for the three V_α products that can be identified with the available antibodies, but it is likely to reflect a general property of αβ T cells (*16*). Thus, our findings, together with the recent report that two TCRγ alleles can be expressed on the same γδ T cell (*17*), show that the "one cell, one receptor" rule is not valid for a portion of both αβ and γδ T cells.

What are the implications of the dual αβ TCR subset? First, it may be more frequently involved in alloreactivity because (i) it has two TCRs rather than one (although each at a lower concentration) and (ii) the non–positively selected receptor may be more prone to see peptides bound to allogeneic MHC molecules (although admittedly self restricted antigen recognition and alloreactivity can be mediated by the same receptor) (*4*, *18*). Second, although the presence of two TCRs on T cells is compatible with central (thymic) tolerance, it could pose a problem for peripheral tolerance when this is based on the inability of tissue cells to stimulate naïve T cells (*19*). Thus, once a dual receptor T cell is activated by a foreign antigen recognized by the first TCR, it may become competent to utilize the second (non–positively selected) TCR to attack the self antigen. This type of cross-reactivity would not be based on structural similarities between foreign and self antigens, but rather on the particular somatic combination of two distinct TCRs.

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10. The mouse immunoglobulin G2a (IgG2a) antibody to human $V_\alpha2$ (clone V2a) was purchased from T Cell Science (Cambridge, MA). The mouse IgG1 antibody to human $V_\alpha12$ (clone 6D6) was provided by M. Brenner (*20*). We produced a mouse IgG1 antibody to human $V_\alpha24$ (*21*). Cells were stained with either antibodies to $V_\alpha2$ plus $V_\alpha24$ or antibodies to $V_\alpha2$ plus $V_\alpha12$, followed by phycoerythrin-conjugated goat antibody to mouse IgG1 plus fluorescein isothiocyanate (FITC)–conjugated goat antibody to mouse IgG2a. In some experiments, T cells that expressed a given V_α were first enriched by sorting, expanded with phytohemagglutinin (PHA), interleukin-2 (IL-2), and irradiated feeder cells, and reanalyzed. Cells stained with two antibodies were sorted on a FACStar Plus (Becton Dickinson) and cloned as described (*22*) by limiting dilution with PHA, IL-2, and irradiated allogeneic PBMCs.
11. RNA extraction and complementary DNA (cDNA) synthesis were performed as described (*23*). We amplified cDNA in 30 μl using 80 ng of one of the TCR V_α-specific primers ($V_\alpha1$, 5′-GGCATTAAGGGTTTTGAGGCTGGA-3′; $V_\alpha2$, 5′-CAGTGTTCCAGAGGGAGCCATTGT-3′; $V_\alpha12$, 5′-TCGTCGGAACTCTTTTGATGAGCA-3′; or $V_\alpha24$ 5′-CTGGATGCAGACACAAAGCAGAGC-3′) [C. Geneveè *et al.*, *Eur. J. Immunol.* **22**, 1261 (1992)] plus 80 ng of the TCR C_α primer (5′-TGCTCTTGAATTCCATAGACCTCATGTC-3′) with the following profile: 15 s at 94°C, 20 s at 60°C, and 20 s at 72°C for 30 cycles in a Perkin-Elmer 480 thermal cycler. The PCR products were fractionated on a 2% agarose gel and isolated with low melting point agarose. The single PCR fragments were directly sequenced with a U.S. Biochemicals Sequenase kit as described [J.-L. Casanova *et al.*, *Nucleic Acids Res.* **18**, 4028 (1990)] with 0.25 pmol of template and 10 pmol of the TCR C_α oligonucleotide 5′-CAGACAGACTTGTCACT-3′.
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14. This ratio can be measured by two-color staining with antibodies to V_α and to CD3. All $V_\alpha24^+$ clones tested show a homogeneous staining with a clear correlation between the level of $V_\alpha24$ and CD3 and a constant ratio. Within the same experiment, this ratio has a fixed high value for the majority of the clones and a lower value for those that are known to express a second V_α chain.
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16. The possibility has to be considered that there could be a mechanism that favored $V_\alpha2$, $V_\alpha12$, and $V_\alpha24$ for dual expression. However, this is unlikely for two reasons. First, rearrangements of both V_α loci occur simultaneously and independently of thymic selection (*12*); rearrangement is therefore expected to be random. Thus, it is unlikely that, upon positive selection of one of the two V_α chains, a bias is introduced for the other unselected V_α chain. Second, the results shown in Fig. 3 indicate that $V_\alpha2$ and $V_\alpha24$ are not overrepresented among co-expressed V_α chains.
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21. A monoclonal antibody (C15, IgG1) was produced from mice immunized with a T cell clone that expressed a $V_\alpha24,J_\alpha Q,V_\beta11$ TCR (*23*). T cells stained by C15 were sorted from peripheral blood and cloned, and the structure of their TCRs was determined by sequencing. All the clones recognized by the C15 antibody express $V_\alpha24$, which can be associated with different J_α segments as well as with different V_β chains. Antibody titration experiments indicate that the affinity of C15 is not influenced by J_α or V_β.
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25. We thank G. De Libero, K. Hannestad, K. Karjalainen, C. Steinberg, and H. von Boehmer for critical reading and comments. M. Brenner kindly provided the human 6D6 antibody. This work is in partial fulfillment of the doctorate in molecular and cellular biology and pathology, University of Padova (E.P.). The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche, Basel, Switzerland.

