Differential Regulation of Cystic Fibrosis Transmembrane Conductance Regulator by Interferon γ in Mast Cells and Epithelial Cells

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Received April 5, 2005; accepted July 26, 2005

ABSTRACT

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-dependent chloride channel in epithelial cells; recently, we identified it in mast cells. Previous work that we confirmed showed that interferon γ (IFNγ) down-regulated CFTR expression in epithelial cells (T84), but by contrast, we found that IFNγ up-regulated CFTR mRNA and protein expression in rat and human mast cells. IFNγ up-regulation of CFTR in mast cells was inhibited by p38 and extracellular signal-regulated kinase (ERK) kinase inhibitors but not a Janus tyrosine kinase (JAK)2 inhibitor, whereas in T84 cells IFNγ-mediated down-regulation of CFTR was JAK2-dependent and ERK− and p38-independent. Furthermore, IFNγ down-regulation of CFTR in T84 epithelial cells was STAT1-dependent, but up-regulation of CFTR in mast cells was STAT1-independent. Thus, differential regulatory pathways of CFTR expression in mast cells and epithelial cells exist that depend upon either p38/ERK or JAK/STAT pathways, respectively. Surprisingly, IFNγ treatment of mast cells inhibited Cl− efflux, in contrast to up-regulation of CFTR/mRNA and protein expression. However, down-regulation of Cl− flux correlated with IFNγ-mediated inhibition of mediator secretion. This and other work suggests that the effect of IFNγ on CFTR expression in mast cells is important for their function.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-dependent Cl− channel that controls transepithelial electrolyte transport, fluid flow, and ion concentrations in the intestine, lungs, pancreas, and sweat glands (Gibson et al., 2003). Over 1200 disease-associated mutations in the cystic fibrosis gene have been reported to the Cystic Fibrosis Genetic Analysis Consortium database (www.genet.sickkids.on.ca/cftr/). Approximately 70% of patients with the disease have a deletion of phenylalanine at amino acid position 508 (F508) that severely decreases CFTR expression in the plasma membrane and compromises permeability to Cl−. CFTR expression is temporally and spatially complex and is regulated by many factors, including cytokines (Besancon et al., 1994; Baudouin-Legros et al., 2005).

In mast cells, several specific Cl− conductances have been identified and linked with degranulation. After antigen stimulation of rat peritoneal mast cells (PMC), there is an increase in Cl− uptake (Romain et al., 1991; Friis et al., 1994). Cl− channel blockers such as 5-nitro-2-(3-phenylpropylamino) benzoic acid inhibit both mast cell Cl− current and degranulation (Romain et al., 1991), whereas diphenylamine-2-carboxylate blocks FcɛRI-stimulated degranulation and forskolin-induced Cl− current in PMCs (Kulka et al., 2002a). Moreover, mast cell-stabilizing compounds cromolyn and nedocromil inhibit mast cell degranulation as well as Cl− ion flux (Alton and Norris, 1996). We have identified CFTR and voltage-gated chloride channel (CIC) family members CIC-2, 3, 4, 5, and 7 in rat mast cells (Kulka et al., 2002a,b), and others have identified CIC3, 5, and 7 in human mast cells (Duffy et al., 2001; Bradding et al., 2003). Thus, given that CFTR in mast cells may be important for their functions, we have studied the regulation of CFTR expression in mast cells.

This work was funded by grants to A.D.B. from the Canadian Institutes of Health Research (CIHR) and to M.D. from the Canadian Cystic Fibrosis Foundation. M.K. was supported by a student voucher from CIHR.

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Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.105.087528.

