Regulation of B-Cell Growth and Immunoglobulin Gene Transcription by Interleukin-6

By Jerome E. Tanner and Giovanna Tosato

Interleukin-6 (IL-6) stimulates growth and immunoglobulin (Ig) secretion in Epstein-Barr virus (EBV)-infected B cells. In this study, we demonstrate that B-cell activation by IL-6 is associated with an initial induction of c-myc, a gene believed to act as a competence factor for increased RNA transcription and DNA replication, and by increases in DNA, RNA, and protein synthesis, as well as cell number. IL-6 increased the levels of Ig mRNA per cell in comparison to a non-cycle-dependent cellular RNA, tubulin. However, two other cell cycle-dependent cellular mRNAs, c-myc and actin, were also induced by IL-6 comparable to Ig mRNAs. Increased levels of Ig mRNA were not due to significant changes in RNA turnover, but appeared to reflect increased levels of RNA transcription. Together, these findings support the notion that IL-6 plays an important role as a stimulator of DNA and RNA synthesis in EBV-activated B cells.

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numbers of Ig plaque-forming cells after 72 hours (4.5-fold stimulation with 100 ng/mL rIL-6, 15-fold increase with 250 ng/mL rIL-6, and 100-fold stimulation at 500 ng/mL rIL-6), whereas addition of LPS (1 μg/mL) resulted in no increased Ig plaques (also data not shown). B cells were assayed for activation and proliferation by metabolic incorporation of [3H]thymidine, [3H]uridine, and [3H]leucine. Briefly, cells were pulsed during the final 4 hours of a 1- to 5-day culture with either 2.5 μCi/ml [3H]thymidine (Dupont, Boston, MA; 6.7 Ci/mmol) or 5 μCi/ml [3H]uridine (New England Nuclear, Boston, MA; 48.6 Ci/mmol), or 5 μCi/ml [3H]leucine (New England Nuclear; 143.5 Ci/mmol), and harvested with a Skatron automatic cell harvester. [3H]leucine-labeled samples were precipitated with 12% trichloracetic acid (TCA) 30 minutes before harvest. Samples were counted on an LKB 1205 beta plate counter (Piscataway, NJ). Stimulation indices for each time point were calculated as mean cpm of IL-6 treated cells/men cpm of untreated cells.

**Induction of growth-responsive transcripts.** Lymphoblastoid cells that had been preincubated for 24 hours at a cell density of 5 × 10⁸ cells/mL in RPMI 1640 medium supplemented with 1 mg/mL BSA (starvation regimen) were cultured either in medium alone (complete Opti-MEM supplemented with 1 mg/mL BSA and 10 mmol/L aprotonin) or in medium supplemented with either rIL-6 (300 ng/mL) or activated monocyte supernatant (1:8 dilution), at a cell density of 3 to 30 × 10⁶ cells/cm² in tissue culture flasks (Costar). Cells were collected at appropriate time points and processed for cytoplasmic dot blot analysis, as described below.

**Measurement of secreted immunoglobulin.** Secreted immunoglobulin was quantitated by an enzyme-linked immunosorbent assay (ELISA), as described. DNA probes used for Northern and cytoplasmic RNA dot blot analysis. Plasmids containing the appropriate probes were isolated by alkaline lysis and CsCl centrifugation. Appropriate DNA inserts were isolated from plasmids by restriction endonuclease digestion and electrophoresis from agarose gels.

The actin probe was a 2.1-kb BamHI fragment containing the actin coding sequence; the C μ probe was a 1.3-kb EcoRI fragment from plasmid pCu0187 containing the human germline heavy chain constant region (kind gifts from Dr Edward Max, Food and Drug Administration, Bethesda, MD); the C γ probe was a 6-kb fragment containing the human gamma 4 constant region; the C θ probe was a 2.6-kb Small fragment containing the human alpha constant region; the C ε probe was a 1.6-kb BamHI fragment containing the kappa constant region; the C θ probe was a 1.0-kb BamHI fragment containing the lambda 2 constant region; and the c-myc probe was a 2-kb EcoRI-ClaI fragment containing exon 3 (all kind gifts from Dr Ian Magrath, National Cancer Institute, Bethesda, MD). Tubulin probes were obtained from the American Type Culture Collection (plasmid ATCC S7104) and Dr Gloria Lee (Harvard Medical School, Boston, MA).

