Mammalian Nitrobenzylthioinosine-sensitive Nucleoside Transport Proteins

IMMUNOLOGICAL EVIDENCE THAT TRANSPORTERS DIFFERING IN SIZE AND INHIBITOR SPECIFICITY SHARE SEQUENCE HOMOLOGY*

(Received for publication, June 19, 1992)

Francis Y. P. Kwong‡¶, Heather E. Fincham‡, Anthony Davies‡†, Nicholas Beaumont‡, Peter J. F. Henderson¶†**, James D. Young‡‡¶§, and Stephen A. Baldwin‡‡

From the ‡Departments of Biochemistry and Chemistry and of Protein and Molecular Biology, Royal Free Hospital School of Medicine, University of London, Rowland Hill Street, London NW3 2PF, United Kingdom, the §Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, United Kingdom, and the ¶‡Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Polyclonal antibodies were raised against the nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter of human erythrocyte membranes. On Western blots of these membranes they labeled the broad "band 4.5" region (average apparent M, 55,000), which contains both the nucleoside and glucose transport proteins. However, they did not recognize the glucose transporter when this was prepared free of nucleoside transporter by expression from a cDNA clone. Their specificity for the nucleoside transporter was confirmed by the ability to immunoabsorb NBMPR- but not cytochalasin B-binding sites from a detergent-solubilized mixture of band 4.5 proteins. Although a large proportion of the antibodies recognized extracellular epitopes, these appeared to be located primarily on the polypeptide moiety of the glycoprotein, as demonstrated by the ability of the antibodies strongly to label the deglycosylated transporter (apparent M, 45,000) on Western blots. The antibodies were species-cross-reactive, recognizing nucleoside transporters from pig and rabbit erythrocytes and from rat liver. The pig protein is similar to the human transporter in its inhibitor sensitivity but is considerably larger (apparent M, 57,000 after deglycosylation). In contrast, the rat protein is similar in size to the human transporter (apparent M, 45,000 after deglycosylation) but much less sensitive to the inhibitors dilazep and dipryridamole. These findings indicate that despite their differences in size and inhibitor specificity, the NBMPR-sensitive nucleoside transporters of these mammalian species are related in amino acid sequence.

Permeation of nucleosides across the membranes of differ-

* This work was supported by project grants from the Cancer Research Campaign and the Medical Research Council of the United Kingdom. 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.

‡ Present address: Murex Diagnostics Ltd., Langley Ct., South Eden Park Rd., Beckenham, Kent BR3 3BS, United Kingdom.

¶ Present address: Dept. of Crystallography, Birkbeck College London, Malet St., London WC1E 7HX, United Kingdom.

† Present address: Dept. of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom.

** Present address: Dept. of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom.

§§ Alberta Heritage Foundation for Medical Research (AHFMR) Medical Scientist.

1 The abbreviations used are: NBMPR, nitrobenzylthioinosine; GLUT1, the human erythrocyte glucose transporter (terminology according to Fukumoto et al., Ref. 29); PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.
to yield a "band 4.5" protein preparation that migrates as a single broad band of average apparent M, 55,000 on SDS-polyacrylamide gels (6). This preparation consists largely of the glucose transporter (~95%) with the nucleoside transporter present as a minor contaminant (~5%). However, we were able to purify the latter by passage of the detergent-solubilized mixture through a column of antibodies specific for the glucose transporter (7). The resultant preparation catalyzed uridine uptake when reconstituted into phospholipid vesicles, and bound ~0.6 mol of NBMPR/mol of polypeptide (7). Here we describe the production and characterization of antibodies against this protein, and their use in establishing that the divergent NBMPR-sensitive nucleoside transporters of a variety of mammalian species probably belong to a single family of homologous transport proteins.

