Characterization of a Novel Na\(^+\)-dependent, Guanosine-specific, Nitrobenzylthioinosine-sensitive Transporter in Acute Promyelocytic Leukemia Cells*

(Received for publication, March 20, 1997, and in revised form, May 13, 1997)

Sheryl A. Flanagan and Kelly A. Meckling-Gill‡

From the Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

NB4 cells are the only bona fide in vitro model of human acute promyelocytic leukemia. We have examined cytidine and guanosine transport in this cell line and characterized a novel guanosine-specific transporter. Cytidine transport occurred predominately by equilibrative nitrobenzylthioinosine (NBMPR)-sensitive (es) transport. In the presence of Na\(^+\), guanosine at various concentrations accumulated at least 6-fold above equilibrium. The initial rate of guanosine transport in Na\(^+\) buffer decreased by 75% with the addition of 1 \(\mu\)M NBMPR and the IC\(_{50}\) for NBMPR inhibition was 0.7 ± 0.1 nM. Replacement of Na\(^+\) with choline also resulted in a 75% decrease in total guanosine transport. The potent inhibition of guanosine transport by NBMPR and the loss of transport in choline suggested that a Na\(^+\)-dependent NBMPR-sensitive transporter was responsible for the majority of guanosine uptake. This concentrative, sensitive transporter is Na\(^+\) dependent with a stoichiometric coupling ratio of 1:1. This novel transporter, referred to as csg, is guanosine-specific with total guanosine transport inhibited by only 50% in the presence of 1 mM competing nucleosides. HL-60, acute myelocytic leukemia cells, do not exhibit csg activity while L1210, murine acute lymphocytic leukemia cells, exhibit csg transport. The presence of the csg transporter suggests an important role for guanosine in particular forms of leukemia and may provide a new target for cytotoxic therapy.

Purine and pyrimidine nucleotides, and their related metabolic products, participate in numerous biological processes. Nucleotides can be synthesized endogenously via \textit{de novo} synthetic pathways and, as a result, are not considered essential nutrients (1, 2). However, a number of tissues including hematopoietic and intestinal epithelial cells are deficient in \textit{de novo} nucleotide synthetic pathways and rely on the salvage of exogenous nucleosides to maintain nucleotide pools and to meet their metabolic demands (3). Nucleoside analogs have important clinical applications in the therapy of neoplastic and viral diseases (4). Most nucleosides and their analogs execute their biological activity intracellularly, but due to their hydrophilic nature do not readily permeate the lipid bilayer. Therefore, the uptake or release of nucleosides and/or nucleoside analogs in mammalian cells is mediated by multiple distinct transport proteins (5, 6). Currently, seven functionally distinct nucleoside transport (NT) processes have been identified. The classification of NT processes is based on functional and pharmacological characteristics including: transport mechanism, \(e = \) equilibrative, \(c = \) concentrative; sensitivity to nitrobenzylkoiso nosine (NBMPR), \(s = \) sensitive, and \(i = \) insensitive; and permeant selectivity.

In general, equilibrative processes are widely distributed among mammalian cells and tissues and exhibit broad permeant selectivity (6–9). There are two discrete equilibrative transporters, NBMPR-sensitive (es) and -insensitive (ei), that have similar kinetic properties, but differ markedly in sensitivity to NBMPR (10–12). Both equilibrative transporters are inhibited by low concentrations of dipyridamole and dilazep (9). The es transporter is inhibited at low concentrations (<1 nM) (10) as a direct result of interaction of NBMPR with high affinity binding sites (\(K_d = 0.1–1 \text{nM} \)) (11, 14), while the ei transporter is not affected by NBMPR up to > 10 \(\mu\)M (10, 12, 13).

Concentrative nucleoside transport processes have been identified in numerous specialized mammalian cells (6). These transporters are insensitive to NBMPR and dipyridamole at concentrations up to 10 \(\mu\)M (9, 15). There is evidence for five subclasses of concentrative transporters that display a complex pattern of overlapping permeant selectivities (5). Due to overlapping permeant selectivities, a complex phenotype can result whereby one nucleoside may be simultaneously transported by more than one process within one cell or tissue. The nucleosides are transported against a concentration gradient into the cell, coupled with the movement of Na\(^+\) down a concentration gradient (16). Thus, these processes are described as inwardly directed Na\(^+\)/nucleoside symporters (17).

There are four major Na\(^+\)-dependent, concentrative, NBMPR-insensitive (ei) transport systems with varying substrate specificities. cif prefers purines and formycin B is the model substrate (18). Two distinct systems prefer pyrimidines and are both referred to as cit. These similar processes are distinguished by the ability to transport guanosine (15, 19), and thymidine is the model substrate for both systems. The fourth ci transporter exhibits broad permeant selectivity and is referred to as cib (6, 20, 21). Both adenosine and uridine are substrates for all four ci transporter types (2). A preliminary report suggested the presence of a concentrative NBMPR-sensitive (cs) transporter in freshly isolated leukemia cells (22), however, this transport process remains to be characterized.

