Human GMP Synthetase

PROTEIN PURIFICATION, CLONING, AND FUNCTIONAL EXPRESSION OF cDNA*

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/GMP synthetase is a key enzyme in the de novo synthesis of guanine nucleotides. Human GMP synthetase has been purified to homogeneity, and a cDNA encoding the enzyme has been isolated from the T-lymphoblastoma cell line, A3.01. The open reading frame encodes a protein of 693 amino acids with a predicted molecular weight of 76,725. The cDNA complements a gua mutant of Escherichia coli, which lacks a functional GMP synthetase and extracts from the transformed E. coli exhibit GMP synthetase activity, which is absent in the parental strain. RNA hybridization analysis shows that human GMP synthetase is encoded by a single 2.4-kilobase message. DNA hybridization analysis suggests that the human GMP synthetase is encoded by one gene. In several human cell lines, the level of mRNA expression is substantially higher in proliferating, transformed cells than in nontransformed cells. In two transformed cell lines, treatment with phorbol ester inhibits proliferation and results in a dramatic down-regulation in the levels of GMP synthetase mRNA and protein.

In the de novo synthesis of purine nucleotides, IMP is the branch point metabolite at which point the pathway diverges to the synthesis of either guanine or adenine nucleotides. In the guanine nucleotide pathway, there are two enzymes involved in converting IMP to GMP: IMP dehydrogenase (EC 1.1.1.205), which catalyzes the oxidation of IMP to XMP; and GMP synthetase (EC 6.3.5.2), which catalyzes the amination of XMP to GMP. Accumulation of guanine nucleotides is not only essential for DNA and RNA synthesis, but it also provides GTP, which is involved in a number of cellular processes important for cell division. GTP hydrolysis is required for microtubule assembly (1), protein glycosylation (2), synthesis of adenine nucleotides (3), protein translation (4), and activation of G proteins (5).

IMP dehydrogenase and GMP synthetase are two of many enzymes involved in cellular metabolism that exhibit elevated levels of activity in rapidly proliferating cells, such as neoplastic and regenerating tissues (6–8). Inhibition of IMP dehydrogenase or GMP synthetase has been shown to result in the inhibition of cell growth (9–14). Because of the antiproliferative effect of IMP dehydrogenase and GMP synthetase inhibitors, both enzymes are potential targets for anticancer and immunosuppressive therapies (11, 15, 16). In fact, in recent clinical trials, a potent inhibitor of IMP dehydrogenase, mycophenolic acid, has shown to be effective in the treatment of transplant rejection and rheumatoid arthritis (16–19). The inhibition of lymphocyte proliferation by mycophenolic acid is closely correlated with the lowering of the intracellular pool of guanine nucleotides (10, 11, 15). Furthermore, the inhibition of proliferation by mycophenolic acid can be blocked by the addition of exogenous guanosine (10, 15, 16). These data suggest that the immunosuppressive activity of mycophenolic acid is a result of the depletion of guanine nucleotide pool. Since GMP synthetase, like IMP dehydrogenase, is crucial for the de novo synthesis of guanine nucleotides, the inhibition of GMP synthetase should also result in the depletion of guanine nucleotides and, therefore, could be a target for immunosuppression and cancer chemotherapy. Inhibiting IMP dehydrogenase and GMP synthetase together may be more potent than inhibiting each enzyme individually in blocking cell proliferation. For these reasons, we are interested in characterizing the GMP synthetase and elucidating the mechanisms important for its regulation.

