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Vesicle-to-Cell Protein Transfer: Insertion of Band 3, the Erythrocyte Anion Transporter, into Lymphoid Cells†

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ABSTRACT: Band 3, the erythrocyte anion transporter, transfers spontaneously between human red cells and model membranes. During incubation of intact erythrocytes with sonicated dimyristoylphosphatidylcholine vesicles, the transporter inserts in functional form and native orientation into the liposome bilayer, with the cytoplasmic segment of the protein contacting the lumen of the vesicle [Newton, A. C., Cook, S. L., & Huestis, W. H. (1983) *Biochemistry 22*, 6110–6117; Huestis, W. H., & Newton, A. C. (1986) *J. Biol. Chem. 261*, 16274–16278]. When band 3–vesicle complexes are incubated with erythrocytes whose native band 3 has been inhibited irreversibly, reverse transfer of the protein restores anion transport capacity to the cells [Newton, A. C., Cook, S. L., & Huestis, W. H. (1983) *Biochemistry 22*, 6110–6117]. Here we report the vesicle-mediated transfer of band 3 to human peripheral blood lymphocytes and to cultured murine lymphoma cells (BL/VL3). Subsequent to incubation with protein–vesicle complexes, both lymphoid cell types exhibit a 2–4-fold increase in the rate of chloride uptake. This enhanced permeability is inhibited ≥98% by the exofacial band 3 inhibitor 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid, consistent with right-side-out insertion of functional band 3 into the lymphoid cell membrane.

Spontaneous transfer of membrane proteins between cells and liposomes (Newton et al., 1983; Huestis & Newton, 1986) offers the prospect of altering the transport or antigenic properties of cells. Liposome-mediated insertion of proteins into intact cell membranes has been accomplished with fusogenic agents such as poly(ethylene glycol) (Eriksson et al., 1985; Baumann et al., 1980), viral envelope proteins (Poste et al., 1980; Volsky et al., 1979), and fusogenic lipids (Correa-Frêire et al., 1984). Insertion of foreign protein also has been achieved by fusing biological membranes with cells (Balakrishnan et al., 1983).

An alternative approach to such membrane modification is the exchange of intrinsic proteins between cells and donor membranes that retain their topological integrity (i.e., do not fuse). Das and co-workers have reported the spontaneous transfer of functional epidermal growth factor receptor from

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hepatic microsomal membranes to a cell line lacking the receptor, under conditions where fusion is unlikely (Bishayee et al., 1982). Cell-to-liposome protein transfer is observed when sonicated vesicles composed of the relatively hydrophilic lipid dimyristoylphosphatidylcholine (DMPC)[1] are incubated with human erythrocytes (Newton et al., 1983; Huestis & Newton, 1986) or cultured murine lymphoma (Newton & Huestis, 1988). Protein–vesicle complexes produced from such interactions are distinct from cell membrane fragments, differing in density, lipid and protein composition, and lumen content. Functional and proteolytic analyses indicate that the proteins in these complexes associate with the vesicle membrane in native orientation. One such transferrable species is the erythrocyte anion transporter, band 3, a transmembrane protein possessing several convenient functional and orientational markers. Vesicle-associated band 3 mediates anion uptake that is inhibited quantitatively by 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) (Newton et al., 1983; Newton & Huestis, 1986) and exhibits proteolytic sensitivities identical with those found in the native cell membrane (Huestis & Newton, 1986). These properties indicate that the transferred protein is inserted into the vesicle bilayer, with the cytoplasmic segment contacting the vesicle lumen.

Spontaneous intermembrane protein transfer from DMPC vesicles to cells, in reverse of the process described above, offers a noninvasive means for insertion of integral membrane proteins into cell plasma membranes. Such insertion was demonstrated at a low but detectable level in erythrocytes whose native band 3 had been inhibited irreversibly (Newton et al., 1983). After incubation with band 3–vesicle complexes, the cells exhibited enhanced, DIDS-sensitive chloride uptake consistent with introduction of a few (one or two) copies of functional band 3 into half of the cells. Chloride uptake into inhibited cells incubated with inhibited band 3–vesicle complexes was indistinguishable from uptake by control cells incubated in buffer.