There are large differences in NT activities from one cell type to another (23). Some cell types such as human erythrocytes express only a single NT system, cs (24), whereas, both cs and ei

* This work was supported by the Natural Sciences Engineering Research Council of Canada and a Departmental Graduate Scholarship (to S. A. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed.

1 The abbreviations used are: NBMPR, nitrobenzylkoisinosine; APL, acute promyelocytic leukemia.
are present in various cell lines, including BeWo human choriocarcinoma cells (25). Other cell lines such as L1210 murine leukemia cells and IEC-6 mouse intestinal epithelial cells, express multiple transporters, including equilibrative and concentrative transporters, simultaneously (18, 15). As well, NT activities within the same cell type have been observed to change with changes in growth state (26, 27), differentiation (21, 32–37), and neoplastic transformation (28). For example, induction of monocyetic (21, 35) or neutrophilic differentiation (33, 34) of HL-60 human leukemia cells leads to a decrease in es nucleoside transport activity and increased Na+-dependent nucleoside transport activity (33–35).

NB4 cells were originally established from the leukemia cells of a patient with acute promyelocytic leukemia (APL) (29). This cell line expresses the specific t(15;17(q22,q11–12)) chromosomal translocation of APL, thus providing a better model for APL than HL-60 cells which were derived from a patient with acute myelocytic leukemia (3) and lack the characteristic chromosomal translocation. In our laboratory, examination of the NT processes present in NB4 cells has revealed a nucleoside transport phenotype different from that of HL-60 cells (31). Analysis of adenosine, thymidine, and uridine transport unveiled the presence of both ei and es transporter systems, and most significantly the presence of a cif transport system (31). This observation was the first report of the presence of cif in a human cell line. To further establish the nucleoside transporter phenotype of NB4 cells, this study directly examined the characteristics of both cytidine and guanosine transport in this cell line. Here we describe the complete characterization of an NBMPR-sensitive, Na+-dependent guanosine-specific transporter (csg), the first to be described in eukaryotic cells.

MATERIALS AND METHODS

Cell Culture—NB4 cells were originally isolated and characterized from a human patient with APL (29). The cells were maintained in Iscove’s modified Dulbecco’s medium (Life Technologies, Inc., Burlington, ON, Canada) with 10% fetal bovine serum (ICN Biochemicals, Inc., Mississauga, ON, Canada), supplemented with penicillin and streptomycin (50 units/ml) and maintained at 37°C in a humidified atmosphere of 5% CO2. The cells were routinely passed in tissue culture flasks (Fisher Scientific, Whitby, ON, Canada) from passage 5 to 40. Cell counts were determined using a Coulter Counter (Model ZM) and cell volume with a Coulter Channelizer 256 (Coulter, Hialeah, FL). Cell viability was determined by a trypan blue dye exclusion assay and cell viability was >85% in all experiments.

Transport Studies—Cell suspensions were harvested during exponential growth (at a density of 8.0–9.0 × 10^6 cells/ml) by centrifuging at 1,000 rpm for 8 min in a Hermle Z 383 K centrifuge (Mandel Scientific Co., Ltd., Guelph, ON, Canada). The resulting pellets were washed twice in 25 ml of Na+-buffer (3 mM K2HPO4, 1.8 mM CaCl2, 1 mM MgCl2, 144 mM NaCl, 20 mM Tris, pH 7.4, osmolality 300 ± 10 osmole) or Na+-replacement choline buffer (3 mM K2HPO4, 1.8 mM CaCl2, 1 mM MgCl2, 140 mM choline Cl, 20 mM Tris, pH 7.4, osmolality 300 ± 10 osmole) and centrifuged at 1,000 rpm for 8 min. The final pellets were resuspended in the appropriate buffer to a final density of 7.0–8.0 × 10^6 cells/ml. The cells were used immediately in the transport assay or after a 15–20 min incubation with 1 μM NBMPR, a nucleoside transport inhibitor.