While there is a substantial amount of published data on the biochemical characterization, enzyme mechanism, cloning, and expression of IMP dehydrogenase (20–24), there is very limited information available for GMP synthetase. Although the cDNA has been isolated from Escherichia coli, Bacillus subtilis, Dictyostelium discoideum and Saccharomyces cerevisiae (25–28), there are no reports regarding the cloning of mammalian GMP synthetases. There are also no reports regarding any homogeneously purified eukaryotic GMP synthetases. Recently, we developed a scheme for purifying GMP synthetase to homogeneity from the human T-lymphoblastoma A3.01 cell line. This purified protein has been used to study the catalytic mechanism of the human enzyme and its interaction with inhibitors. Here we report the cloning and functional expression of the human GMP synthetase gene. We demonstrate that the level of GMP synthetase is down-regulated in cell lines when proliferation is inhibited. These studies provide an important foundation for further work to understand the role of GMP synthetase expression in cell growth and development.

EXPERIMENTAL PROCEDURES

Materials

Restriction endonucleases and all enzymes used for plasmid construction were purchased from New England Biolabs (Beverly, MA); oligo(dT)-cellulose was from Pharmacia Biotech, Inc.; Taq DNA polymerase was from Perkin Elmer Corp.; [α-32P]dCTP was from Amersham Corp.; and [α-32P]UTP was from DuPont NEN. All other chemicals were purchased from Sigma.

Cells and Media

A3.01 cells were a gift from Dr. Thomas Folks (National Institute of Health, Bethesda, MD) (29). The A3.01 cells were cultured in RPMI 1640 media containing 5% fetal bovine serum and 10 µg/ml gentamycin (HyClone, Logan, UT). CEM, HL60, U937, and W138 cells were purchased from American Type Culture Collection (Rockville, MD); Jurkat

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The abbreviation used are: XMP, xanthine 5'-monophosphate; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PCR, polymerase chain reaction; kb, kilobase(s); TPA, 12-O-tetradecanoylphorbol-13-acetate.
AT2465 strain of E. coli was obtained from the E. coli cell culture facility at Syntex. Normal peripheral T-lymphocytes were provided by Stratagene (La Jolla, CA). The AT2465 cells have the presence of formaldehyde and transferred to Hybond N+ (Amersham PA) equilibrated in buffer B (0.1 M potassium phosphate, pH 7.0, 1.7 M ammonium sulfate, and 20% glycerol). GMP synthetase was eluted with a 30-ml gradient from

Sac1

The multiple human tissue blot was purchased from Clontech (Palo Alto, CA). It contained polyclonal antibodies from the following tissues: heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

Isolation, Electrophoresis, and Hybridization of Blotted RNA

Total cellular RNA was isolated according to the method of Chirgwin et al. (32). Polyadenylated RNA was obtained using oligo(dT)-cellulose as described (32). RNA was electrophoresed on agarose gels in the presence of formaldehyde and transferred to Hybond N+ (Amerham Corp.) in 20 × SSPE (1 × SSPE is 10 mm sodium phosphate, pH 7.0, 180 mm NaCl, and 1 mm EDTA) as described (34). A 300-base pair HindIII-SacI fragment from the middle of the coding region of clone 6 was used as a probe unless specified (see Fig. 2). Prehybridization and hybridization with 32P-labeled RNA probes (1 × 106 cpm/ml, Riboprobe system, Promega) were performed at 60°C in 50% formamide, 5 × SSPE, 0.1% SDS, 2 × Denhardt's solution (1 × Denhardt's solution is 0.02% (w/v) each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll), 0.5 mg/ml yeast RNA, and 0.1 mg/ml denatured and sheared salmon sperm DNA. The blots were washed for 1 h at 65°C in 1 × SSPE, 0.1% SDS followed by a 15-min wash at 65°C in 0.1 × SSPE and 0.1% SDS. The multiple human tissue blot was purchased from Clontech (Palo Alto, CA). It contained polyclonal antibodies from the following tissues: heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

Isolation, Electrophoresis, and Hybridization of Blotted DNA

Genomic DNA was isolated from A3.01 cells as described previously (34). Samples of DNA (10 μg each) were digested overnight with enzymes that were either deglycosylated, or that were digested as described previously (34). Transfer was performed as described above for the RNA blots. Prehybridization and hybridization were carried out at 42°C with the same buffers, and radiolabeled probes used for RNA blots. Following hybridization, the blot was washed two times for 30 min at 50°C in 0.1 × SSPE and 0.1% SDS.