EXPERIMENTAL PROCEDURES

Materials—Human erythrocytes used for purification of the nucleoside transporter were prepared from outdated blood obtained from the blood bank of the Royal Free Hospital. Fresh erythrocytes were used for establishment of the sidedness of antibody binding were obtained from N. Beaumont. Human erythrocyte-type glucose transporter (GLUT1), free of erythrocyte nucleoside transporter, was prepared by expression of a cDNA clone, isolated from a human hepatoma cDNA library, in insect cells (Spodoptera frugiperda Clone C) that cells (Spodoptera frugiperda Clone C) using a recombinant baculovirus (9). The resultant insect cell membranes containing the expressed human protein were the kind gift of C.-K. Yi (Royal Free Hospital School of Medicine, University of London). Antibodies raised against the C-terminal region of the human erythrocyte glucose transporter (residues 477-492) were prepared as previously described (10). [4-3H]Cytochalasin B (13.5 Ci/mol), [32P]NBMPR (23 Ci/mol) and [125I]F(ab')2, donkey anti-rabbit IgG were obtained from New England Nuclear (Stevanage, Herts., United Kingdom (U.K.)), Moravek Biochemicals (Brea, CA) and Amersham International (Amersham, Bucks., U.K.), respectively. Preparation of Membranes and Polyacrylamide Gel Electrophoresis—Ghosts and protein-depleted membranes were prepared from washed human, rabbit, and pig erythrocytes by the procedures of Gorga and Lienhard (11). Human band 4.5 proteins were prepared by the method of Cairns et al. (12) and used for the purification of the human nucleoside transporter as previously described (7). A partially purified preparation of the pig erythrocyte nucleoside transporter was made by the procedure of Kwong et al. (13). Typically such preparations contained 1.5 nmol of NBMPR-binding sites/mg of protein and were estimated to be about 20% pure (13). A preparation enriched in rabbit erythrocyte nucleoside transporters was prepared by anion-exchange chromatography on a Q-Sepharose column of protein-depleted solubilized membranes. In brief, protein-depleted membranes (1.5 mg/ml) were incubated with 4°C using 1.35% (w/v) octyl glucoside and then passed through a column of DEAE-cellulose equilibrated in buffer containing 1% detergent. The unretarded fraction, which was routinely enriched 90-fold in NBMPR-binding sites compared with rabbit erythrocyte membranes, was reconstituted by dialysis. Rat liver membranes were prepared as previously described (14).

Preparation and Assay of Antibodies—All preparations of nucleoside transporter used for rabbit immunization were first shown, by their failure to bind cytochalasin B, and lack of reaction with anti-glucagon receptor monoclonal antibodies on Western blots, to be free of apparent contamination with glucose transporters (7). For antibody preparation, samples (100 µg in 0.5 ml of PBS) containing 10% sodium phosphate, 145 mM NaCl, pH 7.2) of the purified protein were emulsified with 1.5 ml of complete Freund's adjuvant and then injected into the inguinal muscle of male New Zealand White rabbits. A second, subcutaneous booster injection (50 µg) in incomplete Freund's adjuvant was made 1 month later. Animals were bled after another 2 weeks. Antiserum was treated for 30 min at 56°C to inactivate complement, and then stored at -70°C. Antibodies were measured by enzyme-linked immunosorbent assay (ELISA) and additional booster injections (identical to the first booster) given when appropriate. Affinity purification of the antibodies from serum was performed by adsorption onto protein-depleted pig erythrocyte membranes (15 µg of membrane protein per assay) for 1 h at 2°C. The membranes were extensively washed by centrifugation and resuspension with PBS and then with PBS containing 800 mM NaCl to remove nonspecifically bound IgG. Anti-nucleoside transporter antibodies were eluted by resuspension of the membranes at ice temperature in 0.2 M glycine-HCl, pH 2.4, followed by centrifugation and immediate neutralization of the supernatant to neutral pH with 2 M Tris. Finally, contaminating albumin was removed from the preparation by chromatography on a column of Affi-Gel blue gel (BioRad) according to the manufacturers' instructions. Purity of the resultant IgG preparation was confirmed by SDS-polyacrylamide gel electrophoresis according to the procedure of Laemmli (15).

ELISA assays of antisera were as previously described, using microtiter plates coated with either 20 ng of synthetic peptide or 400 ng of membranes per well (10). Alkaline phosphate-linked goat anti-rabbit IgG was used as the secondary antibody and p-nitrophenyl phosphate was employed as chromogenic substrate. Colorimetric detection of nucleoside transporters on Western blots also utilized alkaline-phosphatase-linked goat anti-rabbit IgG, as previously described (10). In some experiments (indicated under "Results and Discussion") horse radish peroxidase-labeled donkey anti-rabbit IgG was used to detect bound rabbit IgG on blots using an enhanced chemiluminescence kit (Amersham).