Employing rapid assay technology, the uptake of [3H]guanosine and [3H]cytidine was determined using an inhibitor and oil stop procedure at 22°C (38). A 100-μl volume of transport buffer, containing Na+ or choline buffer with or without 1 μM NBMPR, a known concentration of cold permeant and radioactive permeant was layered over 125 μl of oil (16% Fisher 0121 light paraffin oil and 84% Dow-Corning (Mississauga, ON, Canada) 550 silicone fluid, with a final density of 1.032 g/ml) in a 1.5-ml Eppendorf microcentrifuge tube. The uptake intervals were initiated by the addition of 300 μl of cells incubated in choline or Na+ buffer with or without NBMPR to the microcentrifuge tube, allowing cells to mix with the transport buffer solution for a specific time interval (0, 3, 5, 10, 15, 30, 120, and 300 s) and the interval was ended by the addition of 200 μl of 200 μM cold dilazep, a nucleoside transport inhibitor (a gift from Hoffman La-Roche and Co., Basel, Switzerland). The cells were then pelleted through the oil layer by centrifugation at 14,000 rpm for 15 s using an Eppendorf model 5415C centrifuge. Each time point was performed in triplicate. The transport buffer was aspirated off the oil layer and the tubes were washed twice with water to remove radioactive tracer while preserving the cell pellets. The cell pellets were solubilized by the addition of 300 μl of 5% Triton X-100 for 1 h at 22°C. The contents of the microcentrifuge tubes were then transferred to scintillation vials and 1 ml of scintillation fluid (Fisher Scientific, Whitby, ON, Canada) was added. Radioactivity was determined using a Beckman (Fullerton, CA) LS 7800 liquid-scintillation spectrometer. Time 0 values for uptake were determined by first adding dilazep followed closely by the cell suspension and immediate centrifugation. Cell number and volume were measured immediately after the transport assay was complete. The intracellular volume of NB4 cells was determined from computer generated (TableCurve, Jandel Scientific, Corte Madera, CA) best-fit equations for Na+, Na+-NBMPR, choline, and choline NBMPR treatments. The best-fit equation was chosen and produced a linear response over the first 4 or 5 time points (0, 3, 5, 7, and 10 s). The linear tangent drawn between 0 and 1 s was used as the initial rate estimate for all time courses.

Nucleoside transport processes were distinguished by exploiting the use of NBMPR, an inhibitor of es and transport buffer, and by the use of a Na+-replacement choline buffer. Transport measured in Na+-buffer alone is representative of total transport (A) and measurements made in choline buffer represent total equilibrative transport (es + ei) (C). Transport measured in NBMPR reveals total transport (ei + es) (B), while measurements made in choline NBMPR measure ei (D) only. By subtraction, the individual nucleoside transport processes can be identified. D = ei; C – D = es; B – D = ci; A – B – (C–D) = cs, as shown in Fig. 1.

Kinetic Studies—The kinetic parameters of transport, Km and Vmax were estimated by measuring uptake at various substrate concentrations at a time point that was on the linear portion of the time course and subtracting time 0 uptake. This time point was 7 s for cytidine and 5 s for guanosine. Uptake versus concentration for Na+, Na+-NBMPR, choline, and choline-NBMPR time courses was expressed graphically. By subtraction between curves at each concentration point, as shown in Fig. 1, the ci, es, ei, and cs curves were generated over the same concentration. The untransformed data was fitted to a one-site binding model (GraphPad Prism) and the computer generated K and V for each nucleoside were recorded.

Statistical Analysis—ANOVA followed by the Dunnett’s test was used to compare uptake of [3H]guanosine in the presence of competitors to [3H]guanosine uptake without competition (control) using Instat (GraphPad, San Diego, CA) for personal computers. Significance was reached when p < 0.05.

RESULTS

Time Courses and Initial Rates of Nucleoside Transport—Initial transport rates were estimated from computer-generated best-fit equations (see “Materials and Methods”). The presence of es, ei, cs, and ci transporters were resolved by subtraction of the appropriate time courses. Time courses were performed in Na+ and Na+-free (choline substituted) buffers in the absence or presence of NBMPR to determine the nucleoside transport process profile for each nucleoside studied (Fig. 1). The proportions of each NT system for cytidine and guanosine are given in Table I. Cytidine transport in NB4 cells occurred almost exclusively via es (Table I). The elimination of the Na+ gradient by replacement with choline had no effect on cytidine uptake (Fig. 2, A and B). NB4 cells at 1 μM cytidine uptake reached equilibrium at ~45 s (Fig. 2A) while at 5 μM cytidine reached equilibrium at ~25 s and continued to accumulate (Fig. 2B). A small amount of cytidine transport, 5% of total (Table I), occurred in the choline + NBMPR treatment group. However, this process was unsaturated and likely representative of diffusion.