ABBREVIATIONS: CFTR, cystic fibrosis transmembrane conductance regulator; PMC, rat peritoneal mast cell(s); CIC, voltage-gated Cl− channel; IFN, interferon; JAK, Janus tyrosine kinase; STAT, signal transduction and activator of transcription; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; TNF, tumor necrosis factor; AG-490, α-cyano-(3,4-dihydroxy)-N-benzylcinnamamide tyrphostin B42; SB202190, C6-H4-FN3O; U0126, 1,4-diamino-2,3-dicyano-1,4-bis(2-aminoethylthio)-butadiene; PMA, phorbol 12-myristate 13-acetate; HTB, HEPES Tyrode’s buffer; MC, mast cell; RCMC, rat cultured mast cell(s); FBS, fetal bovine serum; LAD2, Laboratory of Allergic Diseases mast cell line 2; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; JNK, c-Jun NH2-terminal kinase; FAM, 6-carboxyfluorescein; BSA, bovine serum albumin; PCR, polymerase chain reaction; MQAE, N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide; IL, interleukin.
In epithelial cells, IFNγ down-regulates expression of CFTR resulting in a significant decrease in CFTR-mediated Cl− current (Besançon et al., 1994). IFNγ is a member of a family of inducible secretory proteins produced largely by activated T lymphocytes and natural killer cells (Schroder et al., 2004). IFNγ modulates gene expression by activating Janus tyrosine kinase (JAK), resulting in signal transducer and activator of transcription (STAT) 1 binding and phosphorylation. Phosphorylated STAT1 dimerizes and translocates into the nucleus where it binds to γ-activated sequence elements and initiates transcription (Schroder et al., 2004). In addition to the JAK/STAT pathway, IFNγ activates other signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002). With regard to protein kinases (MAPKs) and extracellular signal-transduction proteins such as p38 mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinase (ERK) 1/2 MAPK (Ramana et al., 2002).
for 30 s and then placed into a freezing methanol bath. The bottom of each tube was cut off and placed into a scintillation vial with 48 mM NaOH. Each vial was vortexed for 1 min after which 5 ml of scintillation fluid was added and placed in a Beckman scintillation counter. 

**36Cl** uptake was calculated based on the specific activity of 

**36Cl** in the extracellular medium, calculated as the sum of extracellular 

**Cl** and added **36Cl** (in nanomoles) divided by the radioactivity of the added **36Cl** (in cpm). All values of **36Cl** uptake were corrected for **36Cl** trapped in the extracellular space, which was determined by measuring cpm immediately after **36Cl** addition (50 ± 10.2 cpm).

**N-(Ethoxycarbonylmethyl)-6-methoxyquinolinium Bromide (MQAE) Measurements.** Fluorescence measurements were performed in HTB. Gluconate, Br, or I anions and thus, when Cl leaves the cell, it dissociates from MQAE and fluorescence increases. MQAE fluorescence was excited at 350 nm, and the emission was measured at 450 nm with a PTI spectrofluorimeter (Photon Technology International, London, ON, Canada), using Felix software (version 1.42). All experiments were performed at 37°C. To produce a driving force for Cl efflux, the cells were added to 1 ml of gluconate buffer and MQAE fluorescence was monitored for up to 10 min. Cl efflux was calculated as the initial rate of change of MQAE fluorescence after addition of cells to the gluconate buffer. For quantitative analysis, the data collected in the first 60 s were fitted using linear regression, and the slope was used as a measure of Cl efflux. All traces were normalized to initial baseline reading (buffer, no cells). In some experiments, 10 worm equivalents/ml of **N. brasiliensis** antigen was added to the cell suspension, and changes in fluorescence were monitored for up to 10 min.

**Statistics.** All data are presented as mean of at least three independent experiments with standard error of the mean (S.E.M.). Where indicated, data were analyzed using a paired t test for sample means, analysis of variance, or the Tukey-Kramer multiple comparisons test.

### Results

**Interferon γ Up-Regulates CFTR mRNA and Protein Expression in Rat and Human Mast Cells.** PMA, TNF, and IFNγ down-regulate CFTR expression in epithelial cells (Nakamura et al., 1992; Shen et al., 1993; Besançon et al., 1994). To determine whether CFTR in mast cells was similarly regulated, RCMC were treated with PMA, TNF, or IFNγ for 24 h and CFTR expression was identified by Western blotting as we have done previously (confirmed using isotype controls for flow cytometry, Western blot, and immunohistochemistry; Kulka et al., 2002a). As expected, TNF and IFNγ decreased CFTR expression in T84 cells (Fig. 1A). Surprisingly, however, TNF and IFNγ up-regulated CFTR expression in RCMC. PMA had no detectable effect on CFTR expression in RCMC or T84 (Fig. 1B). Because IFNγ up-regulates STAT1 expression (Hu et al., 2002), membranes were stripped and reprobed with anti-STAT1. As expected, STAT1 protein (visible as a double band, representing STAT1α and STAT1β) was up-regulated in both RCMC and T84 by PMA, TNF, and IFNγ.
Confocal analysis of CFTR expression in T84 cells showed a largely cytoplasmic distribution and as expected from the results of Western blot analysis, the intensity of CFTR staining was decreased after IFNγ treatment (80 ng/ml, 24 h; Fig. 1C). By contrast, CFTR expression in rat PMC was increased after IFNγ treatment and seemed to be associated with granules (Fig. 1D). Studies of nonpermeabilized cells identified some CFTR in a plasma membrane-like distribution on T84, but there was no obvious CFTR with such a distribution on PMC (not shown).