**Northern blot analysis.** Total cellular RNA was extracted according to the method of Chomczynski and Sacchi. Briefly, cells were washed in phosphate-buffered saline (PBS) and suspended in solution D (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7, 0.5% sarcosyl, 0.1 mol/L 2-mercaptoethanol) at a concentration of 1 × 10⁶ cells/mL, followed by phenol-chloroform extraction and two cycles of isopropanol precipitation. The RNA pellet was suspended in Northern blot sample buffer, resolved on 1% formaldehyde-agarose gels, and blotted onto GeneScreen Plus (New England Nuclear), according to the manufacturer’s recommendations. Blots were prehybridized 4 hours at 50°C in 50% formamide, 6 × SSC (1 × SSC = 0.15 mol/L NaCl + 0.015 mmol/L sodium citrate), 1 × Denhardt's solution, 40 mmol/L Pipes, pH 6.5, 100 μg/ml salmon sperm DNA, and 0.5% sodium dodecyl sulfate (SDS), and hybridized using nick-translated probes (10⁶ cpm/mL; Bethesda Research Laboratories). Individual 32P bands were quantitated using a Hoeffer GS 300 densitometer (San Francisco, CA).

**Cytoplasmic RNA dot hybridization.** Cytoplasmic RNA was isolated as described by White and Bancroft. Briefly, cells were washed in cold PBS, suspended at a concentration of 1 × 10⁶ cells per 100 μL of Tris-HCl EDTA pH 7.0, and lysed by the addition of 1/10 vol of 10% NP-40. After 5 minutes incubation at 4°C, nuclei were pelleted by a 3-minute centrifugation in an Eppendorf centrifuge, and the cytoplasmic fractions mixed with equal volumes of 12× SSC and 15% formaldehyde. Samples were heated at 60°C for 15 minutes, and dotted onto GeneScreen Plus as recommended by the manufacturers. Quantitation of Ig heavy and light chains, β-tubulin, or actin RNA was performed as described above. Before rehybridization, blots were stripped of 32P-labeled probe by several washes at 100°C in 0.01 × SSC, 0.01% SDS, as recommended by the manufacturer (DuPont), and residual probe contamination checked by autoradiography.

**Analysis of mRNA turnover.** Starved lymphoblastoid cells were grown for 48 hours either in medium alone or in medium supplemented with either 300 ng/mL rIL-6, or a 1:4 dilution of activated monocyte supernatant at a cell density of 15 to 30 × 10⁶ cells/cm² in 24 cm² tissue culture flasks. Subsequently, 5,6-dicholoro-1-b-D-ribofuranosyl benzimidazole (DRB) was added at a final concentration of 0.1 mmol/L and, at various time points, 4 × 10⁵ cells were removed, washed with PBS, and processed for cytoplasmic RNA dot hybridization. The mRNA turnover rates for IgM heavy, as well as κ and λ light chains, were determined from the slope of the RNA dot-blot signal versus time using linear regression analysis.

**Treatment of starved lymphoblastoid cells with 0.1 mmol/L DRB inhibited [3H]uridine incorporation by greater than 95% within 30 minutes without affecting viability (>95% viable cells 8 hours after drug addition, not shown).**

