Ligand-directed Immunoaffinity Purification and Properties of the One-carbon, Reduced Folate Transporter

INTERSPECIES IMMUNO-CROSS-REACTIVITY AND EXPRESSION OF THE NATIVE TRANSPORTER IN MURINE AND HUMAN TUMOR CELLS AND THEIR TRANSPORT-ALTERED VARIANTS*

Judy H. Chiao‡, Ching-H. Yang‡, Krishnendu Roy‡, Jayashree Pain‡, and F. M. Sirotnak†

From the †Program of Molecular Pharmacology and Therapeutics, Memorial Sloan-Kettering Cancer Center and ‡Graduate School of Medical Sciences, Cornell University, New York, New York 10021

Almost complete purification (>95%) of the 46-kDa murine, one-carbon, reduced folate transporter (RFT) at a recovery of 20% was obtained by ligand-directed immunoaffinity fractionation from transporter overproducing L1210/R83 cells. These cells were labeled with the N-hydroxysuccinimide ester of [3H]aminopterin (AMT), the isolated plasma membrane alkaline washed to remove nonintegral membrane proteins, detergent-solubilized, and RFT-separated on an anti-AMT antibody-protein G-Sepharose column followed by preparative SDS-polyacrylamide gel electrophoresis. Anti-RFT antibody, subsequently derived, differentially blotted (L1210/R83 >> L1210/0) a 46-kDa protein during SDS-polyacrylamide gel electrophoresis of plasma membrane from L1210/R83 and L1210 cells and in L1210/R83 cells after trichloroacetic acid precipitation.

In contrast to that reported for human tumor cells, glycosidase treatment of RFT revealed no common N- or O-linked core oligosaccharides associated with this protein. The same 46-kDa protein at different relative levels was revealed in a Western blot of plasma membrane from other murine tumors. Blotting of plasma membrane from methotrexate resistant, transport defective L1210 cell variants exhibited wild-type levels of a less electrophoretically mobile RFT or greater levels of the same 46-kDa RFT which could not be affinity labeled with N-hydroxysuccinimide-[3H]AMT. The same antibody differentially blotted a 83-kDa plasma membrane protein from human HL-60 and CCRF-CEM cells with different levels of reduced folate transport and affinity labeling of RFT, verifying the conserved nature of this protein consistent with earlier functional studies.

The preferred route for mediated entry of reduced folates and folate analogues through the plasma membrane of tumor cells (reviewed in Refs. 1 and 2) is the one-carbon, reduced folate transport (RFT) system. This transport system is a major determinant of cytotoxicity (2, 3) and of therapeutic responsiveness of tumors (3), and genetic alteration of its biochemical properties has been shown (4) to be a common form of acquired resistance to classical folate analogues in many animal and human tumors. More recent studies utilizing affinity- or photoaffinity labeling have characterized the transporter (RFT) for the one-carbon, reduced folate transport system as an integral membrane protein of molecular mass = 43-46 kDa in murine L1210 cells (5-7), but exhibiting a much higher mass in human CCRF-CEM, K562, and HL-60 cells (8-10). The larger mass of the human transporter appears to relate to its content of core oligosaccharides (8, 9, 11). Further studies on the biochemical properties of this transporter and the regulation of its gene expression have been hampered by the relatively low level of its expression (1) in all of the tumor cells studied and the unavailability of facile methods for its purification. Progress in regard to the former limitation was first documented (12) in our studies describing the isolation by one of two methods of variants of the L1210 cell expressing high levels of the transporter. The isolation of similar variants of human CCRF-CEM and K562 leukemia cells by one of these methods was subsequently reported (8, 13) by others. More recently (10), we applied the same methodology to the isolation of a similar variant of HL-60 cells.

In a preliminary report (14), a method for purification of the RFT from L1210 cells was recently described. These workers utilized affinity purification by streptavidin "capture" of the transporter labeled with a biotinylated folate analogue to obtain microgram amounts of the transporter. More recently, a second approach was described (15) using lectin-affinity chromatography which appeared to be successful when applied to a highly glycosylated reduced folate transporter (8, 11) from an "overproducing" variant of K562 cells. These are extremely interesting approaches with apparent potential for widespread use. However, the application of these approaches in the authors' laboratory for the purification of transporter from L1210 cells "overproducing" the transporter was consistently unsuccessful. As an alternative approach, we now report on the purification of this transporter from an "overproducing" L1210 cell variant by ligand-directed immunoaffinity chromatography. This methodological approach is now described in detail along with data obtained with anti-RFT polyclonal antibodies on the native properties of the transporter and its relative expression and properties in parental and transport altered variants of murine and human tumor cells.