These observations suggest that intermembrane protein transfer is bidirectional and can, under some circumstances, result in selective modification of the transport capacity of intact cells. Protein insertion by this mechanism avoids the use of toxic fusogens [e.g., poly(ethylene glycol)] and the coinsertion of viral proteins, factors that may adversely affect cell viability.

The experiments described in this paper investigate the generality of vesicle-to-cell protein transfer, using DMPC vesicle vectors to insert erythrocyte band 3 into membranes of viable human lymphocytes and murine lymphoma.

Experimental Procedures

Materials

Dimyristoylphosphatidylcholine (DMPC), Histopaque, Trypan blue, and Wright stain were obtained from Sigma Chemical Co. DIDS and IODO-GEN were supplied by Pierce Chemical Co. $Na^{36}Cl$ was from New England Nuclear, $Na^{125}I$ from ICN, and [^{14}C]dipalmitoylphosphatidylcholine ([^{14}C]-DPPC) from Amersham Corp. RPMI 1640 and fetal calf serum were from Gibco, eosinylmaleimide (eosin-MA) was from Molecular Probes, and Sephadex G-25 was from Pharmacia. All other chemicals were of reagent grade. Unless otherwise specified, experiments were conducted in phosphate-buffered saline containing 138 mM NaCl, 6.1 mM Na_2HPO_4, 1.4 mM NaH_2PO_4, 5 mM KCl, 1 mM $MgSO_4$, and 5 mM glucose, pH 7.4 ($NaCl/P_i$ buffer).

Methods

Cells. Human erythrocytes were obtained as described (Bouma et al., 1977) and used within 48 h of collection.

Human peripheral blood lymphocytes were isolated from the buffy coat of 1 unit of freshly drawn blood by centrifugation over Histopaque (Bøyum, 1976). Buffy coat (4 volumes of a 1:1 dilution in $NaCl/P_i$) was underlayered with 1 volume of Histopaque and centrifuged at 300*g* for 30 min, at room temperature. The lymphocytes were pipetted from the Histopaque/plasma interface and diluted in 4 volumes of $NaCl/P_i$ to a final volume of approximately 20 mL. Contaminating erythrocytes were lysed by suspending lymphocytes in at least 3 volumes of 0.87% ammonium chloride solution for 5 min. Lymphocytes were pelleted by centrifugation at 250*g* for 10 min and washed in $NaCl/P_i$ until the supernatant was clear of platelets. Cell viability, as measured by Trypan blue exclusion, was typically >90%. Differential cell counting with Wright stain revealed that lymphocytes accounted for >90% of the leukocytes.

The established T-cell line BL/VL3 was maintained at 37 °C in RPMI 1640 supplemented with 15% fetal calf serum and antibiotics (Lieberman et al., 1979). Cells were harvested by centrifugation (700*g* for 10 min, room temperature) and washed once in $NaCl/P_i$.

Vesicles. Small unilamellar vesicles were prepared by sonication of 24 mM DMPC in $NaCl/P_i$, as described (Bouma et al., 1977). In some experiments, [^{14}C]DPPC (approximately 0.2 μCi mL^{-1}; 112 mCi mmol^{-1}) was included in the sonication medium as a vesicle marker (Huestis & Newton, 1986).