Direct examination of [3H]guanosine transport in NB4 cells revealed the presence of a concentrative, NBMPR-sensitive Na+-dependent transporter. At 5, 50, and 100 μM guanosine, transport in the presence of a Na+ gradient was almost entirely abrogated by the addition of 1 μM NBMPR (Fig. 3, Table I). The replacement of Na+ with choline led to a 70–80% decrease in
Characterization of a Novel Transporter in Leukemia Cells

total uptake. Guanosine reached equilibrium and accumulated 6-7-fold at 50 and 100 μM, and 15-fold at 5 μM over the 5-min time course. Initial rates of \( c_i \) transport, as determined by subtraction of choline NBMPR from Na\(^+\)-NBMPR, were consistently small and whether the rates were representative of facilitated transport was not clear. To measure \( c_i \) directly, i.e. and without subtraction, dipyridamole was added to the Na\(^+\) medium. Accumulation above equilibrium did not occur at 50 or 5 μM guanosine (data not shown). The majority of Na\(^+\)-dependent concentrative uptake was inhibitable by NBMPR, suggesting that concentrative sensitive (c) transport was responsible for the preponderance of total guanosine transport observed in this cell line. We have named this transporter csg.

In a previous study by our group, two high affinity NBMPR-binding sites were identified on NB4 cells (31). To help elucidate whether one of these sites could represent the csg transporter we determined the IC\(_{50}\) of the csg transporter for the preponderance of total guanosine transport observed in this cell line. We have named this transporter csg.

**Kinetics of Transport**—To further characterize the transport of cytidine and guanosine in NB4 cells, kinetic studies were performed. The kinetic parameters of transport were measured at nucleoside concentrations ranging from 1.9 to 500 μM for cytidine and from 1.9 to 125 μM for guanosine. Below 125 μM guanosine the equilibrative system accounted for a small portion of uptake and began to plateau. Beyond 125 μM guanosine, however, uptake via this route began to climb steeply and exceeded the c system. This potential second binding site was not analyzed due to a lack of data points beyond 500 μM, limited by guanosine solubility, and the lack of physiological relevance. The \( K_m \) value for the \( c_i \) transporter was 58 μM for guanosine, and the \( K_m \) values for the es transporter were 112 and 52 μM for cytidine and guanosine, respectively. The transport affinities of both equilibrative systems for guanosine were similar to the \( K_m \) values we previously reported for adenosine, uridine, and thymidine (31), while those for cytidine were approximately 2-fold lower. The \( K_m \) of the csg transporter was estimated at 49 μM, similar to the affinities for both the es and \( c_i \) transporters.

The csg transporter showed the greatest capacity to transport guanosine into NB4 cells, with a \( V_{\text{max}} \) of 1.6 ± 0.2 pmol/μl/s while the equilibrative processes contributed much less at 0.4 ± 0.04 and 0.3 ± 0.04 pmol/μl/s for es and \( c_i \), respectively. Although the affinity of the equilibrative systems for cytidine was low, the es transporter showed a high capacity to transport this substrate as reflected in the high \( V_{\text{max}} \) value. The es \( V_{\text{max}} \) value of 9.58 ± 0.4 for cytidine is about 2-fold greater than the \( V_{\text{max}} \) value of both equilibrative processes for adenosine (31).

**Cation Dependence of the csg Transporter**—The cation specificity of the csg transporter was investigated by substituting Na\(^+\) for other monovalent cations. Time courses were generated using 5 μM guanosine. The uptake of \(^3\)H]guanosine in the presence of 140 mM gradients of the chloride salts, K\(^+\)Cl and Li\(^+\)Cl, and in choline was compared with the uptake demonstrated in a Na\(^+\) gradient. Concentrative transport was reduced by about 10-fold when extracellular Na\(^+\) was replaced with K\(^+\), Li\(^+\), or a choline gradient, indicating high Na\(^+\) specificity of the transporter.

**Effect of Na\(^+\) on Guanosine Uptake**—To investigate the stoichiometric coupling ratio associated with the csg transporter the initial rates (flux) of guanosine at a concentration of 100 μM, as a function of the extracellular Na\(^+\) concentration (0–140 mM) was measured. The relationship of the flux of guanosine versus sodium concentration produced a hyperbolic curve (Fig. 4A), suggesting a ratio of sodium:guanosine of 1:1. This data was further analyzed using the following Hill equation (40),

\[
\text{Flux} = V_{\text{max}}(\text{Na}^+)/[K_{\text{Na}}^+ + [\text{Na}^+])
\]

where \( K_{\text{Na}}^+ \) is the concentration of Na\(^+\) that is able to produce one-half of the maximum rate of nucleoside flux, \( V_{\text{max}} \) refers to the maximum rate of nucleoside flux at saturating concentra-