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Altered Fluid Transport Across Airway Epithelium in Cystic Fibrosis

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In cystic fibrosis (CF), absence or dysfunction of a phosphorylation-regulated chloride channel [CF transmembrane conductance regulator (CFTR)] leads to the loss or reduction of chloride secretion into the airways. Active sodium absorption is also increased in CF, and both of these ion transport changes could alter fluid transport across the airways. Under baseline conditions, cultured human airway epithelia from normal individuals absorbed fluid, and this absorption was increased in epithelia from patients with CF. In normal and CF epithelial cultures fluid absorption was inhibited by amiloride. Adenosine 3′,5′-monophosphate stimulated fluid secretion in normal epithelial cultures but not in cultures from individuals with CF. In contrast, fluid secretion induced by nucleotide triphosphates (uridine triphosphate or adenosine triphosphate) was unaltered in cultures of epithelia from patients with CF, suggesting an approach to the treatment of CF.

The surface of the airway epithelium is covered by a 5-μm layer of fluid (sol) and a blanket of mucus (gel) that are produced by various surface and submucosal gland cells. Cilia on the cells' apical membranes beat within the sol and contact the underside of the mucus blanket, thereby clearing it together with entrapped particulate matter from the lungs. It is believed that in CF this mucociliary clearance is impaired because alterations in CFTR lead to Cl^- impermeability of the luminal membrane, thereby reducing fluid secretion and dehydrating the sol and gel layers (*1*, *2*). In human airways, Na^+ absorption is increased in patients with CF (*3*–*5*), and this may also dehydrate the airway surface and contribute to the pathology.

To directly test whether airway fluid transport is altered in CF, we have made continuous measurements of active ion–linked fluid movement across cultured human airway epithelia. We used primary cultures of nasal (HNE) and tracheal (HTE) epithelia, which have ion transport properties that closely resemble those of

native tissue (5). Fluid transport (J_v) measurements with a signal-to-noise ratio (under baseline conditions) of ≥10/1 were obtained with a double-sided capacitance probe technique that has an accuracy of ±1 nl min^{-1} (6–8). Other investigators have measured J_v in animal airways with various techniques (9–12) that were insensitive to small rates of fluid transport and subject to baseline drift, making the measurements difficult to interpret. Measurements of J_v across normal cultured HNE have been made with a technique different from ours with different quantitative results (13).