Determination of the Sidedness of Antibody Binding to Erythrocyte Membranes—A modification of the procedure of Haspel et al. (16) was employed. Samples of intact human erythrocytes (1.5×106 cells) or protein-depleted membranes (1.5 µg) were incubated with 1 µg of affinity-purified IgG for 1 h at 37°C in 300 µl of 10 mM sodium phosphate, 145 mM NaCl, pH 7.4, containing 1% (w/v) bovine serum albumin, and 1% (v/v) d-glucose. The cells and membranes were then washed three times with the same buffer before resuspension to yield a final volume of 300 µl. Bound IgG was detected by incubation for 15 min at 37°C with 0.2 µCi of [32P]F(ab')2, donkey anti-rabbit IgG, washing three times with buffer, and then counting the pellets in a γ counter.

Immunoadsorption Assays—For immunoadsorption experiments, human erythrocyte membrane bands (50 µg of protein/ml in 50 mM sodium phosphate, 100 mM NaCl, 1 mM EDTA, pH 7.4) were solubilized at 4°C by the addition of octyl glucoside to a concentration of 1% (w/v). Samples (1 ml) of the solubilized proteins were then incubated at 4°C for 1 h, with frequent swirling, with protein A-Sepharose CL-4B to which affinity-purified anti-nucleoside transporter IgG (1.4 mg) or control rabbit IgG (1.4 mg) was bound. After centrifugation to remove the Sepharose, the supernatants were made 2 mM in diethiothreitol and then dialyzed at 4°C against 50 mM sodium phosphate, 100 mM NaCl, 1 mM EDTA, pH 7.4, to remove the detergent. The reconstituted supernatants were then assayed for NBMPR and cytochalasin B binding activity as described below.

General Methods—Cytochalasin and NBMPR binding to membranes and polyacrylamide gel electrophoresis of isolated and denatured samples was measured by equilibrium dialysis as previously described, using a ligand concentration of 40 nM (17, 18). The results of binding experiments are recorded as the means of triplicate assays that differed from the mean by <10%. Protein was measured by the procedure of Lowry et al. (19), except that 0.5% (w/v) SDS was included in order to solubilize membraneous samples. Endoargininase F digestion of both the purified human and pig nucleoside transport proteins was performed as previously described (6). Silver staining of polyacrylamide gels employed the method of Morrissey (20).

RESULTS AND DISCUSSION

Specificity of the Anti-nucleoside Transporter Antibodies—Antiserum prepared from rabbits immunized with the purified human erythrocyte nucleoside transporter reacted strongly with the protein in ELISA, typically yielding half-maximal responses at serum dilutions of greater than 1 in 3000, whereas preimmune serum gave no response (Fig. 1). A detectable, although somewhat lesser, response to the antiserum was seen using plates coated with unfractionated band 4.5 proteins, in which the nucleoside transporter represented only about 5% of the total protein (Fig. 1). In contrast, antisera raised against the unfractionated band 4.5 preparation gave a very strong response to plates coated with this mixture of proteins (results not shown). These findings suggested that the antiserum raised against the purified nucleoside transporter did not recognize, to any great extent, the major band 4.5 membrane protein, human erythrocyte glucose transporter. The anti-
it was important not only to confirm this but also to exclude the possibility that antibodies against the glucose transporter were also present. This was necessary because the two transporters migrate with identical mobility on SDS-polyacrylamide gels (6). Furthermore, the nucleoside transporter used as an immunogen had been purified by subtractive immunoaffinity chromatography from an ~20-fold excess of glucose transporter (7). Although all preparations of the nucleoside transporter used to immunize rabbits were apparently free of glucose transporters, it was possible that they contained denatured glucose transporters undetectable by the cytochalasin B binding assays used to characterize the preparations. In addition they might have contained degradation products of the protein lacking the epitopes recognized by the monoclonal antibodies that were used in Western blotting experiments to check the preparations for the presence of glucose transporter. The presence even of trace amounts of glucose transporter, undetectable in our assays, would present a problem because the glucose transporter appears to be very much more immunogenic than the nucleoside transporter.² No natural human cell type has been described which expresses GLUT1 but not NBMPR-sensitive nucleoside transporters. To assess the possible cross-reactivity of the antibodies with GLUT1 we therefore examined the ability of the antiserum to recognize proteins on Western blots of membranes prepared from insect cells expressing recombinant GLUT1 in the absence of nucleoside transporter. Membranes prepared from insect cells not expressing the recombinant contained no proteins that could be recognized on the blots by either anti-nucleoside transporter serum or anti-GLUT1 serum (Fig. 3). Both the recombinant glucose transporter (GLUT1) and the glucose transporter present in protein-depleted human erythrocyte membranes stained strongly with anti-peptide antibodies against the C-terminal region of the glucose transporter (residues 477–492). However, whereas the human erythrocyte membrane proteins were also recognized by the anti-nucleoside transporter serum, this did not recognize the recombinant GLUT1 (Fig. 3). These findings indicated that antibodies capable of recognizing the denatured glucose transporter were absent or of very low abundance in the antiserum raised against the purified nucleoside transporter.