---

**Table I**

|                          | Total (pmol/mg protein) | pmol/μl/s (% of total) |
|--------------------------|-------------------------|------------------------|
|                          | \( c_s \)                | \( c_e \)                | \( e_i \)                |
| Cytidine 50 μM           | 3.33 ± 0.30             | ND\(^a\)                | 3.19 ± 0.28 (96 ± 8)      | 0.14 ± 0.02 (4 ± 1)     |
| Cytidine 5 μM            | 0.24 ± 0.05             | ND                      | 0.22 ± 0.05 (92 ± 20)     | 0.02 ± 0.01 (8 ± 4)     |
| Guanosine 100 μM         | 2.11 ± 0.21             | 1.91 ± 0.16 (90 ± 8)     | 0.20 ± 0.03 (10 ± 2)      | 0.23 ± 0.03 (11 ± 2)    |
| Guanosine 50 μM          | 1.49 ± 0.19             | 1.04 ± 0.16 (70 ± 11)    | 0.33 ± 0.09 (22 ± 6)      | 0.12 ± 0.04 (8 ± 3)     |
| Guanosine 5 μM           | 0.15 ± 0.01             | 0.13 ± 0.01 (87 ± 7)     | 0.02 ± 0.01 (13 ± 7)      | 0.02 ± 0.01 (13 ± 7)    |

\( a \) \( c_s \), concentrative-sensitive transport.

\( b \) \( e_i \), equilibrative-sensitive transport.

\( c \) \( e_i \), equilibrative-insensitive transport.

\( d \) ND, not detected.
tions of Na$^+$, and $n$ is the Hill coefficient. A plot of flux/[Na$^+$]$^n$ against flux for the correct value of $n$ will yield a straight line.

**Fig. 4**B displays the results of this type of plot of the data in Fig. 4A. A straight line was observed when $n = 1$, $K_{Na^+} = 14.6 \pm 3.8$ mM, and $V_{max} = 2.57 \pm 0.2$ pmol/μl/s, indicating the binding site on the carrier requires the presence of one Na$^+$ molecule.

**Substrate Specificity of the Na$^+$-dependent Concentrative-sensitive Transporter**—The substrate specificity of this novel csg transporter in NB4 cells was investigated by measuring the transport of 100 μM guanosine in the presence of increasing concentrations of other nucleosides, a nucleobase, a nucleoside analog, and nucleoside transport inhibitors. At concentrations of 1 mM, 2’-deoxyguanosine, guanine, uridine, hypoxanthine, adenosine, and inosine significantly inhibited the Na$^+$-dependent cs uptake of guanosine at $5s (p \leq 0.05)$ (Fig. 5) by 35–50%. The other compounds tested including thymidine, xanthine, and ganciclovir, at concentrations of 1 mM, were unable to significantly inhibit Na$^+$-dependent cs guanosine transport. NBMPR and dipyridamole at 1 and 20 μM, respectively, markedly inhibited Na$^+$-dependent cs transport of guanosine by ~90%. When cold guanosine was present at 500 μM (pH 7.1) uptake of [3H]guanosine was inhibited by ~70%. Concentrations greater than 500 μM were unattainable due to guanosine’s low solubility at physiological pH.

**Existence of Na$^+$-dependent cs Transport in Other Cell Lines**—To assess whether other leukemia cell lines exhibit this novel Na$^+$-dependent, concentrative-sensitive transporter for guanosine or csg, we examined two commonly studied cell lines, HL-60 and L1210 cells. Time courses were performed for guanosine uptake at 50 and 100 μM (Table II and data not shown). The initial transport rates were estimated from computer-generated best-fit equations over the linear portion of the time course curve and individual processes were resolved as discussed earlier. Guanosine entered HL-60 cells by both $es$ and $et$ at 50 and 100 μM, but there was no evidence of concentrative-sensitive transport. L1210 cells exhibited Na$^+$-dependent cs guanosine transport at 50 μM guanosine, but this transport process was not easily detected at higher concentrations (Table II).

**DISCUSSION**

In this study, the direct examination of guanosine transport revealed the presence of an NBMPR and dipyridamole-sensitive Na$^+$-dependent, guanosine-specific transporter (csg), the first to be described in eukaryotic cells. Concentrative-sensitive transport (cs) has been previously described in abstract form (22) in freshly isolated human leukemia cells from chronic lymphocytic and acute myelogenous leukemia patients. The characteristics of the csg transporter identified in NB4 cells are different from those of the cs transporter described in freshly isolated leukemia cells. Only adenosine analogs were per-
Characterization of a Novel Transporter in Leukemia Cells

FIG. 4. Sodium-dependent guanosine flux in relation to sodium concentration. The uptake of guanosine (100 μM) was measured in the presence of various extracellular Na+ concentrations (0–140 mM). To obtain the different sodium concentrations, choline was partially substituted to give an osmolarity of 300 ± 10 osmole. Uptake was measured over a full time course (0, 3, 5, 7, 10, 15, 30, 120, and 300 s) and flux at 1 s was determined. A is a plot of guanosine uptake versus Na+ concentration. B is a plot of flux/Na+ versus flux when n = 1. One sodium ion is transported per one guanosine molecule as suggested by the linearity of this plot.

means for the previously described cs transporter, however, the csg transporter does not accept adenosine directly (31) and only 50% of guanosine transport via this system is inhibited in the presence of a 10-fold greater concentration of adenosine. It seems likely that with the differences between this csg transporter and the previously described cs process that other cs processes will be discovered.