We measured J_v and bioelectrical responses from normal HNE cells that were bathed in HCO_3^--buffered physiological saline (8) and then treated sequentially with amiloride and adenosine 3′,5′-monophosphate (cAMP) analogs or nucleotide triphosphates [adenosine triphosphate (ATP) or uridine triphosphate (UTP)] (Fig. 1). During the control period, ~1.6 μl cm^{-2} $hour^{-1}$ (12.5 nl min^{-1}) of fluid was absorbed across the cells, from the luminal to basal side. In vivo, in the absence of other forces affecting fluid movement, this rate of absorption would completely remove the 5-μm sol layer of fluid in the respiratory tract within 20 min. Changing to fresh Ringer solution, which required removing the probes, did not appreciably alter J_v, transepithelial potential (*TEP*), or resistance (R_t).

Luminal application of amiloride, which inhibits active Na^+ absorption by blocking Na^+ conductance in the apical membrane (14), decreased *TEP* and increased R_t (Fig. 1A). In most monolayers (81%) amiloride reduced fluid absorption. In the remaining cases, it transiently induced small rates of fluid secretion that recovered to steady-state amounts similar to the other monolayers treated with amiloride. In the presence of amiloride, ATP, or 8-(4-chlorophenylthio)–cAMP (CPT-cAMP) and 3-isobutyl-1-methylxanthine (IBMX) increased *TEP* and reduced R_t [Fig. 1, A and B, (15)] as expected from the activation of the cAMP- or Ca^{2+}-regulated Cl^- channels that are located in the apical membrane of these cells (16–19). Both secretagogues caused ~1.0 μl cm^{-2} $hour^{-1}$ of fluid secretion. In vivo, this secretory rate could double the depth of the periciliary sol every 30 min. In the absence of amiloride, the effect of cAMP on J_v was variable, but UTP decreased fluid absorption (20).

We also measured fluid transport in CF nasal epithelia that were homozygous or heterozygous for ΔF508 (21). Amiloride reduced J_v to about zero and ATP caused a large transient secretion, but CPT-cAMP and IBMX had no effect on J_v (Fig. 2A). In five monolayers from three CF patients, all with the ΔF508 mutation, cAMP had no effect on J_v, *TEP*, or R_t (22). In a series of similar experiments, amiloride reduced J_v by ~75%, from 4.0 to 1.0 μl cm^{-2} $hour^{-1}$, and the subsequent addition of UTP to the luminal side of the cells caused a large transient secretion (~3 μl cm^{-2} $hour^{-1}$), which declined after ~30 min to