**Affinity Purification of the Anti-nucleoside Transporter Antibodies**—The experiments described above indicated that antibodies against the glucose transporter were absent from the anti-nucleoside transporter serum, or of low abundance. However, a second method of investigation did detect the apparent presence of very low amounts of putative anti-glucose transporter antibodies. This method involved assay of the serum by ELISA, using plates coated with a synthetic peptide corresponding to a region of the glucose transporter known to be highly immunogenic (residues 477–492) (10). Antisera raised against the intact glucose transporter usually contain abundant antibodies capable of recognizing this peptide (10). Some batches of anti-nucleoside transporter serum gave a slight response to the peptides in ELISA, but the absorbance values were much lower than for plates coated with the purified nucleoside transporter (Fig. 1). Similar findings were obtained using plates coated with other immunogenic glucose transporter peptides (results not shown). Reactivity was only apparent when low dilutions of antisera were used, and so it may have been artifactual (Fig. 1). Nonetheless, because the results may have reflected the presence of anti-glucose transporter antibodies, an attempt was made to affinity-purify nucleoside transporter-specific antibodies free of contaminating antibodies in the serum.

² A. Davies and S. A. Baldwin, unpublished observations.
For affinity purification, advantage was taken of the cross-reactivity of the antibodies with the pig erythrocyte nucleoside transporter (see below). Erythrocytes from adult pigs contain no glucose transport activity (22–24). In addition, we showed by Western blotting with a range of anti-peptide antibodies against the human erythrocyte glucose transporter that no immunologically cross-reactive but functionally inactive glucose transport proteins were present in pig erythrocyte membranes (results not shown). Therefore these membranes should bind nucleoside transporter antibodies specifically while being incapable of binding antibodies against the glucose transporter. Adsorption of the anti-nucleoside transporter serum with protein-depleted pig erythrocyte membranes followed by acid elution as described under “Experimental Procedures” section typically yielded ~150 μg of affinity-purified IgG/ml of serum. As shown in Fig. 4, the affinity-purified IgG recognized both pig and human erythrocyte membranes in ELISA but, in contrast to the serum (Fig. 1), did not recognize a microtiter plate coated with the C-terminal peptide of GLUT1 (residues 477–492), even at low dilutions, indicating complete removal of putative anti-glucose transporter antibodies. The fraction of the antiserum which did not become bound to the pig membranes during the purification did recognize the peptide at low dilution (Fig. 4). Identical results (not shown) were also obtained for plates coated with a peptide corresponding to residues 231–246 of the glucose transporter. The unbound fraction had completely lost the ability to recognize pig membranes in ELISA, indicating that all species cross-reactive antibodies had been adsorbed during the purification (Fig. 4). However, very substantial cross-reactivity toward human erythrocyte membranes remained, suggesting that a relatively small sub-population of the anti-human nucleoside transporter antibodies were cross-reactive with the pig protein (Fig. 4).

