onset of GHF1 and GRF expression is correlated with the time at which expansion of the somatotrophic lineage is initiated. Defects in GHF1 expression and function prevent the growth and differentiation of these cells, leading to a complete absence of mature somatotrophs and an almost complete absence of mature lactotrophs. Because the Snell dwarf also has a decreased number of thyrotrophs (*14, 24*), it is possible that, in contrast to previous assumptions, this cell type may also be derived from the somatotrophic lineage. However, it is also possible that thyrotroph differentiation or survival may be dependent on the presence of intact somatotrophs or lactotrophs.

Although proper development requires proliferation of stem cells, as well as various determined progenitors, it is not clear how proliferation and differentiation are coupled during development. Genetic and biochemical evidence indicates that homeodomain proteins are involved in the determination of a cell's fate (*1–3*). Homeodomain genes may regulate the expression of genes that control the *Drosophila* cell cycle (*25*). In addition, another POU protein, the ubiquitously expressed Oct1, is important for viral DNA replication (*26*). Our study provides evidence that a homeodomain protein is required for cell proliferation, in addition to its role in cell fate determination. The regulation of expression of homeodomain proteins by lineage-specific growth factors (*27*) may provide an efficient way of coupling proliferation and differentiation.

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17. The oligonucleotides were added every 72 hours for 6 to 9 days to a final concentration of 50 μM. The medium was changed at days 3 and 6. AS1 is complementary to nucleotide positions 118 to 138 (5′-GAAAGGTTGGCAACTCATTCCCAC-3′), AS3 is complementary to positions 99 to 127 (5′-GGCAACTCATTCCCACAAGAGAGTAGAG-3′), and S1 is identical to positions 1 to 25 (5′-CTCAGAGCCGCCCTGATGTATATATG-3′) of GHF1 mRNA. GH1 is complementary to positions 35 to 59 of GH mRNA (5′-CCAAGCAGTGATCTGTCCACAGGAAC-3′. Rel2 and Rel6 are nonspecific control oligonucleotides.
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28. Saggital sections (200 μm) of the entire pituitary were cut as described (*4*). The sections were stained with propidium iodide (20 μg/ml), and the nuclear fluorescence signal of propidium iodide (Molecular Probes) was examined with an MRC-600 Confocal Imaging system (Bio-Rad, Richmond, CA) on a Zeiss microscope. Immunostaining with antibodies to GHF1 was as described (*4*).
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MHC Class I Deficiency: Susceptibility to Natural Killer (NK) Cells and Impaired NK Activity

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The role of major histocompatibility complex (MHC) class I expression in natural killer (NK) cell target recognition is controversial. Normal T cell blasts from MHC class I–deficient mutant mice were found to serve as target cells for NK cells in vitro, which suggests that MHC class I molecules are directly involved in NK cell recognition. Spleen cells from the mutant mice were deficient in their ability to lyse MHC class I–deficient target cells or NK-susceptible tumor targets, and mutant mice could not reject allogeneic bone marrow. Thus, class I molecules may participate in the positive selection or tolerance induction of NK cells.

NEITHER THE ANTIGENS RECOGnized by NK cells nor their antigen receptors have been identified, though their capacity to destroy various tumor cell lines is well documented. The MHC class I molecules may be involved in recognition by NK cells. Tumor cell variants that are deficient for MHC class I expression are often more susceptible to lysis by NK cells than are MHC class I^+ tumor cells (*1–5*). Because susceptible tumor cells may express tumor antigens recognized by NK cells, it is unclear from these experiments whether the MHC class I deficiency enhances recognition of these antigens or is itself sufficient to render the cells sensitive to NK cells. With the use of cells from mice mutant for β_2-microglobulin (β_2M) (*6–8*) and hence deficient for cell surface class I expression, we directly addressed the role of MHC class I expression in the susceptibility of otherwise normal cells to NK cell lysis in vitro and investigated if MHC class I participates in the development of NK activity in vivo.