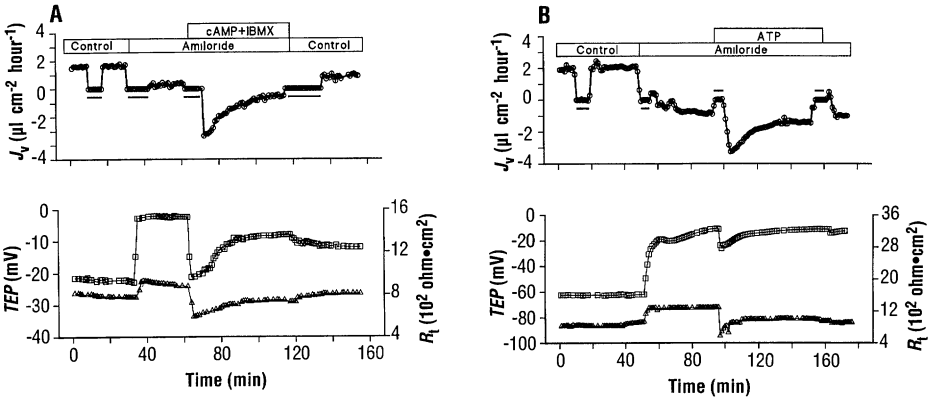

Fig. 1. Effects of amiloride, CPT-cAMP and IBMX, and ATP on J_v (upper traces), *TEP*, and R_t (lower traces) across HNE cultures from normal individuals. (**A**) In the control period, J_v (positive) was absorbed across the monolayer, from the luminal to serosal side, at 1.6 μl cm^{-2} $hour^{-1}$. During the control period the capacitance probes were elevated away from the fluid surface (solid bars). Both sides of the tissue were then perfused with fresh control medium to verify that the solution changes did not induce changes in J_v, *TEP* (squares), or R_t (triangles). Absorption of Na^+ was blocked by the addition of amiloride (100 μM) to the luminal bath; *TEP* was reduced to −3 mV, R_t rose by ~150 ohm · cm^2, and J_v leveled off near zero (t = 50 min) (15). At 65 min, a mixture of CPT-cAMP and IBMX (each 100 μM) was added to the luminal bath in the continued presence of amiloride. This caused a transient secretion of J_v and the expected decrease in R_t and increase in *TEP* (1, 2). After 2 hours, the apical bath was perfused with control medium; J_v increased to 1 μl cm^{-2} $hour^{-1}$ and *TEP* and R_t also returned toward their control values. (**B**) In an epithelial culture from another individual, amiloride again reduced J_v to zero. ATP (100 μM) added to the apical bath produced a steady-state secretion of about −2.2 μl cm^{-2} $hour^{-1}$ and the expected electrical responses (19).

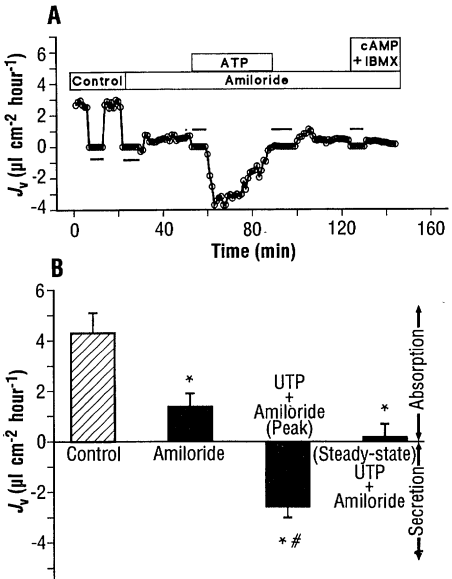

Fig. 2. Effects of amiloride, ATP (or UTP), and the combination of CPT-cAMP and IBMX on J_v in monolayers of cultured CF nasal epithelia. (**A**) The starting J_v was −3 μl cm^{-2} $hour^{-1}$ and *TEP* and R_t were about 10 mV and 1000 ohm · cm^2, respectively. Addition of amiloride (100 μM) to the apical bath reduced J_v to 0.5 μl cm^{-2} $hour^{-1}$; *TEP* and R_t changes were almost identical to those shown in Fig. 1A. Luminal addition of ATP (100 μM) at t = 55 min caused secretion at −3 μl cm^{-2} $hour^{-1}$. The time course and magnitude of this secretion and the electrical changes were similar to those observed in normal HNE (Fig. 1B). J_v returned to the baseline observed in the presence of amiloride alone after ATP was removed. In contrast, the application of CPT-cAMP (100 μM) with IBMX (100 μM) did not have any effect on J_v (or *TEP* or R_t). In four amiloride-treated CF HNE cultures, this mixture of CPT-cAMP and IBMX was added before the nucleotide and produced no changes in J_v, *TEP*, or R_t. (**B**) Summary of six experiments with HNE cultures from four CF patients. Cultures were treated with amiloride, which reduced fluid absorption [*, $P < 0.01$ compared with control, analysis of variance (ANOVA)]; UTP (100 μM) was then added to the luminal bath and induced secretion (#, $P < 0.01$ compared with amiloride alone, ANOVA). The mean steady-state values of *TEP* and R_t in control media and media containing amiloride alone or amiloride with UTP were −39.2 ± 9.5 mV and 1210 ± 219 ohm · cm^2, 2.4 ± 1.8 mV and 1862 ± 444 ohm · cm^2, and −8.1 ± 3.2 mV and 605 ± 75 ohm · cm^2, respectively.