Characterization of the Affinity-purified Antibodies—Evidence that the affinity-purified antibodies were specific for the nucleoside transporter was provided by immunoabsorption experiments. In these, treatment of detergent-solubilized human erythrocyte membrane band 4.5 proteins with affinity-purified antibody immobilized on protein A-Sepharose CL-4B led to the removal of all of the NBMPR-binding sites from the solution, whereas these remained in solution when immobilized control IgG was employed (Table I). It follows that at least a proportion of the affinity-purified antibodies recognized the native conformation of the nucleoside transporter, because the ability to bind NBMPR is lost upon denaturation of the protein. Such a finding is to be expected from the use of native pig erythrocyte membranes as an adsorbant during the affinity purification. In contrast, none of the cytochalasin B binding activity of the band 4.5 proteins was removed by the immobilized anti-nucleoside transporter antibodies, indicating that these did not recognize the native glucose transporter.

In addition to recognizing the native nucleoside transporter, the affinity-purified antibodies also recognized a broad band of average apparent M, 55,000, characteristic of the denatured
**TABLE I**

**Immunoadsorption of detergent-solubilized human erythrocyte band 4.5 proteins**

Removal of NBMPR and cytochalasin B binding activities from a detergent-solubilized mixture of the nucleoside and glucose transport proteins is shown. Solubilized proteins were incubated with either control rabbit IgG or anti-nucleoside transporter IgG, bound to protein A-Sepharose CL-4B, as described under "Experimental Procedures." The ligand binding activities of the supernatants were then measured, after removal of detergent by dialysis.

<table>
<thead>
<tr>
<th>Immunoadsorbent</th>
<th>Ligand binding activity remaining in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBMPR</td>
</tr>
<tr>
<td>Control rabbit IgG</td>
<td>162 pmol sites/mg protein</td>
</tr>
<tr>
<td>Anti-nucleoside transporter IgG</td>
<td>0 pmol sites/mg protein</td>
</tr>
</tbody>
</table>

[^a^] B/F = [bound cytochalasin B]/[free cytochalasin B]. Under the conditions of the assay, this B/F value provides a good measure of the concentration of glucose transporters (17). The results have been normalized to a protein concentration of 1 mg/ml to facilitate comparison and correct for volume changes during reconstitution by dialysis.

nucleoside transporter, on Western blots of the purified transporter and of human erythrocyte membrane band 4.5 proteins (Fig. 5b). The broadness of the transporter band on gels is the result of its glycosylation, and it was of interest to determine whether the antibodies recognized the oligosaccharide or protein moieties of the glycoprotein. Western blotting studies were therefore performed after deglycosylation of the protein by incubation with endoglycosidase F. Silver staining showed that this procedure converted the purified nucleoside transporter and human erythrocyte membrane band 4.5 proteins primarily to species that migrated as a sharper band of apparent M, 45,000 on SDS/polyacrylamide gels (Fig. 5a). This band was stained by the affinity-purified antibodies on Western blots with an intensity similar to that of the fully glycosylated protein, indicating that most, if not all, of the antibodies were directed against the protein rather than oligosaccharide epitopes (Fig. 5b). Identical results were obtained using unfraccionated anti-nucleoside transporter serum (results not shown).

The exposure of the epitopes at the extracellular or cytoplasmic face of the membrane was investigated by measuring the ability of the antibodies to bind to intact human erythrocytes and to protein-depleted human erythrocyte membranes. The latter are known to be unsealed (11), and so both surfaces of the membrane are accessible to antibodies. Control rabbit IgG exhibited little binding either to intact cells or unsealed membranes. Antibodies raised against the C-terminal region of GLUT1 (residues 477-492), which is known to be exposed on the cytoplasmic face of the membrane (10), bound to the unsealed membranes but not to intact erythrocytes (Fig. 6). In contrast, anti-nucleoside transporter antibodies bound to approximately the same extent to either intact cells or membranes, indicating that a substantial proportion of the epitopes are located on the extracellular surface of the membrane, although the additional presence of some cytoplasmic epitopes cannot be excluded (Fig. 6). Similar results were obtained using unfraccionated serum (results not shown).