**RESULTS**

**B-cell activation by IL-6.** Supernatants of monocyte cultures containing IL-6 have been shown to induce DNA synthesis in EBV-immortalized B cells. We now tested whether recombinant IL-6 induces DNA, RNA, and protein synthesis in these cells. To this end, six exponentially growing lymphoblastoid cell lines were first incubated in serum-free medium consisting of RPMI 1640 supplemented with 1 mg/mL BSA at a cell density of 5 × 10⁷ cells/mL (starvation regimen), and then cultured in Opti-MEM culture medium at a cell density of 1.6 × 10⁷ cells/cm² with or without IL-6. Assays for DNA, RNA, and protein synthesis were performed at 24-hour intervals, for a period of 5 days. All six cell lines responded to IL-6 with increased rates of DNA, RNA, and protein synthesis (Fig 1, A to C). Significant increases could be detected by 48 hours, with maximum responses occurring generally at 72 and 96 hours. By 72 hours, a 4.5- to 87-fold increase in DNA synthesis, a 3.5- to 22-fold increase in RNA synthesis, and a 1.6- to 18-fold increase in protein synthesis were measured. These processes were associated with a twofold to sixfold increase in the number of viable cells (Fig 1D). In parallel experiments, cell viability (≥95% at the initiation of culture) averaged 77% ± 6% and 62% ± 8% at 72 hours and 64% ± 7% and 52% ± 7% at 120 hours with and without IL-6, respectively (data not shown). With continued culture up to 5 days, four of the cell lines exhibited...
decreases in DNA, RNA, and protein synthesis rates. This may have been the result of cell crowding and depletion of IL-6 and/or other growth-limiting factors.

Other investigators have reported that IL-6 does not act as a growth factor for B lymphocytes because it failed to promote growth in the EBV-positive cell line CESS. Under the conditions used, we found that IL-6 treatment of CESS cells resulted in a 3.5-, 4.5-, and 5-fold increase in RNA, DNA, and protein levels by 72 hours, respectively (Fig 1A through C). Although rIL-6 caused no significant increase in cell numbers when compared with the original input (10 × 10^6 cells/mL at day 0, 6.8 × 10^6 cells/mL at day 5), CESS cells exposed to IL-6 showed increased viability compared with cells incubated in medium alone; by day 5, untreated and IL-6 treated cells were 20% and 76% viable, respectively. Thus, IL-6 generally promotes DNA, RNA, and protein synthesis in EBV-immortalized B cells, and induces cell division. CESS cells differ from all other cell lines tested in that they fail to proliferate in response to IL-6.

**Induction of c-myc mRNA by IL-6.** c-myc is a nuclear protein closely associated with entry of quiescent cells into the cell cycle and with transcriptional activation. Mitogen-stimulated lymphocytes exhibit a rapid induction of c-myc RNA. When we treated quiescent cells from the lymphoblastoid cell line RY with IL-6 at a cell density of 2.8 × 10^6 cells/cm^2, increases of cytoplasmic c-myc mRNA were observed (Fig 2A). After 20 hours, rIL-6, as well as activated monocyte-conditioned medium containing IL-6, induced 10-fold and fourfold increases in the levels of steady-state c-myc RNA, respectively (Fig 2A). These levels were similar to those detected in logarithmically growing RY cells incubated under optimal culture conditions in medium supplemented with 10% FBS (Fig 2A). In association with increases in c-myc RNA, 20-hour treatment with rIL-6 or monocyte-conditioned medium produced a 1.4- and 2.0-fold increase in cell numbers, respectively (data not shown).

To determine the time of onset and kinetics of c-myc induction, RY cells were grown in the presence or absence of rIL-6, and processed at hourly intervals for Northern blot hybridization. Cytoplasmic levels of c-myc RNA increased within 30 minutes of IL-6 treatment, reached maximum levels of 2.5-fold by 2 hours, and remained elevated during the subsequent 12 hours of observation (Fig 2B). Untreated cells demonstrated no increases in c-myc mRNA during similar periods of incubation, with a maximum 0.4-fold increase in c-myc at 12 hours of culture (Fig 2B). Tubulin mRNA also showed no significant increase in mRNA induction when cultured with IL-6, demonstrating a maximum 0.6-fold increase in mRNA signal intensity (Fig 2B). The level of c-myc induction agrees well with the increase in thymidine incorporation (2.3-fold) (Fig 2B, right). Although the levels of c-myc mRNA induction did vary from experiment to experiment (3- to 10-fold), these results demonstrate an increase in the levels of c-myc mRNA relative to both uninduced cells and tubulin mRNA.