EXPERIMENTAL PROCEDURES

Source of Cells—The isolation and characterization of L1210/R83 variant cells that have increased amounts (30-40-fold) of the RFT has been described previously (6, 12). These cells are maintained in RPMI medium with dialyzed fetal calf serum supplemented with 4 × 10⁻³ M...
L-L5CHO-folateH4, and 6 × 10⁻⁷ M metotrexone. Derivation of human HL-60 or CEM-CCRF cells with elevated levels of one-carbon, reduced folate transport have also been described earlier (9, 10). Maintenance of these lines was in RPMI medium with an amount of L-L5CHO-folateH4 as the sole folate source which will allow maximum growth. Large scale accumulation of L1210/R83 cells in BD2F1 mice has also been described (6). The yield of human tumor cells used in this study was maintained in cell culture (6, 12) in RPMI medium. Transport defective methotrexate resistant variants of L1210 and CCRF-CEM cells were isolated as described (4) and maintained (6, 9) in the presence of this antifolate in RPMI medium.

Transport Methodology, Affinity Labeling, and Analytical SDS-PAGE—Membrane and parental L1210 (13) or CCRF-CEM (6) bacteria were cultured in liquid medium (6) with varying amounts of [³H]MTX in accordance with protocols and processing methodology described previously (6). L1210/R83 cells in Hepes Mg⁺⁺ sucrose buffer (5 × 10⁵ cells/ml) were affinity labeled with 1 µM of the N-hydroxysuccinimide ester of [³H]AMT for 10 min at room temperature in a manner similar to that described by Henderson and Zevely (16). The cells were washed once in Hepes-buffered saline (20 mM Hepes, 140 mM NaCl, 10 mM KCl, 2 mM MgCl₂, pH 7.4) and either stored as a pellet at −70°C or used immediately for plasma membrane isolation (6). Alkaline washed (6) plasma membrane was dissolved in sample buffer and electrophoresed (17) in a 10% polyacrylamide gel.

Preparation of Anti-AMT Monoclonal Antibodies and Western Blotting—CB6F₁ (BalbC × DBA/2J) mice were injected ip with 200–400 µg of anti AMT to keyhole limpet hemocyanin in incomplete Freund's adjuvant and boosted by injection 4 and 8 weeks later by the same antigen in incomplete Freund's adjuvant. Fusion of splenocytes, screening of hybridomas, and cloning was as described in Galfre and Milstein (18) and Hartliow and Lane (19). The antibodies derived were classified as IgG₂a, by isotyping (Southern Biotechnology Associates). Purification of this IgG was by affinity chromatography on a protein G-Sepharose column. Detection of [³H]AMT RFT during Western blotting (18, 19) by successively passing the solution through a column of protein G-Sepharose, the solubilized protein solution was used to resuspend 0.1 M glycine HCl with 0.25% CHAPS (pH 2.2) and collected in 2-ml fractions in tubes containing 1.5 M Tris (pH 11.5). The final concentration of Tris-HCl was 0.15 M (pH 8.2). After the addition of SDS to a final concentration of 2%, the pooled material was concentrated at room temperature using a Stircol (Amicon, Inc.). The concentrated material was loaded onto a Bio-Rad 491 preparative cell (10.5% gel) for electrophoresis using the conditions specified by Laemmli (17). After 11 h of electrophoresis at a constant power of 11 watts, the labeled protein was eluted from the gel during further electrophoresis at a flow rate of 0.8 ml/min and 4 ml fractions were collected in tubes of 10 ml volume. Antiserum obtained 2 weeks after the fourth injection of purifed RFT was used to detect [³H]AMT-linked IgG in the immunoprecipitates. Representative data is shown in Fig. 2 and Table I. Binding of solubilized NHS-[³H]AMT to antibody-protein G-Sepharose was determined to the extent of 70–80% and approx-
material is shown in Fig. 2. This migration profile predicted that the majority of the eluted material would be recovered in the major peak fractions at 46 kDa following preparative electrophoresis. However, the actual recovery of [3H]AMT-RFT in the peak fractions from the Bio-Rad 491 cell usually selected (see Table I and Fig. 3) was considerably less (40%) due to aggregation and proteolysis.