Purification of GMP Synthetase and Enzyme Assay

Step 1: Homogenization—In a typical purification, A3.01 cells (6 × 109 cells) were lysed in 15 ml of buffer A (20 mM Tris-HCl, pH 7.6, 0.1 mM dithiothreitol, 0.5 mM EDTA, and 10% glycerol) by the use of a glass Teflon homogenizer. The homogenate was centrifuged at 15,000 × g for 20 min, and the pellet was discarded. Purification was carried out with the supernatant (cytosol).

Step 2: Ammonium Sulfate Fractionation—Ammonium sulfate was added to the cytosol fraction until 35% saturation, and the precipitated proteins were removed by centrifugation at 20,000 × g for 20 min and discarded. The proteins in the supernatant were precipitated with further addition of ammonium sulfate (60% saturation). These precipitated proteins, which included GMP synthetase, were recovered by centrifugation as above. The protein pellet was dissolved in 12 ml of buffer A. Ammonium sulfate was removed from the protein solution by gel filtration chromatography on PD-10 columns (Pharmacia).

Step 3: DEAE-Cellulose Chromatography—The desalted protein fraction was applied to a 1.5 × 7.3-cm column of DEAE-cellulose (Whatman) equilibrated in buffer A. The enzyme was eluted with a 60-ml gradient from 0 to 0.5 M sodium chloride in buffer A. The fractions containing GMP synthetase activity were pooled.

Step 4: Phenyl-5PW Chromatography—The DEAE-cellulose pool was diluted with an equal volume of 0.2 M potassium phosphate, pH 7.0, 1.7 mM ammonium sulfate, and 20% glycerol. The protein sample was applied to a bacterial column of phenyl-5PW (TosoHaas, Montgomery PA) equilibrated in buffer B (0.1 M sodium phosphate, pH 7.0, 1.7 mM ammonium sulfate, and 10% glycerol). GMP synthetase was eluted with a 40-ml gradient from 1.7 to 0 mM ammonium sulfate in buffer B, and the fractions containing active enzyme were pooled.

Step 5: Mono Q Chromatography—The Phenyl-5PW pool was decontaminated by dialysis against a 50 mM buffer (buffer B, 25% column (Pharmacia) equilibrated with buffer C (20 mM Tris-HCl, pH 8.0, 0.1 mM dithiothreitol, and 20% glycerol). GMP synthetase was eluted with a 30-ml gradient from 0 to 0.3 M sodium chloride in buffer C. The GMP synthetase fractions that contained a single band at 75 kDa on a Coomassie Blue-stained SDS-polyacrylamide gel were pooled. This material is referred to as pure enzyme.

Enzyme Assay during Purification

The spectrophotometric-coupled assay was performed exactly as described by Spector (35). This assay measured the rate of AMP production by GMP synthetase, where the level of AMP was measured through the activity of three auxiliary enzymes. When crude enzyme samples, such as those in steps 1 to 3 of the purification, were examined, this assay method measured high rates of AMP formation even in the absence of XMP. This rate is referred to as background. GMP synthetase activity is represented by the rate of AMP formation in the presence of all substrates subtracted by the rate (background) when XMP is omitted. The enzyme activity determined by this assay is the same as the activity determined by measuring radioactive GMP formation as described below (data not shown).
The nucleotide sequence from residues 40 to 45 was altered to fit with the start site of translation. This base change converted the BglI site at the start site of translation to an NcoI site. The 60-base oligonucleotide which has the sequence 5’-CAT-GGC-3’ was directly ligated into the NcoI/Msal cut vector, and this expression vector was designated NF.6 (NF abbreviates no fusion). A second expression vector, ES.6, was constructed in the same manner, except that two stop codons were inserted in the ligated oligonucleotide 39 base pairs downstream from the start site of translation (ES abbreviates early stop). The nucleotide sequence from residues 40 to 45 was changed from 5’-GGAGGA-3’ to 5’-TGATTA-3’.