Spleen cells from C57BL/6 (B6) mice that had been injected 1 day before with polyinosinic:polycytidylic acid (poly I:C), an agent that induces NK cell activity (*9, 10*), lysed both NK-sensitive YAC-1 tumor cells and concanavalin A–induced T cell blasts (Con A blasts) from (B6 × 129)F_3 mice (H-2^b) homozygous for a mutant β_2M allele (−/− mice) (Fig. 1A). Similar results

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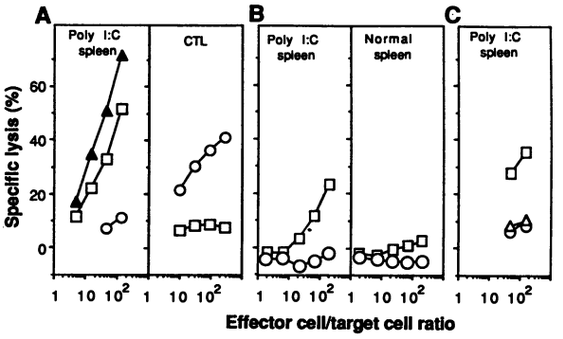

Fig. 1. (**A**) Lysis of β_2M-deficient (−/−) cells by enriched NK cells from poly I:C–pretreated B6 mice, but not by CTLs specific for the male antigen H-Y (*29*). NK cells were enriched by depleting spleen cells of $CD4^+$, $CD8^+$ and IA^{b+} and $J11d^+$ cells (*30*). Cells were titrated for cytotoxic activity (*31*) versus YAC-1 tumor cells (▲), Con A blasts from −/− (B6 × 129)F_3 mice (□), or Con A blasts from +/− (B6 × 129)F_3 littermates (○). (**B**) Lysis of −/− cells by enriched NK cells from poly I:C–pretreated, but not untreated mice. NK cells were enriched from B6 spleen cell populations by depleting IA^{b+} and $J11d^+$ cells (*30*). Target cells were as indicated in (A). (**C**) Restoration of β_2M expression by crossing the human β_2M transgene into −/− mice, renders −/− cells resistant to lysis by enriched NK cells. NK cells were enriched as in B. The target cells were Con A blasts from −/− mice transgenic for a human β_2M transgene (△), Con A blasts from −/− nontransgenic littermates (□) and Con A blasts from +/− nontransgenic littermates (○).

were obtained in 12 of 13 independent experiments with the F_2 and F_3 target cells. To achieve the same lysis of −/− targets as YAC-1 targets, two to nine times more effector cells were required. Con A blasts from MHC class I^+ heterozygous (+/−) mice were barely lysed, if at all (Fig. 1A). Conversely, a polyclonal cytotoxic T lymphocyte (CTL) population specific for the male antigen H-Y efficiently lysed +/− but not −/− male target cells (Fig. 1A).

Spleen cells from mice not pretreated with poly I:C did not significantly lyse either −/− (Fig. 1B) or YAC-1 target cells. These results suggest that mice in our colony have low basal NK activity, and that like NK cells, the effector cells that lyse −/− targets are induced by poly I:C. In all subsequent experiments poly I:C–treated mice were used as a source of effector cells.

That the β_2M mutation itself is responsible for susceptibility to enriched NK cells was shown by the lysis of −/− but not +/+ target cells from inbred 129 mice (*11*) and by examining −/− mice in which surface expression of class I molecules was restored by crossing in a human β_2M transgene (*12*). Expression of the human β_2M transgene reverted −/− target cells to an NK cell–resistant phenotype (Fig. 1C).

Depletion of $CD4^+$ or $CD8^+$ cells from the NK cell–enriched effector population had no effect on the lysis of −/− or YAC-1 cells, whereas depletion of $NK1.1^+$ cells nearly abolished activity on both −/− and YAC-1 target cells (Fig. 2A). Therefore the effector cells are $NK1.1^+CD4^-CD8^-$ cells. Spleen cell populations from three of three severe combined immune deficient (SCID) mutant mice, and two of three wild-type C.B-17 control mice, lysed both −/− and YAC-1 but not +/+ target cells (Fig. 2B). SCID mice have almost no T cells and B cells because of a defect in the gene rearrangement process, but are not deficient in NK cell activity (*13, 14*). Thus, our results indicate that T cell receptors do not participate in the lysis of −/− cells by splenic $NK1.1^+$ cells, 10 to 20% of which bear T cell receptors in normal mice (*15*). Gene rearrangements mediated by the recombinase that rearranges immunoglobulin and TCR genes are apparently unnecessary to generate the receptors used for recognition of −/− cells. Overall, the properties of the effector cells that lyse −/− cells ($NK1.1^+CD4^-CD8^-$ phenotype, normal in SCID mice, induced by poly I:C) are consistent with those of "conventional" NK cells.