NB4 cells exhibit two high affinity NBMPR-binding sites (31). Both binding sites had Kd values in the nanomolar range, 0.1 and 0.35 nM, consistent with the high affinity systems previously described (9). In this study, the NBMPR inhibition of guanosine transport via the csg transporter was 0.7 ± 0.1 nM, suggesting that one of the two NBMPR-binding sites may be that of the csg transporter.

The csg transporter was Na+ dependent, K+ is likely too large, and Li+ too small to emulate Na+ in the csg system. The stoichiometric coupling ratio was determined to be 1:1, analogous to the concentrative insensitive (ci) formycin B selective transporter (cif) and both ci thymidine selective processes (cit) (5).

To provide insight into the structure of this NT membrane protein, permeant selectivity was assessed. In general, the transporter appeared to be highly guanosine specific. Guanosine transport was inhibited by only 55% in the presence of 1 mM competing nucleosides. Other concentrative systems prefer purines (18), pyrimidines (15, 19), or accept both purines and pyrimidines (2). As well, other concentrative systems experience greater than 50% inhibition in the presence of competing nucleosides at concentrations equal to (43) or as little as 2-fold greater (44) than the model nucleoside in question. It appears that the substrate specificity of this novel csg transporter is dependent on both the presence of an intact ribose ring and the existence of particular functional groups. Ganciclovir, although resembling guanosine in its base structure, has a disrupted ribose ring and is unable to compete with guanosine for the transporter. Although there were no statistically significant differences between 2′-deoxyguanosine, inosine, guanine, hypoxanthine, adenosine, or uridine inhibition, 2′-deoxyguanosine appeared to be the most successful competitor of guanosine transport possibly due to the presence of an intact ribose ring and functional groups almost identical to those of guanosine. Guanine, with identical functional groups to guanosine, but without the ribose ring, appeared to compete with less success than 2′-deoxyguanosine. Studies of the cs transporter have shown the transporter to be highly stereoselective strongly preferring the 1-enantiomer over the l-enantiomer (45) with the ability of a molecule to be transported greatly decreased or elevated by loss or substitution of the 3′hydroxyl residue (46). Therefore, manipulation of molecule structure has the potential to alter transport capacity significantly.

We examined two commonly studied leukemia cell lines, HL-60 and L1210 cells: only L1210 cells exhibited concentrative-sensitive transport. From these experiments it appears that concentrative-sensitive guanosine transport is not unique to human leukemia cell lines. HL-60 cells were isolated from a patient with acute myelocytic leukemia rather than APL (NB4) (30). Several differences between HL-60 cells and NB4 cells have already been reported by this laboratory (31, 47). The csg transporter appears to be another distinguishing feature of the NB4 cell line.

Why do NB4 cells express this guanosine-specific transporter? Guanylate ribonucleotides are involved in a variety of biological roles including, RNA and DNA synthesis, and are required for the function of small GTP-binding proteins (such as Ras) and heterotrimeric G proteins involved in signal transduction (2, 48, 49). GTP pools appear to be rate-limiting in DNA biosynthesis in rat liver and hepatomas (48). NB4 cells were observed to transport less guanosine when harvested in

FIG. 5. Substrate specificity of the cs transport in NB4 cells. Transport of 100 μM guanosine was measured in the presence of 1 mM competing nucleosides, a nucleobase, a nucleoside analog, or nucleoside transport inhibitors. Uptake was measured after a 5-s incubation of the cells with the transport buffer (Na+ buffer, 100 μM guanosine, and the competitor). The flux of guanosine observed in the presence of 1 mM concentration of the various competitors is expressed as a percentage of guanosine flux measured in the absence of competing compounds. 2′-deoxyguanosine, inosine, hypoxanthine, guanine, adenosine, uridine, NBMPR, and dipyridamole significantly inhibited guan uptake. The bars represent the mean ± S.E. of data obtained in at least three separate experiments. Ganciclovir A, 1 mM ganciclovir + 100 μM guanosine; ganciclovir B, 1 mM ganciclovir + 5 μM guanosine.
Initial transport rates for guanosine transport in various leukemia cell lines

| Lines  | Initial Transport Rates (pmol/mg cell/min) | % of Total Initial Transport Rates |
|--------|-------------------------------------------|-----------------------------------|
| HL-60 cells | 1.20 ± 0.2 | 1.1 ± 0.2 |
| L1210 cells | 2.25 ± 0.5 | 0.90 ± 0.1 |

- a: Concentrative-sensitive transport.
- b: Equilibrative-insensitive transport.
- c: Equilibrative-sensitive transport.
- d: ND, not determined.

plateau phases (data not shown) and therefore it is conceivable that the csg transporter provides a means of ensuring that an adequate supply of dGTP is available to the cell to sustain DNA replication and excessive proliferation. In a hematopoietic cell, such as the NB4 cell, de novo synthesis is limiting and the salvage via membrane transporters is likely to be critical.