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a steady-state value not significantly different from zero (Fig. 2B). These results show that fluid secretion can still be activated by UTP and ATP in cultures from CF patients, probably through the Ca^{2+}-activated Cl^- conductance, which is unimpaired in CF (*16, 17*).

When compared with their nasal counterparts, cultured HTE (Fig. 3) from normal individuals or patients with CF showed a very similar pattern of J_v and electrical (*TEP*, R_t) responses to amiloride, cAMP, and triphosphate nucleotides (*23*). In six HTE cultures from patients with the ΔF508 mutation, CPT-cAMP plus IBMX (100 μM each) had no effect on J_v, *TEP*, or R_t (*22*).

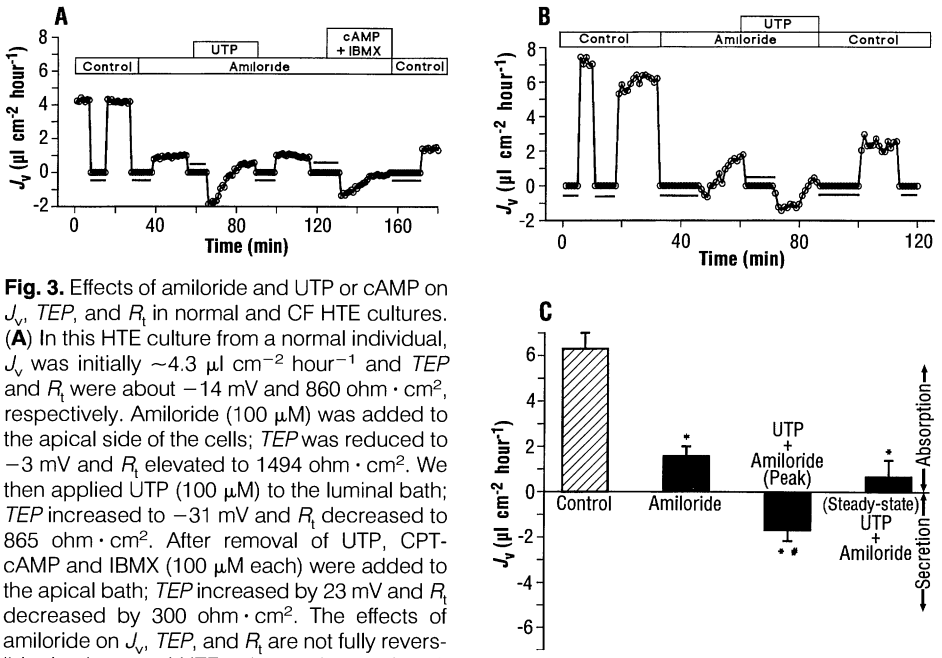

Fig. 3. Effects of amiloride and UTP or cAMP on J_v, *TEP*, and R_t in normal and CF HTE cultures. (**A**) In this HTE culture from a normal individual, J_v was initially ~4.3 μl cm^{-2} hour^{-1} and *TEP* and R_t were about −14 mV and 860 ohm · cm^2, respectively. Amiloride (100 μM) was added to the apical side of the cells; *TEP* was reduced to −3 mV and R_t elevated to 1494 ohm · cm^2. We then applied UTP (100 μM) to the luminal bath; *TEP* increased to −31 mV and R_t decreased to 865 ohm · cm^2. After removal of UTP, CPT-cAMP and IBMX (100 μM each) were added to the apical bath; *TEP* increased by 23 mV and R_t decreased by 300 ohm · cm^2. The effects of amiloride on J_v, *TEP*, and R_t are not fully reversible. In six normal HTE cultures, the starting J_v was 4.4 ± 0.6 μl cm^{-2} hour^{-1} and was reduced to 1.2 ± 0.5 μl cm^{-2} hour^{-1} by apical amiloride (100 μM). Application of CPT-cAMP plus IBMX (100 μM each) caused a significant transient secretion of −0.8 ± 0.1 μl cm^{-2} hour^{-1}. In another group of six HTE cultures, the starting J_v was 4.3 ± 0.5 μl cm^{-2} hour^{-1} and was reduced to 1.0 ± 0.4 μl cm^{-2} hour^{-1} by apical amiloride. UTP caused a significant transient secretion of −1.8 ± 0.4 μl cm^{-2} hour^{-1}. (**B**) In this CF culture, the starting J_v was ~7 μl cm^{-2} hour^{-1}, and *TEP* and R_t were ~−24 mV and 1700 ohm · cm^2, respectively. J_v was slightly altered (14%) by a change to fresh medium, but there