Because −/− cells are susceptible to lysis by NK cells, we examined the status of NK cells in these animals. In comparison to their +/− or +/+ littermates, enriched NK cells from poly I:C-pretreated −/− mice were significantly and reproducibly diminished in their ability to lyse −/− or YAC-1 target cells (Fig. 3A); the lysis of −/− targets was consistently more diminished than was the lysis of YAC-1 targets.

Irradiated −/− mice ($H\text{-}2^b$) were also unable to reject allogeneic (BALB/c, $H\text{-}2^d$) bone marrow grafts, whereas +/− littermates rejected the BALB/c marrow grafts (Fig. 3B). Because previous studies show that $NK1.1^+$, $CD8^-$ cells mediate rejection of allogeneic $H\text{-}2^d$ marrow by irradiated $H\text{-}2^b$ mice (*16*), the present studies suggest that class I–deficient mice are deficient in active NK cells that destroy allogeneic as well as class I–deficient cells.

The diminished activity of NK cells from −/− mice was not due to the improved husbandry of the −/− animals, because the +/− and +/+ littermates were raised under identical conditions, often in the same cage. These results suggest that at least a component of NK activity, responsible for lysis of YAC-1 and −/− targets, is masked, inactive, or absent in MHC class I–deficient mice. Flow cytometric analysis revealed, however, that $NK1.1^+$ cells are present in −/− mice in normal numbers (2 to 3% of spleen cells), indicating that the presence of $NK1.1^+$ cells does not depend on MHC class I expression (Fig. 4).

Our data provide evidence that MHC class I deficiency is sufficient to render nontransformed cells sensitive to NK cell lysis. Thus, the likely explanation for our earlier finding that −/− bone marrow transplants fail in irradiated, MHC-matched normal mice (*17*) is that the stem cells are lysed, rather than inhibited (*18*). The specific destruction of MHC class I–deficient cells by NK cells implicates class I molecules or molecules that interact with class I molecules as the ligands for at least a subset of NK cells (*1, 17*). If class I molecules are themselves recognized, it must be proposed that this binding inhibits rather than activates the lytic program of NK cells, since normal β_2M expression renders cells resistant to NK cell–

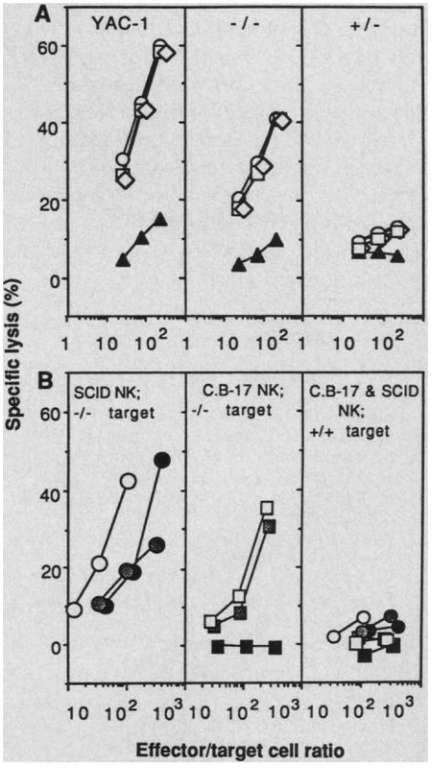

Fig. 2. (**A**) Effector cells that lyse −/− or YAC-1 targets are $NK1.1^+CD4^-CD8^-$ cells. Effector cells were pretreated (*32*) with complement and monoclonal antibodies (MAbs) to NK1.1 [MAb PK136 (*33*)] (▲), CD4 (MAb GK1.5) (◇), CD8 [MAb AD4(15)] (□), or no antibody (○) and the surviving cells were tested for cytotoxic activity on the indicated targets. (**B**) Spleen cells from three of three individual scid mice (circles) and two of three isogenic C.B-17 mice (squares) lyse −/− targets (left panels) but not +/− targets (rightmost panel). The mice were pretreated with poly I:C (100 μg).

mediated lysis. Alternatively, class I molecules may mask or inhibit the expression of specific antigens that are recognized by NK cell receptors and that, when recognized, activate the lytic program (*4, 5, 19*).