To test for complementation of the guanosine requirement, AT2465 cells that were transformed with NF.6 or ES.6 were spread on M9 minimal medium plates with or without guanosine (100 μg/ml). All plates were supplemented with 0.4% glucose, 0.2% casamino acids, 20 μg/ml IPTG, and 100 μg/ml of the following: thiamin, glutamine, histidine, arginine, inosine, biotin, 2'-deoxyuridine, and ampicillin. To determine GMP synthetase activity, single colonies were selected and inoculated in LB media containing 100 μg/ml ampicillin. Cultures were allowed to grow to mid-log phase, and then IPTG was added to a final concentration of 20 μg/ml. Cells were harvested 3 h after the addition of IPTG.

Determination of Enzyme Activity in Transformed E. coli Cells

Cells were resuspended in lysis buffer (20 mM Tris-HCl, pH 7.6, 0.5 mM dithiothreitol, 0.2 mg/ml lysozyme, 2 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA, and 10% glycerol) and lysed at 37 °C for 10 min. Genomic DNA was sheared by passing the lysate through a 27 gauge needle three times to four times. The cytosolic fraction was separated from the membranes by centrifugation at 15,000 x g for 15 min. GMP synthetase activity in the cytosol was determined by measuring the formation of [γ-32P]GMP from [γ-32P]UMP (50 mCi/mmol, Moravek, Brea, CA). The assay mixture contained 75 mM Tris-HCl, pH 7.8, 10 mM MgCl2, 2 mM ATP, 1 mM [γ-32P]UMP (10 mCi/mmol), 5 mM glutamine and 50 mM dithiothreitol. Typically, 5 μl of cytosol was added to 25 μl of assay mix, and the reaction was allowed to proceed at 40 °C for 15 min. The reaction was stopped by the addition of 6 μl of quench solution, which contained 250 mM EDTA and 82.5 mM each of GMP and XMP as carriers. GMP and XMP were separated by thin layer chromatography as described (37). Briefly, the quenched reaction mixtures (10 μl) were streaked onto 2.5 cm x 20 cm strips of polyethyleneiminecellulose plates (Macherey-Nagle, Germany). The strips were developed in 2 M formic acid. The position of GMP was visualized by ultraviolet light and marked. The GMP band was excised, and the amount of [32P]GMP was determined by liquid scintillation counting. Protein concentration was determined by Bradford analysis (Bio-Rad).

Generation of Anti-GMP Synthetase Antibody

The HindIII-Sacl fragment used for RNA blots was subcloned into the bacterial expression vector pGEX-2T (Pharmacia) and expressed as a glutathione transferase fusion protein in SURE bacteria. This fusion protein was isolated using a glutathione-Sepharose column according to the manufacturer’s instructions. A 1-liter culture yielded 1.5 mg of protein, which was used to immunize rabbits according to an approved protocol. Purified human GMP synthetase was used in immunoblot analysis to test for antibody production.

Immunoprecipitation and Immunoblot Analysis

Cells were lysed in a buffer consisting of 25 mM Hapes, pH 7.3, 150 mM NaCl, 1% Nonidet P-40, 10 μg/ml leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride. Lysates (600 μg of protein each) were immunoprecipitated at 5 °C for 18 h with 20 μl of GMP synthetase antisera and 75 μl of 50% protein A-Sepharose (final volume, 500 μl). As controls, identical samples were run in parallel with preimmune sera. The immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P (Millipore) membranes. The membrane blots were incubated with the GMP synthetase antisera (1:500 dilution) for 18 h at room temperature, followed by incubation with peroxidase-conjugated protein G (Calbiochem). The protein bands of interest were then visualized using the enhanced chemiluminescence (ECL) detection system (Amersham Corp.).