was no effect on *TEP* or R_t. When amiloride (100 μM) was applied to the apical side of the cells, *TEP* was reduced to −5 mV and R_t was elevated to 3400 ohm · cm^2. Addition of UTP (100 μM) to the apical bath caused a transient secretion of ~1 μl cm^2 hour^{-1}. (**C**) Summary of six experiments with HTE cultures from three CF patients. Cultures were treated with amiloride, which reduced fluid absorption (*, $P <$ 0.01 compared with control, ANOVA); UTP (100 μM) was then added to the luminal bath and induced secretion (#, $P < 0.01$ compared with amiloride alone, ANOVA). The mean *TEP* and R_t values are given in Fig. 4.

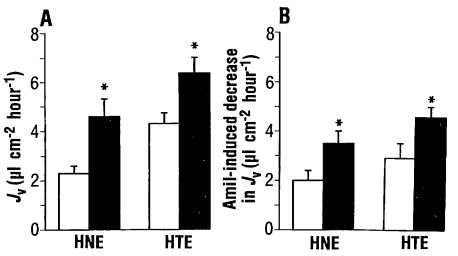

Fig. 4. (**A**) Comparison of baseline unstimulated J_v for both HNE and HTE from individuals with or without CF. For HNE: 6 cultures from 4 CF patients and 20 cultures from 11 normal individuals. For HTE: six cultures from three CF patients and eight cultures from four normal individuals. Data are expressed as mean ± SEM and asterisks indicate $P < 0.05$ between normal and CF (unpaired Student's *t* test). Black bars, CF; white bars, normal. The mean *TEP* and R_t for CF HNE in a slightly larger set of cultures (n = 9, six patients) were −42.4 ± 6.8 mV and 1140 ± 131 ohm · cm^2, respectively; in comparison, the *TEP* and R_t in 20 cultures from 11 normal individuals were −24.9 ± 4.3 mV and 1072 ± 259 ohm · cm^2. In eight HTE cultures from four CF patients the *TEP* and R_t were −52.5 ± 6.9 mV and 1240 ± 265 ohm · cm^2, and in eight cultures from four normal individuals the corresponding values were −30.8 ± 7.2 mV and 1123 ± 406 ohm · cm^2. In both HNE and HTE, *TEP* was significantly greater in CF compared with normal (*, $P < 0.05$) as previously reported (*3, 4*). (**B**) Comparison of the amiloride-induced decrease in J_v in HNE and HTE cultures from individuals with or without CF (n = 6 to 14, *, $P < 0.05$ between normal and CF, unpaired Student's *t* test).

In HTE cultures from CF patients, luminal amiloride inhibited fluid absorption by ~85%; the subsequent addition of UTP caused a significant ($P < 0.01$) transient secretion that returned to a steady-state value near zero after ~30 min (Fig. 3C).