In Table I, we compare the total protein recovered at each purification stage. We also compare the disintegrations/min of radioactivity per mg of protein at each stage. From these values, we estimate that approximately 500-fold purification was required to obtain highly purified RFT from the alkaline washed plasma membrane of these cells and that final recovery was in the range of 20%. Although the data suggest that complete purity was achieved, in actual practice, the purity is probably only in the range of 96–98%. Following SDS-PAGE (slab gel) of this purified material, duplicate transblots were stained with Coomassie Blue or immunoblotted with anti-AMT monoclonal antibody using ECL detection. In each case a single protein band (approximately 46 kDa) was delineated (Fig. 4) on the blot.

Immunoblotting and Size of Native RFT in Parental and Transport-elevated L1210/R83 Cells—Purified RFT from L1210/R83 cells was utilized to prepare anti-RFT polyclonal antibody in rabbits. The serum after absorption with nonspecifically labeled proteins (see above) was used to immunoblot plasma membrane from parental and L1210/R83 cells following SDS-PAGE. The ECL results in Fig. 5A revealed an immuno-reactive band at 46 kDa in both cases with an extremely large differential in intensity favoring L1210/R83 plasma membrane. A similar blot with preimmune serum from the same rabbit did not blot (data not shown) a 46-kDa protein. Minor protein bands in a higher molecular mass range on the gels observed during blotting of material derived from L1210/R83 cells but not L1210 cells with either anti-AMT or anti-RFT immune serum are also seen with purified RFT which was size selected during purification. This reflects aggregation of RFT seen also after affinity labeling (6) of RFT overproducing L1210/R83 cells. These results verify that the absorbed antiserum is highly specific for RFT but does not entirely rule out the possibility that minor amounts of antibodies derived against other membrane proteins that were size selected along with RFT were also present in this serum. Size determination of RFT in L1210/R83 derived plasma membrane, whether by affinity labeling or immunoblotting, was always carried out with purified plasma membrane. Despite the fact that protease
SDS-PAGE of crude membrane preparations obtained by rapid immunoblotting this protein with anti-RFT antibody following proteolytic fragment. Therefore, to more rigorously verify that inhibitors (6) were routinely used during preparation of these Western blotting with anti-RFT polyclonal antibody. 

In plasma membrane of L1210 and L1210/R83 cell detected by 46-kDa when obtained with plasma membrane (Fig. 5A), trichloroacetic acid precipitate (Fig. 5B), and crude membrane (Fig. 5C), or when purified (Fig. 5C). Blotting with antibody of trichloroacetic-acid-precipitated L1210/R83 cells did not detect RFT (Fig. 5B) ostensibly because of its low content in the precipitate of whole cell material.

Analysis for the Extent of Glycosylation of Purified RFT—As in prior (5, 23, 24) studies with human tumor RFT, the availability of anti-RFT antibodies greatly facilitates analytical studies of this protein that requires SDS-PAGE. Accordingly, we (23) analyzed the murine RFT for both N- and O-linked glycosidase. In the former case, aliquots of purified transporter from L1210/R83 cells were treated with N-glycanase (24) in the presence of proteinase inhibitors (6). In the latter case, aliquots of the same protein were treated with O-glycanase following treatment with neuraminidase (11, 24) in the presence of the same proteinase inhibitors. In both instances, no change in the relative rate of migration of RFT as shown by immunoblotting and ECL detection was discerned (Fig. 6) following SDS-PAGE. As a control for these analyses, we treated under the same conditions (30, 31) three glycosylated proteins, ferritin, α-acid p-glycoprotein, and transferrin with N-glycanase and determined their migration after staining with Coomassie Blue. In every case, a difference in the rate of migration of these proteins after treatment with glycanase was seen (Fig. 4) during SDS-PAGE.