Phorbol Ester Treatment of Cells

U937 and HL60 cells were incubated at a density of 0.5 x 10⁶ cells/ml and treated with 20 ng/ml of 12-0-tetradecanoylphorbol-13-acetate (TPA). Control cells were treated with vehicle (ethanol) in parallel. Three days later, the cells were harvested and divided. One half of the cell pellet was used to prepare RNA for Northern analysis, and the other half was used to prepare cell extracts for immunoprecipitation.
Table I

<table>
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<th>Fraction</th>
<th>Total Protein (mg)</th>
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<th>Specific Activity (μmol AMP/ min mg)</th>
<th>Purification Fold</th>
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Table II

Sequence of tryptic peptides of human T lymphocyte GMP synthetase

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</tr>
<tr>
<td>2</td>
<td>ALNQEQQVIAVXINGPM</td>
</tr>
<tr>
<td>3</td>
<td>VINAARSYNTXZLIPSED</td>
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<tr>
<td>5</td>
<td>XNEBVXGMNLK</td>
</tr>
<tr>
<td>6</td>
<td>VIEPLDFIKDEVR</td>
</tr>
<tr>
<td>7</td>
<td>XPFPGGLAI</td>
</tr>
<tr>
<td>8</td>
<td>XFIITSDMGipaTPGNEIXV</td>
</tr>
<tr>
<td>9</td>
<td>IIMDLTSDKGGTE</td>
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</table>

Fig. 2. Restriction map of GMPs.6 and probes. The region of hatched bars indicates the position of the longest open reading frame. The scale gives length in base pairs. The black bars mark the length and position of the PCR fragment per.288A. The open bars mark the length and position of the cDNA fragments used as probes in Northern and Southern hybridization analysis. The arrows mark the position and direction of oligonucleotide primers 2S and 8A in the 5' to 3' orientation.

40.8% identical to the Dictyostelium and E. coli enzymes, respectively (Fig. 4A).

The sequence of GMPs.6 also shares sequence similarity with other G-type glutamine amidotransferases with the highest degree of similarity spanning amino acids 69–118 and 176–197 of GMPs.6 (Fig. 4B). These sequences may be involved in glutamine binding and amide transfer. A conserved cysteine residue is thought to be involved in catalysis by forming a glutamyl γ-thioester intermediate (42–45).

Functional Expression of GMP Synthetase—in order to determine if the GMPs.6 clone was functional, we tested it for expression by inhibition of a guaA mutation in the AT2465 strain of E. coli. This strain lacks a functional GMP synthetase and does not grow in minimal medium unless guanosine is present (Ref. 31 and data not shown). An expression vector was constructed by subcloning the sequence spanning bases 1–3909 of GMPs.6 into pTRC.99A, which is a plasmid expression vector that contains an ampicillin-resistance marker and the tetracycline promoter, which is inducible by IPTG. This plasmid was designated NF.6 and contained the entire open reading frame and all of the 3' but none of the 5'-untranslated sequences. As a negative control, NF.6 was modified to contain two stop codons 39 bases downstream from the start site of translation. This plasmid was designated ES.6 and should produce a truncated protein. Both ES.6 and NF.6 were transformed into AT2465 E. coli, and the cells were allowed to grow on minimal medium plates containing ampicillin and IPTG with or without guanosine. Cells that were not transformed did not produce colonies on either plate due to the absence of ampicillin resistance in the parental AT2465 cells (not shown). Fig. 5 shows that while both NF.6- or ES.6-transformed cells produced colonies in the presence of guanosine, only NF.6-transformed cells formed colonies in the absence of guanosine. This result showed that GMPs.6 contained a cDNA that complements an E. coli guaA mutation.