Nucleoside transport processes appear to be regulated during cellular differentiation (21, 33). HL-60 cells induced to differentiate to neutrophils by treatment with dimethyl sulfoxide (53), retinoic acid, or tiazofurin (51). There has considering the efficacy of intracellular targeted cytotoxic agents. It is possible that the intracellular phosphorylation of the nucleoside often exceeds the extracellular phosphorylation of the nucleoside influx. Thus, the intracellular free nucleoside concentration attains a very slow steady state (7). At 50 μM cytidine, very little accumulation of the nucleoside occurred, suggesting that the intracellular phosphorylation reaction was saturated at this concentration.

In general, dNTP and NTP levels are higher in actively dividing tumor cells than in untransformed dividing cells (41). For example, proliferating rat hepatoma 329DA cells in cultured monolayers have a 6.5-fold greater dCTP pool size over normal regenerating rat liver (42). The large accumulation of cytidine in the NB4 leukemia cell line may be a reflection of the increased requirement for dCTP and CTP to perform DNA replication thereby allowing for rapid and extensive proliferation.

In conclusion, we have identified a new Na⁺-dependent guanosine-specific nucleoside transporter that may play a role in leukemia development, cell proliferation, and myeloid differentiation. This transporter strongly prefers guanosine over other physiological permeants and may provide a new target for chemotherapy.