Immunoblotting of RFT in Parental and Transport-defective Murine Tumor Cells—Using the absorbed anti-RFT serum, immunoblotting of alkaline washed plasma membrane from a series of parental and methotrexate-resistant, transport-defective, murine tumors was carried out. The ECL data in Fig. 7A delineated a single 46-kDa protein band following SDS-PAGE of plasma membrane from L1210, P388, Ehrlich, M5076, and S180 cells. Moreover, the different intensity of the blots indicated that there was some variability in levels of immunoreactive RFT found in each case. All these cells display one-carbon, reduced folate transport as determined in a functional assay (25, 26).

The ECL data shown in Fig. 7, B and C, also delineate a single protein band in the vicinity of 46 kDa following SDS-PAGE of plasma membrane from several independently isolated transport defective L1210 cell variants. All of these variants have markedly lower levels (Table II) of one-carbon, reduced folate transport in comparison to parental L1210 cells and in contrast to the latter, trans-stimulation (2) by internalized L-SCH-O-folateH4 of [3H]MTX influx could not be demonstrated. Also, variants L1210/R26A–D have markedly lower levels (Table II) of one-carbon, reduced folate transport as determined in a functional assay (25, 26).

Inhibitors (6) were routinely used during preparation of these membranes, the possibility exists that the RFT detected was a proteolytic fragment. Therefore, to more rigorously verify that the native size of RFT was, in fact, in the vicinity of 46 kDa, we immunoblotted this protein with anti-RFT antibody following SDS-PAGE of crude membrane preparations obtained by rapid sonication and centrifugation at 0 °C as a trichloroacetic acid precipitate of total cellular protein. Data in Fig. 5, A–C show that the size of L1210/R83-derived RFT blotted is the same (46 kDa) that size of L1210/R83-derived RFT blotted is the same (46 kDa) when obtained with plasma membrane (Fig. 5A), trichloroacetic acid precipitate (Fig. 5B), and crude membrane (Fig. 5C), or when purified (Fig. 5C). Blotting with antibody of trichloroacetic-acid-precipitated L1210/R83 cells did not detect RFT (Fig. 5B).

Fig. 5. Relative amount and electrophoretic properties of RFT in plasma membrane of L1210 and L1210/R83 cell detected by Western blotting with anti-RFT polyclonal antibody. Following SDS-PAGE, purified RFT, RFT in crude or plasma membrane, or trichloroacetic acid precipitate of parental or variant (L1210/R83) L1210 cells was transblotted to nitrocellulose and immunoblotted with anti-RFT antibody. A, 2-μg sample of plasma membrane from L1210 and L1210/R83 cells. B, 7-μg sample of trichloroacetic acid precipitate of L120 and L1012/R83 cells. C, 5-μg sample of crude membranes from L1210/R83 cells or 8 ng of purified RFT. Additional details are given in the text or legend to Fig. 1.

Table I Summary of data on the purification of the one-carbon, RFT from L1210/R83 cells

| Fraction      | Total protein | Total activity | Affinity-labeled protein | Recovery |
|---------------|---------------|----------------|--------------------------|----------|
|               | mg            | dpm/mg (×10^6) | μg                       | %        |
| Na₂CO₃/EDTA   |               |                |                          |          |
| Washed        | 100           | 0.0057         | 198                      | 1.00     |
| Immunoeluate  | 0.352         | 0.814          | 94                      | 142      |
| SDS-PAGE      | 0.040         | 3.05           | 39                      | 532      |
| Eluate        |               |                | 20                      |          |

a Calculated from the specific activity of [3H]AMT and total specific dpm in the 45-47-kDa fraction of protein attained during SDS-PAGE.
b Recovery reflects the loss of dpm occurring during each purification step and the fraction size selected for the subsequent step.
classes as shown in Fig. 7, B and C, and Table II. The variants L1210/R26A–D exhibit levels of RFT somewhat higher than parental L1210 cells. However, RFT in all of these variants migrates during SDS-PAGE at a rate slightly reduced relative to parental cell RFT, so that an aberrant molecular size was obtained for each which was approximately 10% higher than that determined for parental cell RFT (46 kDa). Variants L1210/R24 and 25 were even more unusual. RFT in these variants was not affinity labeled by NHS-[3H]AMT (Fig. 2, Table II) but was found in the plasma membrane, based upon the ECL data, at levels 4- (L1210/R24) to 8-fold (L1210/R25) higher than in parental L1210 cells.