Current data do not distinguish these two hypotheses. However, the present findings seem likely to be related to the recessive susceptibility of allogeneic or parental marrow grafts to rejection by NK1.1$^+$ cells (*17, 20–22*) and to the recessive susceptibility of human T cell blasts to lysis by human cloned NK cell lines (*23*). The concordant reduction in −/− mice of both the NK-mediated lysis of −/− cells and the rejection of allogeneic marrow, is further evidence for a relationship between these phenomena. The simplest model to explain both of these phenomena is that NK cells have receptors for some or all of the self class I molecules normally expressed by cells, and that engagement of these receptors by the class I molecules on a cell inhibits the lysis of this cell by NK cells (*17, 22*). Failed expression of one or all of these molecules may therefore render a cell sensitive to NK cell–mediated lysis.

MHC class I deficiency is apparently important for the susceptibility of some tumor cell lines to lysis by NK cells (*2, 4, 5, 24*) but not others (*25, 26*). Therefore, so-called NK activity is likely comprised of two or more independent activities, not all of which are influenced by MHC expression. It remains unclear whether all NK cells, or just a subset of them (*27, 28*), can lyse −/− cells or mediate other types of NK activity. In the present studies, lysis by NK cells of −/− targets was generally less efficient than that of YAC-1 targets, but this may be due to differential expression of accessory molecules or other molecules that affect susceptibility to lysis.

The deficiency of NK activity in −/− mice implicates class I MHC in the development of at least a major component of NK activity, that which is responsible for destruction of both allogeneic and class I–deficient cells. The role that MHC class I plays in development will remain unclear without more data on NK cell recognition. The expression on −/− cells of NK-specific antigens, normally masked, might render NK cells tolerant in −/− mice. Because the frequency of NK1.1$^+$ cells is not reduced in −/− mice, tolerance may involve anergy rather than deletion, or only a minor subset of NK1.1$^+$ cells, responsible for lysis of −/− cells, may be deleted from the NK1.1$^+$ population.

Alternatively, if recognition of self MHC class I alleles provides a negative signal to NK cells, a "positive selection" step during NK cell development may operate to define self MHC class I. In the absence of any class I molecules, the MHC-related component of NK activity may not develop. The undiminished frequency of NK1.1$^+$ cells might be explained if these cells are largely trapped in a nonfunctional, immature stage, or if only a minor subset of NK1.1$^+$ cells, responsible for the MHC-related component of NK activity, does not develop.

Other possiblilities to explain the diminished NK activity in −/− mice are that class I interactions are necessary to induce NK cell cytolytic activity or that class I molecules serve as part of the NK receptor. It is also possible that −/− cells in the effector population inhibit the activity of −/− NK cells by serving as "cold target" competitors. This possibility seems unlikely, by itself, to account for the diminished lysis of −/− cells, because it implies the chronic lysis of autologous cells in −/− mice; no hemopoietic deficiencies or other signs of autoimmune reactivity, however, are seen. The MHC class I–deficient mice should be useful in determining the mechanism by which class I expression modulates NK activity.