We next examined the transformed cells for the presence of GMP synthetase enzyme activity. Fig. 5 shows that GMP synthetase activity was present in NF.6 transformed cells and absent in ES.6 transformed cells. Furthermore, the activity was induced by the presence of IPTG. There was no detectable GMP synthetase activity in untransformed cells (not shown). These findings confirm the complementation results and demonstrate that we have cloned a cDNA encoding a functional human GMP synthetase.

Analysis of GMP Synthetase mRNA—GMP synthetase mRNA in A3.01 cells was examined by RNA hybridization analysis. To determine whether there were multiple messages for GMP synthetase, we probed the RNA blots with three different cDNA fragments covering the 5' end (5'-Sty1), middle (HindIII-Sac I) and 3' end (BglII-3') of the coding region (Fig. 2). All three probes hybridized to a single message of 2.4 kb in samples containing poly(A)-selected RNA (Fig. 6A and data not shown). From these studies, we concluded that there was a single message 2.4 kb in length coding for GMP synthetase in A3.01 cells.

Using the HindIII-SacI probe, we then examined a variety of human cell lines for the presence of GMP synthetase mRNA (Fig. 6B). All cell types examined showed a single message of 2.4 kb for GMP synthetase, the same as in A3.01 cells. However, the level of message varied dramatically between transformed and nontransformed cells. All of the leukemic and lymphoid cell lines appeared to have similar levels of message (Fig. 6B, lanes 4–9). The level of expression of these lines was significantly higher when compared with the two nontransformed cell lines (Fig. 6B, lanes 1 and 2) and peripheral T-lymphocytes (Fig. 6B, lane 3).

In another experiment, we examined a variety of human tissues for GMP synthetase message. Similar to the cell lines, we found a single 2.4-kb message in all tissues examined (data not shown). In summary, these results suggest that there is one message for GMP synthetase in human cells.

Analysis of Genomic DNA for GMP Synthetase—We next examined human genomic DNA to ascertain whether there may be more than one gene coding for GMP synthetase. DNA from A3.01 cells was examined by Southern hybridization analysis using the HindIII-SacI probe. Fourteen different restriction endonucleases were used and many gave single bands that hybridized with the probe (data not shown). These results are consistent with a single gene for GMP synthetase in the human genome.

Alteration of Expression Level of GMP Synthetase—The elevated level of GMP synthetase mRNA in the transformed cells compared with the nontransformed cells suggests that an increase in the activity of GMP synthetase is required for increased levels of metabolic activity associated with proliferation. It can be proposed that when cells are continuously proliferating, as with transformed cells, the requirement for guanine nucleotides is high, hence the expression of enzyme is elevated. Conversely, when proliferation is arrested, the re-
Alteration of Expression by Inhibition of Proliferation

Fig. 3. Nucleotide and predicted amino acid sequences of human GMP synthetase. Amino acid residues are represented in single-letter code. Lines and numbers in parentheses mark the position of sequences corresponding to the tryptic peptides determined from the purified native enzyme. Boxes mark the position of sequences corresponding to the oligonucleotide primers. Nucleotide residue 1 represents the start site of translation.
Fig. 4. Similarity between human GMP synthetase and other amidotransferases. The residues that are identical to the human GMP synthetase sequence are underlined and in boldface. The arrow marks the conserved cysteine residue. A, the predicted protein sequence of human GMP synthetase is aligned with that of other G-type amidotransferases. B, the putative glutamine binding domain of human GMP synthetase is aligned with that of other G-type amidotransferases.