DISCUSSION

The results demonstrate that ligand-directed immunoaffinity fractionation with preparative SDS-PAGE is a reasonable approach to the purification of RFT from L1210 cells. Also, the availability of an L1210 cell variant overproducing RFT to a very substantial extent (6) contributed greatly to our realizing adequate amounts of purified RFT for further studies. Although this method of purification has inherent limitations with regard to the maximum degree of purification attainable, it seems likely that the RFT consistently recovered will continue to be greater than 95% in purity. No indication of more substantial amounts of impurity, i.e. manifested as multiple protein bands in the size selected region, was obtained either by direct staining of protein or immunoblotting following SDS-PAGE. Conversely, since most integral plasma membrane proteins are glycosylated, significant amounts of such proteins as contaminants would have been visible after migration away from glycanase-treated RFT during SDS-PAGE.

The amount of RFT generated by the above methodology was very adequate for eliciting polyclonal antibody formation in rabbits. Western blotting with this immunoadsorbed antibody preparation of plasma membrane from L1210 and L1210/R83 cells detected the 46-kDa RFT in relative amounts commensurate with higher one-carbon, reduced folate transport and proteases particularly when overproduced in transport elevated variants (10).
NHS-AMT affinity labeling of RFT in L1210/R83 cells (6). Of interest as well were results showing that these antibodies also blotted RFT in plasma membrane of other tumors of different histologic origin and an 83-kDa RFT in plasma membrane from HL-60 and CCRF-CEM cells. Again, the relative extent of blotting was similar to the relative level of one-carbon, reduced folate transport and specific NHS-AMT labeling found (9, 27, 28) in parental and variant CCRF-CEM cells. These results on relative tissue content and interspecies cross-immunoreactivity of RFT lend considerable support to the notion we have expressed earlier (1), that one-carbon, reduced folate transport is a conserved property among tumor cells of different species.

These and earlier studies from the authors laboratory (6) and others (5, 7, 29) utilizing alternative methods of size estimation suggested that L1210 cell RFT exists in the plasma membrane as a 43- to 46-kDa protein in a form with little or no core oligosaccharides attached (14). In the latter case, this was concluded from an experiment examining (14) the effect of N-glycanase on the migration of stained purified RFT during SDS-PAGE. We have extended these studies in greater detail to show that immunologically delineated RFT has no detectable N- or O-linked oligosaccharides attached. These are in sharp contrast to results of very extensive prior studies (11, 15, 23) by others revealing N- and O-linked oligosaccharides on RFT in plasma membrane of human leukemia cells (K562 and CCRF-CEM). Most importantly, these studies showed that N-glycanase treatment of plasma membrane from cells grown in tunicamycin markedly reduced the molecular size of RFT to approximately 50 kDa, a size similar to that delineated for murine tumor RFT.

The availability of antibody to RFT has made it possible (15) (this study) to examine in more detail the properties of RFT in tumor cell variants with defective one-carbon, reduced folate transport that are resistant to classical folate analogues. The variants examined here revealed interesting differences in the amount and physical properties of RFT in addition to the functional modifications documented in Table II. One group of independently isolated L1210 cell variants (L1210/R26A-D) exhibited an isoform of RFT with a modest, but consistent, decrease in electrophoretic mobility compared to wild-type RFT. Two other variants (L1210/R24 and R25) exhibited markedly increased amounts of RFT despite the fact that these 46-kDa proteins could not be affinity labeled with NHS-AMT. Prior studies (7, 29) of similar transport defective variants of the L1210 cell detected no differences in the amount or physical properties of RFT, while a transport-defective variant of CCRF-CEM cells exhibited slightly elevated amounts of a more rapidly migrating isoform of RFT during SDS-PAGE. In contrast, the transport-defective, variant of CCRF-CEM cells examined here and the variants of K562 cells examined by immunoblotting by others (15) showed only lower levels of RFT in the plasma membrane. Thus, it would appear that impaired function of one-carbon, reduced folate transport in tumor cell variants with acquired resistance to folate analogues can result from a variety of alterations affecting both the structure and amount of RFT.