Cell-to-Vesicle Band 3 Transfer. Protein–vesicle complexes were prepared by incubating erythrocytes (typically 4 mL) with sonicated DMPC vesicles (typically 3 mL of 24 mM DMPC) for 1 h at 37 °C. Cells were removed by centrifugation (3000*g*, 5 min), and band 3–vesicle complexes were isolated from membrane fragments and unaltered sonicated vesicles by density gradient centrifugation, as described (Huestis & Newton, 1986). Resuspended in buffer (generally 2 mL), the isolated protein–vesicle fraction contained 2–6 mM DMPC. Radioiodination of band 3–vesicle complexes was achieved by IODO-GEN catalysis (Markwell & Fox, 1978). Protein–vesicle complexes obtained by incubation of 6 mL of vesicles (24 mM DMPC) with 10 mL of packed erythrocytes were isolated and resuspended to 2 mL in buffer. The suspension was transferred to an IODO-GEN-coated vial (150 μg), and 23 μCi of $Na^{125}I$ was added. The vial was incubated at room temperature for 10 min with occasional swirling. Unreacted iodide was removed by passing the vesicles through two successive Sephadex G-25 columns (9 mL of resin/column). Vesicle-bound band 3 was inhibited irreversibly by reaction with eosin-MA, a monovalent inhibitor that, unlike DIDS, does not cross-link and aggregate the protein–vesicle complexes. Protein–vesicle complexes (typically 1.5 mL; 5 mM DMPC) were incubated with 0.7 mM eosin-MA in $NaCl/P_i$ buffer at 22 °C for 1 h in the dark (Nigg & Cherry, 1979). Unreacted eosin-MA was removed by passing the sample through a Sephadex G-25 column (9 mL of resin).

Vesicle-to-Cell Band 3 Transfer. Human peripheral blood lymphocytes (200 μL) were incubated at 37 °C for 1 h with (a) 3 mL of band 3–vesicle complexes (1 mM DMPC) or (b) 3 mL of $NaCl/P_i$. Cells were centrifuged at 250*g* for 10 min, and the cell pellets were resuspended in 6 mL of buffer and divided into a 4-mL and a 2-mL aliquot. The two samples

[1] Abbreviations: DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; eosin-MA, eosinylmaleimide; Tris, tris(hydroxymethyl)aminomethane.

were centrifuged and the cell pellets resuspended in 3 mL of $NaCl/P_i$ (larger sample) or 1.5 mL of 0.2 mM DIDS in $NaCl/P_i$.

Cultured murine lymphoma were incubated at cytocrit 5–11, final volume of 3 mL, with (a) band 3–vesicle complexes, (b) eosin-MA-inhibited band 3–vesicle complexes, or (c) $NaCl/P_i$ buffer. DMPC concentrations in samples a and b were 2–5 mM in various experiments. After 1 h of incubation at 37 °C, cells were pelleted (700*g*, 10 min), washed 2 times in 50 volumes of $NaCl/P_i$, and resuspended to 6 mL in buffer (cytocrit 2–6). As evidenced by Trypan blue exclusion, the viability of the lymphocytes was not affected detectably by this treatment.

Anion Permeability Measurements. Chloride-flux measurement was initiated by addition of 2 μCi of $Na^{36}Cl$ to a rapidly stirring cell suspension (cytocrit 2–6, in $NaCl/P_i$ ± 0.2 mM DIDS) maintained at 22 °C. At times specified in the legend to Figure 1, aliquots (500 μL for human lymphocytes; 250 μL for murine lymphoma) were transferred into 1 mL of $NaCl/P_i$ containing 0.2 mM DIDS, on ice. Cells were pelleted (8800*g* for 2 min) and washed in 1 mL of 0.2 mM DIDS (once for human lymphocytes; twice for murine lymphoma). Cell pellets were resuspended in water (0.5 mL for human cells; 0.2 mL for murine cells). Aquasol (10 mL) was added to the entire sample, which then was analyzed by liquid scintillation counting.