A

<table>
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B

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1. Requirement for guanine nucleotides is lowered, and the expression of enzyme is reduced. We were interested in the possibility that the expression of GMP synthetase would be down-regulated if transformed cells were induced to change their metabolic state. To investigate the effect of arresting proliferation on GMP synthetase expression, we examined U937 and HL60 cells. Both of these cell lines undergo differentiation following treatment by phorbol esters. Phorbol esters are known to cause these cells to adhere to plastic surfaces, stop dividing, and differentiate into macrophage-like cells (46-48). U937 and HL60 cells were treated with TPA for 3 days. During this time, the majority of the cells had adhered to the tissue culture flasks and had not proliferated since the start of treatment, indicating that growth had been arrested. Vehicle-treated control cells continued to divide and remained in suspension during the treatment. After the third day, the differ-
entiated adhered cells (TPA-treated) and the undifferentiated nonadhered cells (vehicle-treated) were harvested. Total RNA was prepared and examined for the presence of GMP synthetase mRNA (Fig. 7A). The TPA treatment dramatically reduced the levels of GMP synthetase mRNA where the levels in the differentiated U937 and HL60 cells were 14 and 3% of those in the proliferating cells, respectively. Cell extracts from the same treatment groups were examined for the presence of GMP synthetase protein by immunoblot analysis (Fig. 7B). Similar to the mRNA, the level of GMP synthetase protein in HL60 cells following TPA treatment was reduced to 28% of the untreated control cells. These results show that the expression of GMP synthetase is sensitive to cell proliferation and that the arrest of proliferation causes a decrease in enzyme expression at both the mRNA and protein levels.

**DISCUSSION**

Our data provide irrefutable evidence that we have obtained a cDNA that encodes a functional human GMP synthetase protein. Since obtaining the data presented here, we have successfully expressed the cDNA in baculovirus-infected insect cells. The recombinant enzyme has been purified and characterized and has identical kinetic and biochemical properties as the native human enzyme.3

Cloning of IMP dehydrogenase cDNA lead to the discovery that there are two separate genes in human coding for IMP dehydrogenase (type I and type II) (22). The two isoforms are identical in sizes and similar in their sequences and biochemical properties (20). Unlike the data for IMP dehydrogenase, RNA and DNA hybridization experiments indicate a single message and one gene for human GMP synthetase (Fig. 6 and data not shown). However, these findings do not preclude the existence of isoforms of GMP synthetase created by posttranslational modification. Although the two isoenzymes of IMP dehydrogenase are similar proteins, their messages are clearly distinguishable and their expression levels are differentially regulated (20, 22, 24, 29). The type I IMP dehydrogenase expression is constitutive, and it does not vary between normal leukocytes and transformed leukemic cells, while the type II expression is elevated in leukemia cells. Furthermore, type II mRNA is down-regulated in differentiated HL60 cells. Similar to the type II IMP dehydrogenase, GMP synthetase expression is also elevated in transformed cells and down-regulated in differentiated HL60 cells (Figs. 6 and 7). Considering the tandem cooperation between the two enzymes, it is likely that there are common elements in the regulation of their expression.

Recent studies suggest that inhibiting enzymes in the de novo synthesis pathway of purine and pyrimidine nucleotides can result in the inhibition of immune cell functions. This has proven true with the inhibition of IMP dehydrogenase by mycophenolic acid (11, 15, 16) and the inhibition of dihydroorotic acid dehydrogenase by Brequinar (50–52). Since accumulation of guanine nucleotides is crucial for cell proliferation, it is likely that the inhibition of enzymes downstream of IMP dehydrogenase would also be effective in inhibiting immune cell functions. GMP synthetase activity is elevated in many tumor tissues and
rapidly proliferating cells (6–8). Here we demonstrate that the expression of mRNA is dramatically elevated in transformed cell lines compared with nontransformed cell lines (Fig. 6B). This suggests that the increase in enzymatic activity is a result of increased protein expression. Furthermore, inhibition of proliferation causes down-regulation of GMP synthetase expression at both the mRNA and protein levels (Fig. 7). Therefore, it seems apparent from the collective data that GMP synthetase would likely be a successful target for cancer chemotherapy and immunosuppression. For this reason, we are currently investigating the regulation of GMP synthetase gene expression and its role in cell growth and development.

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REFERENCES

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