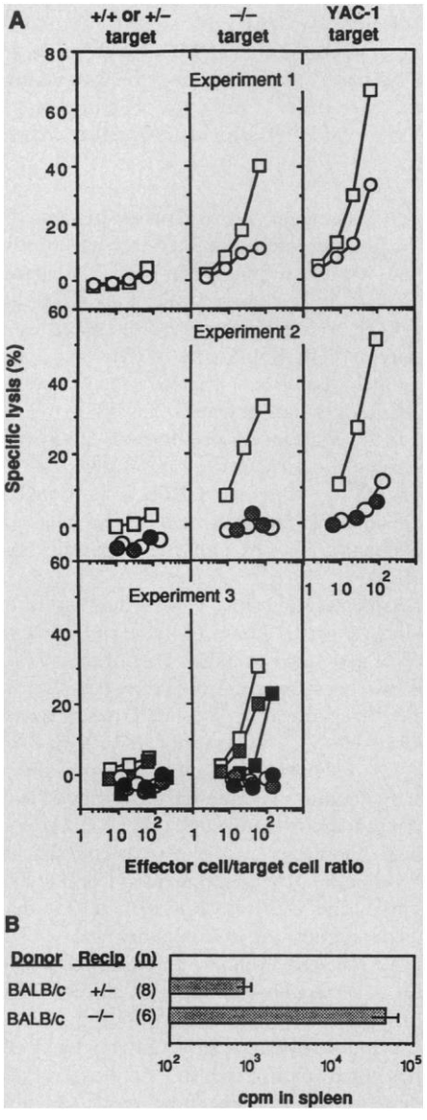

Fig. 3. (**A**) Lysis of YAC-1, −/−, +/+, or +/− targets by spleen cells from +/+ (squares) and −/− (circles) (B6 × 129)F_3 mice. Three experiments are shown. NK cells were enriched from spleen cell populations (from poly I:C pretreated mice) by depleting B cells with anti-IA^b and complement treatment (experiments 2 and 3) or depleting IA^{b+}, J11d$^+$, CD4$^+$, and CD8$^+$ T cells by treatment with MAbs plus complement (*30*). In experiment 1, enriched NK cells were prepared from a pool of spleen cells from four −/− or two +/− and two +/+ mice. The NK cells were tested for their ability to lyse −/− or +/+ targets from inbred 129 mice (*11*), or YAC-1 tumor cells. In experiments 2 and 3, enriched NK cells from individual −/− or +/+ mice were tested for their ability to lyse (B6 × 129)F_2 and F_3 target cells. The target cells were YAC-1 tumor cells and −/− or +/− Con A blasts in experiment 2, and −/− or +/+ Con A blasts in experiment 3. (**B**) Irradiated +/− but not −/− H-2^b mice reject BALB/c bone marrow allografts. Rejection is assessed by failure of 5 × 10^6 T cell–depleted transplanted bone marrow cells to proliferate in the spleens of irradiated (940 rads) recipient mice 5 days after transplantation, by determining ^{125}IUdR incorporation (*17*).

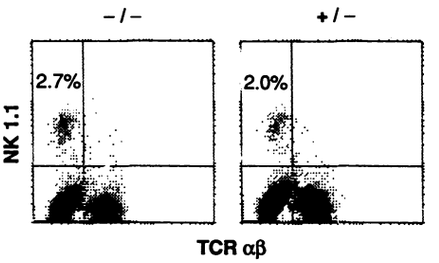

Fig. 4. Unfractionated spleen cells from untreated −/− and +/− mice contain 2 to 3% NK1.1$^+$ cells. Spleen cells from B6 backcross mice (*34*) were stained with MAbs to NK1.1 and TCRβ. In another experiment, 2.3% of +/− and 2.2% of −/− spleen cells stained with anti-NK1.1. Without enrichment of NK1.1$^+$ cells it is difficult to address the status of the minor TCRβ$^+$ NK1.1$^+$ population.

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11. One example of an experiment employing target cells from 129 inbred mice can be seen in Fig. 3, experiment 1. The 129 inbred line was initiated by breeding the original mosaic founder animal (*6*) with 129/Sv mice, and interbreeding the +/− offspring; since the embryonic stem cell line is of 129/Sv origin, this procedure yields inbred 129 −/− animals.
12. M. Zijlstra, unpublished data showing restoration of class I expression by the human β_2M transgene (*35*).
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30. To activate NK cells in vivo, mice were inoculated intraperitoneally one day before an experiment with poly I:C (100 μg) (*9, 10*). To enrich NK cells, spleen cells were depleted of non-NK cells bearing various markers with the use of MAbs plus complement [a mixture of guinea pig and rabbit sera (*36*)]. MAbs to IA^b (BP107), heat stable antigen (J11d), CD4 (GK1.5), and CD8 [AD4(15)] were used as indicated. After the depletions, viable cells were isolated on a Ficoll gradient.
31. The cytotoxic cell assay was as described (*36*) except that medium containing a serum substitute (AIM V medium, Gibco, Gaithersburg, MD) was employed for preparing the Con A blast target cells and in the assay cultures. However, when the assay was performed with serum or with AIM V medium on the same day, no difference was observed in the lysis of −/− cells by enriched NK cells.
32. Spleen cells from B6 mice pretreated with poly I:C were first depleted of B cells by treating the cells with anti-IA^b plus complement (*30*) and isolating the surviving cells on a Ficoll gradient. Aliquots of these cells were treated with antibodies plus complement (*36*). Following the complement treatment, viable cells were isolated on a Ficoll gradient, washed, and tested for lysis of YAC-1 tumor cells, or −/− or +/− Con A blast targets from (B6 × 129)F_2 and F_3 mice as indicated. Cell numbers were not adjusted for the numbers of cells killed by antibody and complement, so the effector/target ratio in these panels refers to input effector cell number.
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34. The staining experiment in Fig. 4 employed third-generation backcross mice of (B6 × 129)F_1 +/− mice to B6 mice; it was necessary to use B6 backcross mice, because the B6 strain but not the 129 strain expresses the NK1.1 allele. Viable nucleated spleen cells from the indicated mice were isolated on a Ficoll gradient, incubated with biotinylated MAbs (PK136) to NK1.1 (*33*), washed, and stained with phycoerythrin-streptavidin (Southern Biotechnology, Birmingham, AL) and fluorescein-conjugated MAb H57-597, specific for TCRβ antibody (*37*). The stained cells were analyzed on an Epics C flow cytometer and the data presented on a logarithmic scale. Dead cells were excluded by their forward and right angle light scattering characteristics.
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38. We thank P. Krimpenfort, A. Berns, and H. L. Ploegh for the human β_2M transgenic mice, R. Kubo for the anti-TCRβ hybridoma, M. Bosma for the SCID mice used to initiate our colony, R. West and J. Loring for excellent technical assistance, C. Byron for advice on poly I:C induction of NK cells, and J. Allison, D. Asarnow, and I. Correa for critical reading of the manuscript. Supported by NIH grants to D.R. (RO1 AI30171) and R.J. (R35 CA44339) and by a grant from the W. M. Keck Foundation to D.R.. M.Z. is supported by a Fan Fox & Leslie R. Samuels Foundation Fellowship from the Cancer Research Institute.