**Steady-state levels of immunoglobulin mRNA after IL-6 treatment.** IL-6 has been shown to increase immunoglobulin (Ig) production in EBV-immortalized lymphocytes, in Staphylococcus aureus activated cells, and in pokeweed mitogen-activated B cells. Experiments were performed to determine whether IL-6 promotes Ig secretion by augmenting Ig mRNA, and whether this induction is specific for Ig mRNAs. To this end, we have examined IL-6-induced expression of Ig mRNAs after IL-6 treatment, and compared these levels with mRNA levels of β-tubulin, a gene generally not induced by cell growth, and with mRNA of actin, a growth-responsive gene. The six cell lines used in these experiments, AVM, 322, RY, TB, TO, and CESS, are representative of lymphoblastoid lines expressing IgM, IgG, and IgA heavy chain with either κ or λ light chains.
Fig 2. Induction of \(c\)-\(myc\) mRNA by IL-6. (A) Starved lymphoblastoid cells (RY), plated at 2.8 \(\times\) 10^6 cells/cm^2, were cultured either in medium alone (control), in medium supplemented with either rIL-6 (260 ng/mL), or in monocyte-conditioned supernatant (1:8 dilution), or 10% FBS. Cytoplasmic RNA was extracted from these cells either at time 0 or after 20 hours of culture, serially spotted at twofold dilutions onto Genescreen Plus, and probed for \(c\)-\(myc\) RNA. The top dot for each condition reflects RNA derived from 1 \(\times\) 10^6 cells.

(B, left) Starved lymphoblastoid cells (RY) that were plated at 5 \(\times\) 10^5 cells/mL and cultured either in medium alone (control) or in medium containing rIL-6 (400 ng/mL) were processed for Northern blot or [3H]thymidine-labeled during the final 4.5 hours of incubation. Northern blots were probed for \(c\)-\(myc\) or tubulin, scanned, and relative signal intensities calculated. The results are shown as stimulation indices over time in relation to time zero signal, which is arbitrarily given a stimulation index of 1. Myc in control cultures (+), myc in cultures containing IL-6 (O), and tubulin in cultures containing IL-6 (D).

Proliferation rates, as measured by [H]thymidine incorporation for the RY cells cultured with and without IL-6 and processed for Northern Blot are shown in B (right).

Two of the cell lines, AVM and TB, are polyclonal; the remaining lines are oligoclonal. Starved B cells were grown for 72 hours in the presence or absence of rIL-6 at a cell density of 3 \(\times\) 10^7/cm^2, and then assayed for the indicated mRNA levels. Results from two experiments (Table 1) show that IL-6 treatment was associated with increased mRNA levels of both Ig (heavy and light chains) and actin. In contrast, IL-6 treatment was not associated with increased tubulin mRNA levels. Although there was some variability in mRNA expression on exposure to IL-6 among

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Abbreviation: ND, not done.

*The indicated cell lines, precultured for 24 hours in starvation medium consisting of RPMI 1640 supplemented with 1 mg/mL BSA at a cell density of 5 \(\times\) 10^5 cells/mL, were cultured for 72 hours in culture medium consisting of Opti-MEM supplemented with 1 mg/mL BSA, 10 mmol/L aprotinin, and 300 ng/mL IL-6 or 1:8 dilution of monocyte supernatant (MS) at a cell density of 3 \(\times\) 10^7 cells/cm^2.

†Total RNA stimulation indices were determined as the ratio of mean cpm in triplicate cultures with or without IL-6. [H]uridine was added during the final 4.5 hours of culture. Actin, tubulin, and heavy and light chain RNA stimulation indices were determined, after hybridization with the appropriate \(^3^P\)-labeled probe, by densitometry measurements as the ratio of signal for cells cultured with or without IL-6.