Transfer of Radioiodinated Band 3. Radioiodinated protein–vesicle complexes (5 mL containing on the order of 5×10^{14} band 3 molecules in approximately 0.5 mM DMPC) were incubated with human lymphocytes at a cytocrit of 4, for 1 h at 37 °C. Lymphocytes were washed and stored at 4 °C overnight in 50 mL of $NaCl/P_i$, and the plasma membrane was isolated by differential centrifugation, as follows (Snary et al., 1976). Lymphocytes were suspended in 15 mL of 1 mM $NaHCO_3$ plus 0.5 mM $CaCl_2$ and disrupted with 25 strokes in a Dounce homogenizer. Lysate was centrifuged at 500*g* for 20 min to pellet nuclei and mitochondria. Further centrifugation of the supernatant (12800*g* for 20 min) yielded a microsomal pellet, which was then suspended in bicarbonate lysis medium containing 40% (w/v) sucrose. This suspension was layered under a solution of 30% (w/v) sucrose in the same buffer and centrifuged at 54450*g* for 4 h. The interfacial band (plasma membrane) was removed, diluted with 40 mM Tris plus 110 mM KCl, pH 7.5, and pelleted by centrifugation at 45000*g* for 1 h at 4 °C. The washed plasma membrane was analyzed by gel electrophoresis (Ames, 1974) and by autoradiography using Kodak X-Omat film and intensifying screens (Du Pont).

Results

Sonicated DMPC vesicles were incubated with human erythrocytes and the resultant protein–vesicle complexes separated from membrane fragments by sucrose density gradient centrifugation of the cell-free supernatant (Huestis & Newton, 1986). Vesicle-to-cell protein transfer was effected by incubation of band 3–vesicle complexes with lymphoid cells for 1 h at 37 °C, and cells were isolated by centrifugation and washed. Control cells were incubated in buffer and isolated.

Chloride transport into the isolated lymphocytes was measured at 22 °C. Normal human peripheral blood lymphocytes exhibit a fast and slow component of chloride uptake (Negendank, 1984). In the experiment outlined in Figure 1A (open circles), the fast component had an apparent rate coefficient of 0.2 min^{-1} and accounted for approximately 4% of the total chloride taken up at equilibrium. The major, slower component of uptake exhibited an apparent rate coefficient of 0.002