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Demonstration That CFTR Is a Chloride Channel by Alteration of Its Anion Selectivity

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Expression of the cystic fibrosis transmembrane conductance regulator (CFTR) generates adenosine 3′,5′-monophosphate (cAMP)–regulated chloride channels, indicating that CFTR is either a chloride channel or a chloride channel regulator. To distinguish between these possibilities, basic amino acids in the putative transmembrane domains were mutated. The sequence of anion selectivity of cAMP-regulated channels in cells containing either endogenous or recombinant CFTR was bromide > chloride > iodide > fluoride. Mutation of the lysines at positions 95 or 335 to acidic amino acids converted the selectivity sequence to iodide > bromide > chloride > fluoride. These data indicate that CFTR is a cAMP-regulated chloride channel and that lysines 95 and 335 determine anion selectivity.

CYSTIC FIBROSIS (CF) (*1*) IS CAUSED by mutations in the gene encoding CFTR (*2–6*). Amino acid sequence analysis and comparison with other proteins (*3, 7, 8*) suggest that CFTR consists of two repeats of a unit containing six membrane-spanning segments and a putative nucleotide binding domain (Fig. 1). The two repeats are separated by a large polar segment called the R (regulatory) domain, which contains multiple potential phosphorylation sites. The predicted topology of CFTR, with the exception of the R domain, resembles that of a number of other membrane proteins, such as the multiple drug resistance P-glycoprotein, the yeast STE6 gene product, and several bacterial periplasmic permeases (*3, 7, 8*).

Chloride transport by epithelial tissues is abnormal in patients with CF (*9*); apical membrane Cl^- channels do not open in response to an increase in intracellular cAMP. When CFTR is expressed in cell types that do not normally express CFTR or have cAMP-regulated Cl^- channels (*10, 11*), cAMP-regulated Cl^- currents are generated. The simplest interpretation of these results is that CFTR is a cAMP-regulated Cl^- channel, but it is possible that CFTR could regulate endogenous, previously silent Cl^- channels. The notion that CFTR is a Cl^- channel has been controversial because CFTR does not resemble any known ion channels but most resembles a family of energy-dependent transport proteins (*3, 7, 8*). To test whether CFTR is a cAMP-regulated Cl^- channel, we used site-directed mutagenesis to change the properties of CFTR.

As permeating ions flow through an ion-selective pore, they sense the electrostatic forces generated by amino acids that line the pore. This interaction between amino acids and the permeating ions determines ionic selectivity (*12*). We reasoned that if CFTR is a Cl^- channel, then changing positively charged amino acids in CFTR to negatively charged amino acids might alter ionic selectivity. Similar strategies have been used in K^+ channels, the nicotinic acetylcholine receptor, and the mitochondrial voltage-dependent anion-selective channel (*13*).

We mutated amino acids within the putative membrane-spanning sequences (M1 through M12) (Fig. 1) identified by Riordan and co-workers (*3*). On the basis of their hydropathy and the prediction that they form α helices, these sequences are

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