Ig secreted in the culture supernatant was measured by ELISA using a polyvalent anti-human Ig reagents. The results reflect the ratios of Ig secreted in triplicate cultures with or without IL-6.
the cell lines, each of the six EBV-infected cell lines displayed an increase in the amount of actin mRNA that ranged between 1.2- and 4.5-fold (Table 1). In the same experiments, Ig heavy chain mRNAs were induced by 1.1- to 4.6-fold (Table 1). Light chain mRNA similarly showed, in general, a twofold to threefold increase when compared with untreated cells (Table 1). These values agree well with the overall levels of IL-6 induced cellular RNA synthesis, as determined for each cell line in parallel experiments (Table 1). Ig mRNA induction by IL-6 was associated with increased levels of secreted Ig during the 72-hour period (Table 1). It should be noted that the levels of total cellular RNA induction by IL-6 in these experiments are lower than those observed in Fig 1. This reflects the higher cell densities used here during IL-6 stimulation (1.6 \times 10^6 to 3 \times 10^4 cells/cm^2), and the greater concentration of autocrine growth factors competing with exogenous IL-6 for cell stimulation.

The differences in actin and Ig heavy and light chain mRNAs between untreated and IL-6 treated cells could not be attributed to differences in cell viability. In a representative experiment, after 72 hours, untreated cells and IL-6-treated cells were 80% and 94% viable, respectively. Together, these experiments suggest that induction of Ig secretion by IL-6 may be regulated, at least in part, at the RNA level. Beta-tubulin, a cellular gene generally unresponsive to cell growth, showed no significant induction following IL-6 treatment (Table 1).

Previous experiments involving CESS cells indicated that IL-6 induces a significant increase in \( \gamma \)-chain mRNA, with little increase in light chain mRNA. Using this cell line, we have found that both \( \gamma \) heavy chain and \( \lambda \) light chain mRNAs were induced by approximately twofold with IL-6 stimulation. Actin mRNA showed similar induction levels in this cell line (Table 1).

Thus, lymphoblastoid cells generally respond to IL-6 stimulation with increased expression of Ig and other mRNAs, but not \( \beta \)-tubulin. Because actin, unlike \( \beta \)-tubulin, is a growth-responsive gene, these findings support the view that induction of Ig mRNA is a reflection of cell growth stimulated by the cytokine.

**Ig mRNA stability.** The observed increase in levels of Ig mRNA due to IL-6 could be the result of increased gene transcription, increased mRNA stability, or both.

DRB, a specific inhibitor of RNA polymerase II chain initiation, was used to measure cytoplasmic Ig mRNA half-lives. A comparison of the rates of RNA turnover in IL-6-treated and untreated cell cultures allows one to establish whether IL-6 augments Ig mRNA by increasing its stability. The cell line RY was chosen for its ability to produce significant Ig levels following IL-6 addition. RY cells treated with rIL-6 at a cell density of 3 \times 10^6 cells/cm^2 showed no significant changes in mRNA stability when compared with untreated cells. As shown in Fig 3A, linear regression slopes of heavy chain mRNA differed by only 8% (slopes of −0.046 for untreated and −0.050 for rIL-6-treated cells). In addition, linear regression slopes of a light chain mRNA differed by only 21% (slopes of −0.055 for untreated and −0.069 for rIL-6-treated cells) (Fig 3B).

Cells treated with activated monocyte conditioned supernatant (Mono SNT), although showing only a 16% difference in \( \lambda \) light chain mRNA as compared with untreated cells (Mono SNT slope of −0.066), demonstrated a 30% increase in Ig heavy chain mRNA turnover (Mono SNT slope of −0.065). This 30% increase in heavy chain turnover may be due, in part, to cytokines other than IL-6 present in the monocyte supernatant, which would modify Ig turnover rates.

From these experiments, we conclude that the increases in Ig mRNA steady-state levels seen in B cells exposed to IL-6 are not due to a significant increase in the levels of mRNA stability.

