition, WEHI-231 cells can adapt to LPS. After prestimulation for 12 h with LPS, WEHI-231 cells are no longer resistant to anti-IgM-induced effects (76). The kinetics of LPS protection of WEHI-231 from growth arrest parallels the kinetics of cyclin G2 mRNA induction. If WEHI-231 cells are stimulated simultaneously with LPS and anti-IgM, cyclin G2 mRNA expression is repressed until 14 h later. Then cyclin G2 mRNA accumulation begins and parallels the decrease in the S-phase population seen thereafter. The rise in cyclin G2 levels in the presence of LPS can be augmented if BCR-stimulated WEHI-231 cultures are pre-treated with LPS for 14 h prior to the addition of anti-IgM, and these prestimulated cultures contain fewer S-phase cells and more G1-phase-arrested cells than simultaneously costimulated cultures. Thus it appears that a balance between the amount of cyclin D2 and cyclin G2 mRNAs is an important indicator of the protection provided by LPS to immature B cells challenged by B cell receptor ligation. When the balance is tipped so that cyclin G2 transcripts accumulate to high levels and cyclin D2 declines to near or below the amount present in untreated cells, growth arrest ensues. This hypothesis is supported by the observation that inhibition of anti-IgM-mediated growth arrest through CD40 stimulation resulted in a similar decrease in cyclin G2 message and a slight enhancement in cyclin D2 message levels; the rise in cyclin D2 later declining and cyclin G2 rising as the protection afforded by CD40 ligation decreased.

Our investigation suggests that cyclin G2, and perhaps the related cyclins G1 and I, may play a role that is antagonistic to the function of proliferative cyclins. The cloning of murine cyclin G2 and characterization of its expression in various murine tissues provides the basis to further investigate the function of cyclin G2 expression during growth arrest and tissue differentiation by controlled over-expression in different rodent cell lines and the generation of transgenic mice. Production of antibodies specific for cyclin G2 for immunohistochemical studies of selected tissues as well as for the identification of proteins associated with cyclin G2 by immunoprecipitations should now be possible.

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