The recent isolation of cDNAs from L1210 (30) and hamster ovary (31) cell recombinant cDNA libraries, which restore MTX transport in transport-defective cells, has potentially important implications for one-carbon, reduced folate transport in tumor cells. It is of interest, therefore, that our results further verify that the molecular size of native RFT expressed in L1210 cells is in the neighborhood of 46 kDa, while pRFC-1 derived from the L1210 cell cDNA library and a related hamster ovary cell cDNA appear to code for a protein of 58 kDa. An NHS-[3H]AMT affinity-labeled protein of 46 kDa was previously delineated (6) among the plasma membrane proteins from L1210/R83 cells during molecular sieve chromatography, most likely ruling out that the 46-kDa RFT reflects an aberrancy in electrophoretic mobility. This discrepancy with regard to the molecular size of RFT remains unexplained at this time, but it dearly has implications for the identity of the protein coding for pRFC-1. Therefore, further work will be required before the exact relationship between pRFC-1 and its hamster and human homologue and RFT is understood, and we present these comments and our findings in this regard with no bias intended.

Acknowledgment—The samples of plasma membrane from the various sublines of CCRF-CEM cells were provided by Doctor G. Jansen, Department of Oncology, Free University of Amsterdam, The Netherlands.

TABLE II

| Cell line | \( K_m \) | \[^{3}H\text{MTX} \text{influx} V_{max} \) | Trans-stimulation | RFT content (variant parental) |
|-----------|-----------|---------------------------------|------------------|--------------------------------|
| L1210     | 4.35 ± 0.7 | 7.84 ± 1.1                      | 120              | 1.0                            |
| L1210/R26A| 3.75 ± 0.6 | 0.16 ± 0.005                    | 0                | 1.0                            |
| L1210/R26B| 4.10 ± 1   | 0.14 ± 0.003                    | 0                | 1.7                            |
| L1210/R26C| 4.25 ± 0.8 | 0.18 ± 0.004                    | 0                | 2.2                            |
| L1210/R26D| 3.95 ± 0.7 | 0.15 ± 0.003                    | 0                | 1.6                            |
| L1210/R24 | 16.5 ± 2  | 0.52 ± 0.1                      | 0                | 1.5                            |
| L1210/R25 | 15.9 ± 3  | 0.09 ± 0.02                     | 0                | <0.05                          |

\(^{a}\) Cells were preincubated in transport buffer for 5 min with or without 10 \( \mu M \) L-SCHO-folate \( \text{H} \), washed, and resuspended with 1 \( \mu M \) \[^{3}H\text{MTX} \) in the same buffer for varying periods of time at 37°C.

\(^{b}\) Determined by radioactive counting of \[^{3}H\text{AMT-RFT} \) after SDS-PAGE.

\(^{c}\) Determined by densitometry of individual ECL-RFT blots.

FIG. 8. Relative amount and properties of RFT in the plasma membrane of human leukemia cells and their variants as detected by Western blotting. The indicated amounts of purified plasma membrane from CCRF-CEM cells and its transport elevated (CEM/7A) or transport reduced (CEM/T) variants and HL-60 cells were run as a 43-kDa protein (

---

Abbreviations:
- MTX: Methotrexate
- RFT: One-carbon, Reduced Folate Transporter
- NHS-AMT: N-hydroxysuccinimide-aminomethylthiazole
- CEM: Human leukemia cell line
- HL-60: Human promyelocytic leukemia cell line
- K562: Human erythroleukemia cell line
- CCRF-CEM: Human leukemia cell line
- SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
- ECL: Enhanced chemiluminescence
- L-SCHO: L-succinyl-N-hydroxy-L-ornithine
REFERENCES