**Acknowledgment**—We thank Dr. Laura Nagy for suggestions regarding preparation of the manuscript.

**REFERENCES**

1. Carver, J. D., and Walker, W. A. (1995) *Nutr. Biochem.* 6, 58–72
2. Voet, D., and Voet, J. G. (1990) in *Biochemistry* (Sawicki, D., Silberman, M., and Steifel, J., eds) pp. 740–768, John Wiley and Sons, New York
3. Fox, H., and Kelly, W. W. (1978) *Annu. Rev. Biochem.* 47, 655–686
4. Perigaud, C. G., Gosselin, G., and Imbach, J.-L. (1992) *Nucleosides & Nucleotides* 11, 903–945
5. Coss, C. E. (1995) in *Drug Transport in Antimicrobial and Anticancer Chemotherapy* (Georg papadakis, N. H., eds) pp. 403–451, Marcel Dekker, New York
6. Belt, J. A., Marina, N. M., Phelps, D. A., and Crawford, C. R. (1993) Adv. Enzyme Regul. 33, 235–252
7. Plagemann, P. G. W., and Wohlhueter, R. M. (1980) *Curr. Top. Membr. Transp.* 14, 225–330
8. Gati, W. P., and Paterson, A. R. P. (1989) in *Red Blood Cell Membranes: Structure, Function, and Clinical Implications* (Agre, P., and Parker, J. C., eds) pp. 635–661, Marcel Dekker, Inc., New York
9. Plagemann, P. G. W., Wohlhueter, R. M., and Wold, W. D., (1988) *Biochim. Biophys. Acta* 947, 405–443
10. Belt, J. A. (1983) *Mol. Pharmacol.* 24, 479–484
11. Belt, J. A. (1984) in *Biochem. Biophys. Acta* 774, 39–52
12. Belt, J. A., and Noel, L. D. (1985) *Biochem. J.* 232, 681–688
13. Jarvis, S. M., and Young, J. D. (1980) *Biochem. J.* 196, 377–385
14. Belt, C. E., Gaudette, L. A., and Paterson, A. R. P. (1974) *Biochim. Biophys. Acta* 345, 1–10
15. Vijayalakshmi, D., and Belt, J. A. (1988) *J. Biol. Chem.* 263, 19419–19423
16. Jarvis, S. M., William, T. C., Lee, C. W., and Cheeseman, C. I. (1989) *Biochem. Soc. Trans.* 17, 448–450
17. Lee, C. W., Cheeseman, C. I., and Jarvis, S. M. (1988) *Biochim. Biophys. Acta* 942, 139–149
18. Crawford, K. A., Ng, C. Y. C., Noel, L. D., and Belt, J. A. (1990) *J. Biol. Chem.* 265, 9732–9736
19. Gutierrez, M., and Giacomini, K. M. (1993) *Biochim. Biophys. Acta* 1149, 202–208
20. Wu, X., Yuan, G., Brett, C. M., Hui, A. C., and Giacomini, K. M. (1992) *J. Biol. Chem.* 267, 8113–8118
21. Lee, C. W., Sokoloski, J. A., Sartorelli, A. C., and Handschumacher, R. E. (1991) *Biochem. J.* 274, 85–90
22. Paterson, A. R. P., Gati, W. P., Vijayalakshmi, D., Cass, C. E., Mant, J. J., Young, J. D., and Belch, A. R. (1993) *Proc. Am. Assoc. Cancer Res.* 34, 14
23. Jarvis, S. M., Hammond, R. C., Paterson, A. R. P., and Clanachan, A. S. (1982) *Biochem. J.* 208, 83–88
24. Woldfendin, C., and Plagemann, P. G. W. (1987) *Biochim. Biophys. Acta* 903, 18–30
25. Bouma, C. E., Hughe, D. L., and Cass, C. E. (1992) *Biochem. J.* 288, 987–996
26. Jakobs, E. S., Van Os-Corby, D. J., and Paterson, A. R. P. (1990) *J. Biol. Chem.* 265, 22210–22216
27. Meckling-Gill, K. A., Guillbert, L., and Cass, C. E. (1993) *J. Cell. Physiol.* 155, 530–538
28. Meckling-Gill, K. A., and Cass, C. E. (1992) *Biochem. J.* 282, 147–154
29. Lanotte, M., Martin-Theroumier, V., Najman, B., Balserini, P., Valenti, F., and Berger, R. (1991) *Blood* 77, 1080–1086
30. Dalton, W. T., Jr., Ahearn, M. J., McCladie, K. B., Freireich, E. J., and Trujillo, J. M. (1988) *Blood* 71, 242–247
31. Roevers, K. I., and Meckling-Gill, K. A. (1996) *J. Cell. Physiol.* 166, 593–600
32. Lee, C. W. (1994) *Biochem. J.* 300, 407–412
33. Sokoloski, J. A., Sartorelli, A. C., Handschumacher, R. E., and Lee, C. W. (1991) *Biochem. J.* 280, 515–519
34. Goh, L.-B., Sokoloski, J. A., Sartorelli, A. C., and Lee, C. W. (1995) *Biochem. J.* 294, 693–697
35. Shen, S.-P., Cleareld, J. S., Hambell, A. B., Wiemann, M. C., Parks, R. E., Jr., and Stoeckler, J. D. (1986) *Cancer Res.* 46, 3449–3455
36. Fidu, M. D., and Morand-Portugal, M. T. (1993) *Cell. Mol. Neurobiol.* 13, 493–502
37. Jones, K. W., and Rylett, R. J., and Hammond, J. R. (1994) *Brain Res.* 660, 104–112
38. Paterson, A. R. P., Harley, E. R., and Cass, C. E. (1984) *Biochem. J.* 224, 1001–1008
39. Burrs, N. S., and Cass, C. E. (1986) *J. Cell. Physiol.* 128, 375–382
40. Segel, I. H. (1976) *Enzyme Kinetics*, Wiley, New York
41. Traut, T. W. (1994) *Mol. Cell. Biochem.* 140, 1–22
42. Jackson, B. C., Lui, M. S., Borizjki, T. J., Morris I. I. P., and Weber, G. (1980) *Cancer Res.* 40, 1286–1291
43. Williams, T. C., and Jarvis, S. M. (1991) *Biochem. J.* 274, 27–33
44. Jarvis, S. M. (1989) *Biochim. Biophys. Acta* 979, 132–138
Characterization of a Novel Transporter in Leukemia Cells

45. Gati, W. P., Dagnino, L., and Paterson, A. R. P. (1989) *Biochem. J.* **263**, 957–960
46. Zimmerman, T. P., Mahony, W. B., and Prus, K. L. (1987) *J. Biol. Chem.* **262**, 5748–5754
47. Bhatia, M., Kirkland, J. B., and Meckling-Gill, K. A. (1995) *Biochem. J.* **308**, 131–137
48. Weber, G. (1988) *Adv. Enz. Reg.* **27**, 405–433
49. Sokoloski, J. A., Blair, O. C., and Sartorelli, A. C. (1986) *Cancer Res.* **46**, 2314–2319
50. Wright, D. G. (1987) *Blood* **69**, 334–337
51. Tricot, G. J., Jayaram, H. N., Nichols, C. R., Pennington, K., Lapis, E., Weber, G., and Hoffman, R. (1987) *Cancer Res.* **47**, 4988–4991
52. Bhatia, M., Kirkland, J. B., and Meckling-Gill, K. A. (1994) *Leukemia* **8**, 1744–1749
53. Pilz, R. R., Huvar, L., Scheele, J. S., Van den Berghe, and Boss, G. R. (1997) *Cell Growth & Differ.* **8**, 53–59