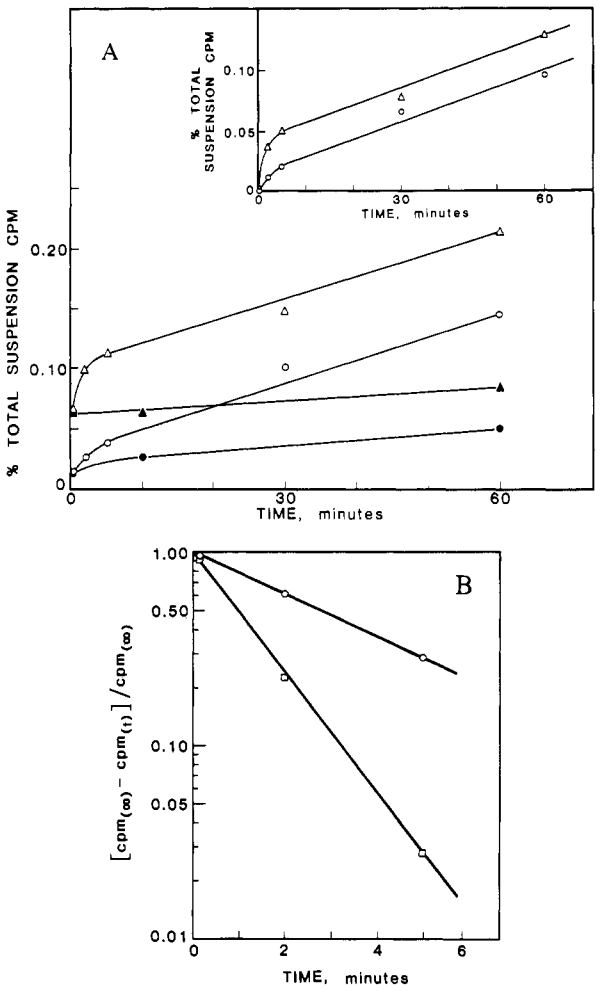

FIGURE 1: (A) Chloride uptake at 22 °C into human peripheral blood lymphocytes after incubation with band 3–vesicle complexes: lymphocytes incubated with buffer (○); lympocytes incubated with band 3–vesicle complexes (△). Filled symbols show chloride uptake in the same cell samples in the presence of 0.2 mM DIDS. Inset: DIDS-sensitive uptake of control lymphocytes (○) and band 3 treated cells (△), obtained by subtracting the DIDS-insensitive components. (B) Accelerated chloride uptake into lymphocytes treated with band 3–vesicle complexes. Data from panel A are fit to the function $cpm_t = cpm_0 + cpm_\infty(1 - e^{-kt})$, where the parameters cpm_0, cpm_∞, and k are obtained from least-squares analysis. DIDS-insensitive background (closed symbols in panel A) has been subtracted from all data; cpm_0 is zero. Fast component of chloride exchange in control lymphocytes (○); band 3 mediated component in vesicle-treated lymphocytes (□), obtained by subtraction of the control fast component (panel A inset, △ − ○).

min^{-1}. [The fast component does not arise from contaminating platelets, whose rate of chloride uptake is 2 orders of magnitude lower than the fast component (not shown) and whose chloride space is too small to account for the chloride equilibrated at the fast rate.] Total chloride uptake into control human lymphocytes was inhibited approximately 80% by DIDS (Figure 1A, filled circles).

Human lymphocytes that had been incubated with band 3–vesicle complexes showed increases in both the rate and extent of the fast flux component. These increases are evident in the rapid DIDS-sensitive component of chloride uptake, shown in the inset to Figure 1A. The amount of chloride equilibrated at the fast rate increased to account for an additional 3.6% of the chloride entrapped in the cells at equilibrium, and the rate of the fast component increased 4-fold relative to buffer-treated cells. Subtracting the fast uptake exhibited by control lymphocytes from the fast uptake of band 3 treated cells, a rate coefficient of 0.8 min^{-1} is obtained

Table I: Analysis of Chloride Uptake in Cultured Murine Lymphoma

lymphoma	rate of chloride uptake (min^{-1}) −DIDS[a]	+DIDS
+functional band 3–vesicle complexes	0.4 ± 0.1	0.009
+eosin-MA-inhibited band 3–vesicle complexes	0.2[b]	0.010
+buffer	0.24 ± 0.07	0.005

[a] Values represent average, with standard deviation, of three experiments. [b] Average of two values.

(Figure 1B, squares). This contrasts with a value of 0.2 min^{-1} for control cells (Figure 1B, circles). The slow component of uptake was unaffected by treatment with band 3–vesicle complexes. [Figure 1A indicates that more ^{36}Cl was associated with vesicle-treated cells than buffer-treated cells at $t = 0$; this may reflect a change in the viability of a small population of cells during the vesicle treatment. Incubation with eosin-MA-inhibited band 3–vesicle complexes also increased the chloride associated with cells at $t = 0$ but did not affect the rate of anion transport compared to buffer-treated cells (not shown).]

In similar experiments, cultured BL/VL3 murine lymphoma were incubated with protein–vesicle complexes containing functional or eosin-MA-inhibited erythrocyte band 3. In contrast to human lymphocytes, chloride uptake into the murine lymphoma cells displayed single-component kinetics, with an apparent rate coefficient of 0.2 min^{-1} at 22 °C. Treatment of the cells with functional band 3–vesicle complexes increased the rate of this uptake by 70%, from $k = 0.24 \pm 0.07$ min^{-1} to $k = 0.4 \pm 0.1$ min^{-1} (Table I). In lymphoma incubated with vesicles containing inhibited band 3, the chloride-flux rate was indistinguishable from buffer-treated controls. Chloride flux in both control and band 3–vesicle-treated samples was inhibited ≥98% by DIDS (Table I).

Incorporation of band 3 into the plasma membrane of human lymphocytes was investigated further by examining the isolated plasma membrane of cells treated with radioiodinated protein–vesicle complexes. Superficial radioiodination of band 3–vesicle complexes labeled band 3, a protein migrating with an apparent molecular mass of 26 kDa, and, to a lesser extent, proteins comigrating with glycophorin A (88 kDa), glycophorin B (41 kDa), and a broad band migrating between 50 and 60 kDa (Figure 2, lane 1).