**Species Cross-reactivity of the Anti-nucleoside Transporter Antibodies**—In addition to recognizing the human erythrocyte nucleoside transporter, the anti-nucleoside transporter serum also reacted with partially purified rabbit and pig erythrocyte nucleoside transporters in ELISA, although the absorbances obtained using microtiter plates coated with these antigens were somewhat lower than for plates coated with the human protein (Fig. 7). On Western blots of the rabbit transporter (results not shown) the antiseraum weakly stained a broad band of average apparent M, 55,000, identical in its mobility to the rabbit nucleoside transporter identified by photo-affinity-labeling with NBMPR (25). No protein bands were labeled on blots of membranes prepared from cow erythrocytes (not shown), which do not exhibit detectable carrier-mediated transport of nucleosides (2). However, on blots of pig erythrocyte membrane fractions, strong labeling was obtained of a band with an apparent M, of 64,000, identical to that of the protein identified as the nucleoside transporter by photoaffinity labeling with NBMPR (6). The intensity of the staining paralleled the nucleoside transporter content of the samples, 1 μg of the partially purified pig transporter staining more intensely than 9 μg of protein-depleted pig erythrocyte membranes (Fig. 2). Additional, if indirect, evidence for cross-reactivity with the pig transporter was provided by the success of the affinity purification strategy described above, in which it was possible to isolate antibodies capable of recognizing the human transporter by adsorption onto pig erythrocyte membranes. It follows that the antibodies isolated in this fashion must be capable of recognizing the native pig erythrocyte nucleoside transporter. The affinity-purified antibodies also
recognized the denatured pig nucleoside transporter on Western blots, both before and after deglycosylation with endoglycosidase F (Fig. 5).

After deglycosylation the immunoreactive pig transporter migrated as a fairly sharp band with a mobility on SDS-polyacrylamide gels identical to that seen for the deglycosylated transporter in photoaffinity labeling experiments (6). Its apparent Mₐ of 57,000 was considerably greater than that of the deglycosylated human erythrocyte nucleoside transporter (Mₐ 45,000) (Fig. 5). However, the observation that the anti-nucleoside transporter antibodies recognized both the deglycosylated pig and human proteins indicates that they must be related in amino acid sequence, despite their difference of 12,000 in apparent Mₐ. The pig and human proteins also exhibit similarities in their sensitivity to inhibition by the vasodilators dipyridamole and dilazep (2). In contrast, the transporters of rat tissues are more resistant to these inhibitors (3, 4, 26, 27), but exhibit an apparent Mₐ similar to that of the human protein after deglycosylation (5). It was therefore of interest to examine the reactivity of the anti-human nucleoside transporter antibodies toward the nucleoside transporters of rat tissues. Rat liver was therefore examined, because it contains an abundance of nucleoside transporters, unlike rat erythrocytes (4, 14). The antibodies were found to recognize a protein that migrated as a fairly broad band of average apparent Mₐ 60,000 on Western blots of a crude membrane fraction prepared from rat liver (Fig. 8). Labeled bands of higher apparent Mₐ, probably represent oligomers of the transporter (Fig. 8). Control IgG showed no labeling. The Mₐ of the major labeled band was similar to that previously reported for nucleoside transporters from a variety of rat tissues, identified by photolabeling (3, 4, 5, 14). This finding suggests that despite its different sensitivity to inhibitors, the rat liver nucleoside transporter is related in sequence to the human and pig proteins.

**CONCLUSIONS**

In the present report we have described the production of polyclonal antibodies that specifically recognize the human erythrocyte nucleoside transporter, but not the glucose transporter. These antibodies are currently being used in our laboratories to screen cDNA libraries for clones encoding nucleoside transport proteins. We envisage that they will also be of great use in probing the distribution of nucleoside transporters in different tissues. We have already shown that the antibodies are not specific for the erythrocyte, but also recognize nucleoside transporters from other human tissues including the placenta (28). Interestingly, in the latter tissue the syncytiotrophoblast brush-border nucleoside transporter is recognized by the antibodies, but the kinetically similar basal membrane transporter is not, indicating the existence of two distinct NBMPR-sensitive transporters in this tissue (28).

In addition to recognizing NBMPR-sensitive nucleoside transporters from various human tissues, the antibodies were also found to recognize the nucleoside transporters of rabbit and pig erythrocytes, and of rat liver. As discussed in the
Introduction, these nucleoside transporters differ considerably from one another in size and inhibitor sensitivity. Their recognition by the anti-human erythrocyte nucleoside transporter antibodies indicates that despite such differences these transporters share common structural features, and so are likely to employ similar mechanisms for nucleoside translocation. Additional evidence for this conclusion, based upon enzymic cleavage studies, is presented in a separate report.3

REFERENCES