**Northern blot analysis of immunoglobulin mRNA.** Since IL-6 treatment of lymphoblastoid cells caused both an increase in RNA synthesis, as measured by [\( \text{H} \)]uridine incorporation, as well as an increase in steady-state levels of Ig mRNA, we tested whether induction of Ig mRNA by IL-6 was selective, or associated with an overall increased transcriptional response, due to cell growth. If induction of
Ig mRNA was due to a specific stimulation of Ig gene transcription by IL-6, rather than a consequence of an overall stimulation of cellular transcription, one would expect to see an increased Ig mRNA signal by Northern blot analysis as compared with other genes. To eliminate any increased signal contributed by cell growth, we elected to compare Ig mRNA levels with those of the growth-responsive gene actin. Total cellular RNA was collected at daily intervals from lymphoblastoid cells cultured either in medium, in medium plus rIL-6, or in medium containing activated monocyte supernatant. Subsequently, equal amounts of RNA were processed for Northern blot analysis, and hybridized with an actin probe, as well as with Ig heavy and light chain probes.

As shown in Fig 4, the levels of actin and Ig heavy or light chain mRNAs were comparable in cells grown in the presence or absence of IL-6, indicating no specific Ig gene induction by IL-6.

Since Ig and actin mRNA signals did not differ significantly in IL-6-treated and untreated cells when equal amounts of RNA were loaded per gel, and since Ig and actin mRNA did increase on a per cell basis (Table 1), it is likely that increases of Ig mRNA with IL-6 reflect an overall increase in cellular RNA transcription due to cell growth.

DISCUSSION

IL-6 was originally reported to be a differentiation factor for activated human B cells inducing increased Ig production, but no cell proliferation. Subsequent studies have indicated that supernatants of activated monocytes contain IL-6, as well as highly purified natural and recombinant IL-6, promote growth in EBV-immortalized B lymphocytes when these are grown under culture conditions known to minimize the growth-promoting effects of autocrine growth factors. The present study confirms that IL-6 is a growth factor for EBV-immortalized B cells, and extends these results, demonstrating that IL-6 transcriptionally activates a number of growth-responsive genes. Five distinct EBV-induced B-cell lines treated for 72 hours with rIL-6 demonstrated significant increases in DNA, RNA, and protein synthesis, as well as cell numbers. In addition, the Ig heavy and light chains, as well as the c-myc and actin genes, but not the growth-unresponsive gene tubulin, were transcriptionally activated by IL-6.

Earlier experiments that used the EBV-infected lymphoblastoid cell line CESS concluded that IL-6 did not promote growth, but acted strictly as a B-cell differentiation factor. We have also noted the absence of cell proliferation in CESS cells following IL-6 treatment, but we did observe a threefold to fourfold increase in the levels of DNA and RNA, a 1.5-fold increase in protein synthesis, and a fourfold increase in cell viability in these cells. Because we have observed cell proliferation induced by IL-6 in five other independently derived B-cell lines, secreting all three major Ig isotypes, we feel that cell growth is a typical response to IL-6 in most EBV-immortalized B cells. The failure of CESS to proliferate to IL-6 may represent an abortive response to IL-6.

Whether the sustained c-myc expression seen here is due to maintenance of elevated expression throughout the cell cycle, or to recruitment of additional resting B cells, is unknown. It should be noted that quiescent fibroblasts, as well as T and B lymphocytes, show similar induction patterns. In these cells exposed to mitogens and/or growth factors, c-myc mRNA is first detected within 30 minutes and reaches maximal levels within 2 to 3 hours.

Like other cytokines, IL-6 may act primarily during the transition from G0 to G1, by increasing transcription of c-myc, as well as of other competence genes. Evidence in support of this view comes from studies showing both a G0 to G1 progression of hematopoietic stem cells following addition of IL-6, and a G1 growth arrest of murine IL-6-dependent hybridomas following removal of IL-6. IL-6 favors B-cell immortalization by EBV, and this may be due, in part, to IL-6 induction of these other competence genes. Because the B cells used in the present study were immortalized by EBV, further studies are needed to determine whether IL-6 induces c-myc and G0 to G1 progression in normal B cells.