Plasma membrane was isolated from human peripheral blood lymphocytes after incubation with radioiodinated protein–vesicle complexes. Three bands were apparent by autoradiographic analysis of polyacrylamide gels: one migrating with an average molecular mass slightly lower than that of vesicle band 3, a second with an apparent molecular mass of 28 kDa, and a broad band of molecular mass 50–60 kDa (Figure 2, lane 2).

Discussion

Both human and murine lymphoid cells exhibit increased anion permeability after incubation with functional band 3–vesicle complexes. The enhanced uptake is inhibited quantitatively by the specific band 3 inhibitor DIDS. Because DIDS inhibits at the external, but not cytofacial, surface of the transporter, this suggests that the increased anion permeability is mediated by band 3 inserted in native orientation into the cell membrane.

The increased anion permeability of vesicle-treated lymphocytes is not likely to arise from band 3–vesicle complexes adsorbed to the cell surface. Recovery of [^{14}C]DPPC-labeled vesicles indicates that more than 95% of the band 3–vesicle complexes are removed from human lymphocytes in the postincubation centrifugation and washing. The chloride space in any remaining vesicles (0.002% of suspension volume) is an order of magnitude lower than the increased uptake (0.04% of suspension chloride). Additionally, chloride uptake in band 3–vesicle complexes is extremely rapid ($t_{1/2}$ <10 s at 0 °C), too fast to account for the rate increase observed in lymphocytes at 22 °C (Newton et al., 1983; Newton & Huestis, 1986).

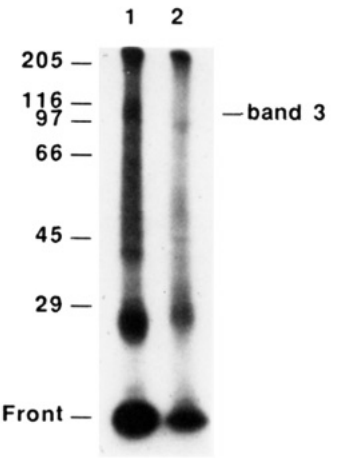

FIGURE 2: Autoradiogram of polyacrylamide gel (10%) of human peripheral blood lymphocyte plasma membrane isolated from cells treated with radioiodinated band 3–vesicle complexes: radioiodinated band 3–vesicle complexes (lane 1); lymphocyte plasma membrane isolated from cells treated with band 3–vesicle complexes (lane 2).

The increased anion permeability of lymphocytes after treatment with protein–vesicle complexes could reflect nonspecific vesicle-induced perturbations of membrane permeability. However, incubation of murine lymphoma with inhibited band 3–vesicle complexes does not alter their rate of chloride uptake. Moreover, increased uptake arising from a nonspecific permeability defect would not be subject to inhibition by the specific band 3 inhibitor DIDS. Thus, the increased lymphocyte anion uptake conferred by incubation with band 3–vesicle complexes is consistent with right-side-out insertion of functional band 3 into the plasma membrane of the cells.

Plasma membrane isolated from lymphocytes incubated with radioiodinated protein–vesicle complexes contains three major radiolabeled proteins. It is unlikely that these proteins derive from contaminating protein–vesicle complexes. The isolation procedure employed would not be expected to copurify protein–vesicle complexes with plasma membrane: the former bands at a density corresponding to 15% sucrose (Huestis & Newton, 1986), while the latter is recovered from the interface of 30% and 40% sucrose. Additionally, proteins in the lymphocyte plasma membrane fraction do not comigrate precisely with the vesicle-bound species on polyacrylamide gels and thus are not representative of protein–vesicle complexes (Figure 2).

Although unlikely in the absence of catalyst, the iodinated bands in the lymphocyte plasma membrane could represent native proteins labeled by trace amounts of free iodide. However, superficial radioiodination of human peripheral lymphocytes results primarily in the labeling of a 70-kDa protein; no iodinated bands in the 28- and 90-kDa range are visible by autoradiography (data not shown).