We compared the mean values for baseline J_v data from primary cultures of HNE and HTE from individuals with or without CF (Fig. 4A). The bioelectric data for all experiments are summarized in Fig. 4. Epithelia from patients with CF show hyperabsorption of fluid and loss of secretion compared with epithelia from normal individuals. This provides direct support for the hypothesis from bioelectric data (*1–5*) that the clinical pathology associated with CF stems in part from a reduction in the fluid lining the respiratory tract. The amiloride-induced decrease in J_v was significantly greater in CF HNE and HTE cultures ($P <$ 0.05), suggesting that the hyperabsorption of fluid in CF is mediated by apical membrane Na^+ channels (Fig. 4B).

The increase in Na^+ absorption in CF supports the notion that CFTR may regulate the Na^+ transport pathway, perhaps by modulating the activity of the appropriate channel and pump proteins or by regulating their delivery to the apical and basolateral membranes (*24*). Finally, our results indicate that fluid secretion can be induced across CF airway epithelia, a finding that could have therapeutic implications.

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made on either side of the epithelium. The control Ringer contained 113 mM NaCl, 26 mM $NaHCO_3$, 5.6 mM glucose, 5 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 1.0 mM NaH_2PO_4 and was bubbled with 95% O_2–5% CO_2 to pH 7.4.
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15. In 20 HNE monolayers from 11 normal individuals, the means (± SEM) of baseline J_v, *TEP*, and R_t were 2.3 ± 0.3 μl cm^{-2} $hour^{-1}$, −24.8 ± 7.8 mV, and 1072 ± 259 ohm · cm^2, respectively. In 14 of these monolayers (eight individuals), amiloride decreased J_v and *TEP* to 0.3 ± 0.2 μl cm^{-2} $hour^{-1}$ and −3.9 ± 1.4 mV, respectively, and increased R_t to 1651 ± 329 ohm · cm^2 (steady-state); all the changes are significant ($P < 0.01$ or 0.05, unpaired Student's *t* test). In six of the amiloride-treated monolayers (four individuals), the mixture of CPT-cAMP and IBMX significantly ($P < 0.01$) reduced J_v and R_t. The peak values were −1.4 ± 0.4 μl cm^{-2} $hour^{-1}$ and 781 ± 44 ohm · cm^2; *TEP* increased to −25.3 ± 8.2 mV; J_v then returned to a steady-state level of −0.4 ± 0.3 μl cm^{-2} $hour^{-1}$. In six other amiloride-treated monolayers (four individuals), ATP significantly ($P < 0.01$) reduced J_v and R_t. The peak values were −2.9 ± 0.5 μl cm^{-2} $hour^{-1}$ and 618 ± 183 ohm · cm^2. *TEP* increased to −20.3 ± 1.7 mV and J_v was −0.6 ± 0.2 μl cm^{-2} $hour^{-1}$ in the steady state.
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20. In the absence of amiloride, CPT-cAMP and IBMX (100 μM each) either increased, decreased, or had little effect on J_v, *TEP*, and R_t in normal HNE cultures ($n = 5$). These responses are not due to variations among cultures because all of the cultures from these individuals showed secretory responses to CPT-cAMP plus IBMX in the presence of amiloride. The effect of cAMP on J_v may be determined by the balance of its effects on Na^+ absorption and Cl^- secretion (*1–3, 13*). In cultures from normal individuals and patients with CF, apical addition of UTP (100 μM) reduced J_v, increased *TEP*, and decreased R_t. In three (two normal, one CF) of the seven cultures examined, J_v was actually reversed from absorption to a small transient secretion in response to UTP; in the other four cultures (two normal, two CF) absorption was reduced.
21. Nasal polyps were obtained from four CF patients (all males; mean age, 10 ± 2 years) and genotyped as follows: three ΔF508/ΔF508 and one ΔF508/R560T. Both mutations are associated with a severe phenotype [B. Kerem *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8447 (1990)].
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23. Tracheas were obtained from three CF patients (two males, one female; mean age, 33 ± 4 years) and were genotyped as follows: one ΔF508/ΔF508, one ΔF508/unknown, and one ΔF508/R560T.
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Structure-Function Analysis of the Ion Channel Selectivity Filter in Human Annexin V