Following a 3-day culture with 300 ng/mL of rIL-6, the levels of extracellular Ig produced by EBV-immortalized B cells increased by an average of threefold when compared
with untreated cells. These levels of Ig induction are similar to those previously reported with CESS cells.\textsuperscript{4,8} In the present experiments, analysis of Ig mRNA in the cell lines exposed to IL-6 showed a twofold to fourfold enhancement on day 3. This increase of Ig mRNA was accompanied by an overall increase of cellular RNA transcription, and was not associated with significant changes in Ig mRNA turnover rates. In addition, IL-6-treated cells exhibited no specific induction of Ig gene transcription when compared with a growth-responsive gene, actin. The levels of IL-6 induced Ig transcription observed here are comparable to those observed in other systems, such as in Ig-secreting plasma cells, when compared with mature resting B cells, and in LPS-stimulated murine splenocytes, when compared with unstimulated splenocytes.\textsuperscript{6,8,7}

The observation that IL-6 stimulates a variety of growth-responsive genes in EBV-immortalized B cells contrasts with a previous report.\textsuperscript{27} Using a clone of the EBV-infected cell line CESS selected on the basis of high surface expression of IgG1, Raynal et al reported that IL-6 treatment was associated with a specific induction of γ1 (β) and λ mRNAs.\textsuperscript{27} In the present studies, uncloned CESS cells treated with IL-6 exhibited a fourfold induction of cellular RNA (Fig 1), a 1.6- to 2-fold induction of γ, λ, and actin mRNAs (Table 1), but no induction of tubulin mRNA (Table 1). It is possible that selected clones of CESS cells differ from the uncloned line in their response to IL-6. It is also possible that IL-6 treatment of the selected CESS clone was associated with induction of a number of mRNA in addition to γ and λ, but not of glyceraldehyde-3-phosphate dehydrogenase, used as the control.\textsuperscript{7}

In this study, IL-6 showed no differential effects among IgM, IgG, and IgA-committed B cells. All three were responsive to IL-6. This is in contrast to other interleukins, such as IL-4 and IL-5, which display preferential effects on IgE and IgA secretion. Whether IL-6 might preferentially affect certain IgG subclasses similar to the effect of interferon gamma on IgG2a was not addressed here. Whether IL-6 increases Ig production as a result of a direct action at the Ig loci or indirectly is unknown. In our experiments, IL-6 increased the amount of Ig mRNA per cell in comparison to a growth-unresponsive cellular mRNA, β-tubulin, but also resulted in the induction of a growth-responsive gene, actin, and in the stimulation of DNA, RNA, and protein synthesis. This could suggest that G, entry and perhaps continued cell cycling is critical for Ig production in these cells. Such conclusions would be consistent with a number of previous studies in which blockade of B-cell proliferation by hydroxyurea resulted in loss of Ig secretion.\textsuperscript{46} Although no increase in cell numbers was seen in CESS cells stimulated by IL-6 to secrete Ig, RNA synthesis was induced, and the degree of induction was comparable to that of Ig secretion. Previously, it was reported that Ig production by CESS was not inhibited by hydroxyurea, an inhibitor of DNA synthesis.\textsuperscript{7} However, the levels of RNA or DNA synthesis were not reported. Furthermore, the levels of drug added in these studies were 100 times lower than those found to be effective by others.\textsuperscript{46} Additional experiments using higher concentrations of hydroxyurea with CESS may resolve this issue.

The results presented here demonstrate that Ig production induced by IL-6 is generally associated with cell proliferation and stimulation of expression of other cell cycle-dependent genes. While additional studies are required for further understanding how IL-6 mediates Ig production, the observation that IL-6 is a nonselective B-cell stimulator will help in designing future experiments. Hopefully, understanding the control of Ig secretion by IL-6 will provide insight into the regulation of B-cell growth.

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Regulation of B-cell growth and immunoglobulin gene transcription by interleukin-6

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