It is more likely that the iodinated bands in the lymphocyte plasma membrane are proteins transferred from the protein–vesicle complexes. The band migrating with a molecular

weight slightly less than that of vesicle band 3 may be glycophorin or may comprise a population of anion transporter more susceptible to reverse transfer because of lower levels of glycosylation. Alternatively, the shift in apparent molecular weight may reflect lymphocyte-mediated modifications (for instance proteolysis) of foreign band 3. Similarly, the band with an apparent molecular mass 1 or 2 kDa greater than that of the 26-kDa vesicle protein may comprise a population of this protein more amenable to reverse transfer.

A broad radioiodinated band comigratory with the erythrocyte glucose transporter is visible in the 50–60-kDa range on gels of both protein–vesicle complexes and target lymphocytes. In contrast, vesicles incubated with superficially radioiodinated erythrocytes contain gel bands migrating in this molecular weight range that are detectable by protein stain but contain little radiolabel (Huestis & Newton, 1986). Thus, if the transferred protein is the glucose transporter, its disposition in the vesicle bilayer is such that radioiodination sites normally less accessible in the erythrocyte membrane are exposed. This protein appears to insert into the lymphocyte plasma membrane at an efficiency comparable to band 3 transfer (Figure 2).

Vesicle-to-cell protein transfer is several orders of magnitude less efficient than the reverse cell-to-vesicle protein transfer. Approximately 1% of erythrocyte band 3 molecules transfer to recipient DMPC vesicles (at a ratio on the order of 10^5 vesicles per cell), while only 1 or 2 molecules per cell, representing approximately 0.001% of the vesicle-bound band 3, are reinserted into the erythrocyte membrane (Newton et al., 1983). Insertion of band 3 into human peripheral lymphocytes is even less efficient. Under the conditions employed here, vesicle treatment increases the cell space equilibrating with chloride at the fast rate from 4% to 8% of the total chloride space. Assuming a random distribution of inserted band 3 molecules (that is, molecules are delivered one at a time to a random sample of cells), this increase is consistent with delivery of 1 or 2 anion transporters to 4% to 8% of the cells.[2] Vesicle-to-cell transfer may be energetically less favorable as a result of electrostatic or steric barriers present in cell membranes that the vesicle-bound protein must overcome during insertion. Indeed, analysis of the plasma membrane of human peripheral blood lymphocytes incubated with radioiodinated band 3–vesicle complexes suggests that the population of band 3 that transfers to the cells may be less heavily glycosylated than the bulk vesicle transporter.

The insertion of functional proteins into the plasma membrane of cells offers a unique opportunity for investigating the role of specific proteins in cellular functions. Incorporation of transmembrane proteins is of particular interest because of their roles in transmembrane signal transduction, cellular recognition, and membrane permeability. Although the efficiency of vesicle-to-cell protein transfer is low, insertion of very few proteins is required to change the transport or antigenic properties of cells. With appropriate manipulations of vesicle composition (for instance, alteration of vesicle surface charge to enhance cell–vesicle contact), or through minor modification of vesicle proteins (such as removal of bulky carbohydrate residues), it may be possible to employ vesicle-to-cell protein transfer as a specific, noninvasive tool for altering the surface properties of cells.

[2] If the inserted band 3 is distributed randomly in the lymphocyte population such that reconstituted cells have one or two inserted transporters, it mediates chloride transport at an anomalously high rate. The turnover rate of individual band 3 molecules in the erythrocyte membrane (on the order of 10^{-7} min^{-1} per band 3/μm^3) is much lower than the rate observed for band 3 reconstituted into lymphocytes (0.8 min^{-1} per band 3/cell, corresponding to on the order of 10^{-3} min^{-1} per band 3/μm^3). Perhaps the transporter compensates for limiting concentrations of band 3 by accelerating the turnover rate per transporter. Indeed, Volsky and co-workers (1979) observed that when 10^5 band 3 molecules were reconstituted into Friend erythroleukemic cells (by viral protein mediated fusion), anion exchange was catalyzed at a rate 2–3-fold faster per band 3 molecule than for erythrocyte-bound band 3. Thus, the low transporter number and the nonnative environment may have affected the turnover rate of lymphocyte-incorporated band 3.

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