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Electrophysiology and structural studies were performed on an annexin V variant containing a mutation of glutamic acid–95 to serine in the center of the pore region. The mutation resulted in a lower single channel conductance for calcium and a strongly increased conductance for sodium and potassium, indicating that glutamic acid–95 is a crucial constituent of the ion selectivity filter. There were only minor differences in the crystal structures of mutant and wild-type annexin V around the mutation site; however, the mutant showed structural differences elsewhere, including the presence of a calcium binding site in domain III unrelated to the mutation. Analysis of the membrane-bound form of annexin V by electron microscopy revealed no differences between the wild type and mutant.

Annexin V belongs to a family of calcium- and phospholipid-binding proteins (*1*) and forms voltage-dependent calcium channels in planar lipid bilayers (*2*). Our analysis of human annexin V by x-ray crystallography (*3*) revealed a hydrophilic pore in the center of the protein that is filled with a chain of water molecules and that we tentatively identified as the ion-conduction pathway (*3*). We introduced a Glu→Ser mutation at amino acid 95, which is located within the pore, and have analyzed the mutant protein for both functional (ion selectivity) and structural changes.

We prepared phospholipid bilayers from liposomes (*4*) in the inside-out configuration of the patch-clamp technique (*5*). Annexin V was added to the bath solution and bound to the bilayers at the tip of the patch pipette. We recorded single channel currents through wild-type annexin V and the Glu→Ser^{95} mutant channels at different pipette potentials with Ca^{2+} in the pipette and Na^+ in the bath solution (Fig. 1, A and B). Although the mutation caused only a slight decrease in Ca^{2+} currents through the protein, the Na^+ currents were strongly increased. In addition, the voltage dependence of the gating kinetics differed for the two channels (*6*).

We next quantified the single channel conductances and the ion selectivity. For the wild-type and Glu→Ser^{95} annexins, respectively, the Ca^{2+} conductances were 29.5 ± 1.6 pS and 22.0 ± 2.2 pS, the Na^+ conductances were 24.7 ± 1.2 pS and 132.5 ± 4.5 pS, and the reversal potentials were −21.0 ± 1.8 mV and +5.9 ± 1.0 mV (Fig. 1C). The calculated permeability ratio (P_{Ca}/P_{Na}) was 3.75 for the wild-type annexin and 0.71 for the mutant, indicating that the mutant channel had lost selectivity for Ca^{2+} versus Na^+ ions. Similar changes were observed for K^+: For the wild-type and the Glu→Ser^{95} annexins, the K^+ conductances increased from 21.2 ± 1.9 pS to 96 ± 12 pS and the reversal potential shifted from −23.2 ± 1.9 mV to +2.1 mV.

Comparison of the crystal structures of wild-type annexin (*3*) and the Glu→Ser^{95} mutant (*7*) revealed only small differences around the mutated residue (Fig. 2A). In the wild-type molecule, Glu^{95} was hydrogen-bonded across the pore to Arg^{271} and His^{267} (*3*) located in module 1 (consisting of domains I and IV), interactions that are absent in the Glu→Ser^{95} mutant. In contrast, the mutated residue Ser^{95} interacted only with Tyr^{91} in module 2 (consisting of domains II and III). That limitation may diminish the steric hindrance for an ion passing through the pore. Both residues are involved in hydrogen bonds to water molecules.

When the Na-Ca gradient in the single channel measurements was reversed, the inward rectification of the ion currents (Fig. 1C) changed to an outward rectification of

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