1. Sirotnak, F. M. (1985) Cancer Res. 45, 3992–4000
2. Goldman, I. D., and Matherly, L. H. (1986) Int. Enycl. Pharmacol. Ther. 118, 283–302
3. Sirotnak, F. M., and DeGraw, J. I. (1984) in Folate Antagonists as Therapeutic Agents (Sirotnak, F. M., Burdall, J. J., Esninger, W. D., and Montgomery, J. A., eds) Vol. 2, pp. 43–91, Academic Press, New York
4. Sirotnak, F. M., Moccio, D. M., Kelleher, L. E., and Goutas, L. J. (1981) Cancer Res. 41, 4447–4452
5. Price, E. M., and Fresh, J. H. (1987) Biochemistry 26, 4757–4763
6. Yang, C-H., Sirotnak, F. M., and Mines, L. S. (1988) J. Biol. Chem. 263, 9703–9709
7. Schuetz, J. D., Matherly, L. H., Westin, E. H., and Goldman, I. D. (1988) J. Biol. Chem. 263, 9840–9847
8. Matherly, L. H., Czajkowski, C. A., and Angeles, S. M. (1991) Cancer Res. 51, 3432–3437
9. Fresh, J. H., Ratman, M., McAlinden, T. P., Prasad, K. M. R., Williams, F. E., Westerhof, G. R., Schornagel, J. H., and Jansen, G. (1992) Adv. Enzyme Regul. 32, 17–31
10. Yang, C-H., Pain, J., and Sirotnak, F. M. (1992) J. Biol. Chem. 267, 6628–6634
11. Matherly, L. H., Angeles, S. M. (1994) Biochem. Pharmacol. 47, 1094–1098
12. Sirotnak, F. M., Moccio, D. M., and Yang, C-H. (1984) J. Biol. Chem. 259, 13139–13144
13. Jansen, G., Westerhof, G. R., Jarmuszewski, M. J. A., Kathmann, I., Rijksen, G., and Schornagel, J. H. (1990) J. Biol. Chem. 265, 18272–18277
14. Fan, J., Vitols, K. S., and Huennekens, F. M. (1991) J. Biol. Chem. 266, 14862–14865
15. Matherly, L. H., Angeles, S. M., and Czajkowski, C. A. (1992) J. Biol. Chem. 267, 23253–23260
16. Henderson, G. B., and Zevely, E. M. (1984) J. Biol. Chem. 259, 4558–4562
17. Laemmli, U. K. (1970) Nature 227, 680–685
18. Galfre, G., and Milstein, C. (1983) Methods Enzymol. 73, 3–46
19. Hartlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 511–553, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
20. Towbin, B., Stachelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
21. Peterson, G. L. (1983) Methods Enzymol. 91, 95–119
22. Cashmore, A. R., Dreyer, R. M., Horwath, C., Kini, J. O., Coward, J. K., and Bertino, J. R. (1980) Methods Enzymol. 66, 457–468
23. Matherly, L. H., Czajkowski, C. A., and Angeles, S. M. (1991) Cancer Res. 51, 3420–3426
24. Maley, F., Trimble, R. B., Tarentino, A. L., and Plummer, T. H. (1989) Anal. Biochem. 180, 195–204
25. Sirotnak, F. M., and Donbach, R. C. (1976) Cancer Res. 36, 1151–1158
26. Sirotnak, F. M., Moccio, D. M., Goutas, L. J., Kelleher, L. E., and Montgomery, J. A. (1982) Cancer Res. 42, 924–928
27. Jansen, G., Westerhof, G. R., Jarmuszewski, M. J. A., Kathmann, I., Rijksen, G., and Schornagel, J. H. (1989) Cancer Res. 49, 2455–2459
28. Schuetz, J. D., Westin, E. H., Matherly, L. H., Pincus, R., Swardlow, P. S., and Goldman, I. D. (1989) J. Biol. Chem. 264, 16261–16267
29. Dixon, K. H., Lanpher, B. C., Chiu, J., Kelley, K., and Cowan, K. H. (1994) J. Biol. Chem. 269, 17–20
30. Williams, F. M. R., Murray, R. C., Underhill, T. M., and Flintoff, W. R. (1994) J. Biol. Chem. 269, 5810–5816
Judy H. Chiao, Ching-H. Yang, Krishnendu Roy, Jayashree Pain and F. M. Sirotnak

J. Biol. Chem. 1995, 270:29698-29704.
doi: 10.1074/jbc.270.50.29698

Access the most updated version of this article at http://www.jbc.org/content/270/50/29698

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 20 of which can be accessed free at http://www.jbc.org/content/270/50/29698.full.html#ref-list-1