To further characterize up-regulation of CFTR in mast cells, CFTR mRNA expression after IFNγ dose-response and time-course treatments was analyzed in RCMC, human LAD2, and T84 (Fig. 2). Quantitative PCR analysis confirmed that IFNγ (10 ng/ml) significantly (p < 0.05) up-regulated CFTR mRNA expression in RCMC by 3 h after treatment, and the magnitude of this up-regulation was 39 ± 13% at 12 h. In LAD2 MC, the up-regulation of CFTR was statistically significant by 8 h of IFNγ treatment and by 12 h was 54 ± 17% greater than in untreated cells (Fig. 2A). Significant up-regulation of CFTR was induced in mast cells within 8 h with as little as 1 ng/ml (RCMC) or 10 ng/ml (LAD2) of IFNγ (Fig. 2B).

By contrast, IFNγ significantly decreased CFTR mRNA expression in T84 cells within 3 h (Fig. 2, A and B) and by 12 h the magnitude of decrease was 49 ± 4% at 12 h of treatment. The IFNγ effect was dose-dependent such that 1, 10 and 100 ng/ml IFNγ decreased CFTR mRNA expression in T84 cells by 23, 50, and 83% after 8 h (all statistically significant decreases compared with the untreated group).

Western blot analysis showed that by 6 h, CFTR expression was increased compared with untreated RCMC and by 24 h, CFTR expression was significantly up-regulated (Fig. 2C). In T84 cells, a decrease in CFTR protein expression was observable at 6 h and remained low at 24 h (Fig. 2D). In both T84 and RCMC, STAT1 protein was up-regulated after 6 h of treatment and remained elevated up to 24 h.

**IFNγ Up-Regulation of CFTR Is Inhibited by MAP Kinase Inhibitors.** To determine which IFNγ signaling pathways were activated in mast cells compared with epithelial cells, RCMC were treated with IFNγ, and whole cell lysates were analyzed for phosphorylated STAT1, p38, ERK, and JNK (Fig. 3). IFNγ activated STAT1, ERK, and p38, but not JNK phosphorylation. STAT1 was activated at 5 min and remained activated for up to 30 min. ERK2 (bottom band) was constitutively activated, but phosphorylation of both ERK1 (top) and ERK2 (bottom) was induced after 5 min. p38 was activated at 15 min, later than STAT1 or ERK.

To determine whether JAK/STAT1, p38 or ERK signaling pathways were involved in IFNγ-mediated up-regulation of CFTR, RCMC, LAD2, and T84 were treated with IFNγ in the presence of a JAK2 inhibitor, AG-490; a p38 kinase inhibitor, SB202190; and an ERK MAPK inhibitor, U0126. In RCMC and LAD2, AG-490 did not affect IFNγ-mediated up-regulation of CFTR protein expression, but both SB202190 and U0126 partially inhibited IFNγ-mediated up-regulation of CFTR (Fig. 4). By contrast, in T84 cells, AG-490 blocked IFNγ-mediated down-regulation of CFTR. Membranes were stripped and rebotted with anti-STAT1 to compare regulation of another IFNγ-responsive protein. STAT1 up-regulation in RCMC was sensitive to AG-490 but in human LAD2 cells, STAT1 up-regulation was inhibited by AG-490, SB202190, and U0126. T84 cells were similar to the RCMC in that STAT1 up-regulation was only blocked by AG-490 but

![Fig. 2. Quantitative PCR analysis of CFTR expression in RCMC, LAD2, and T84 after different times and doses of IFNγ treatment (A and B). Asterisks represent statistical significance as determined by Student's t test compared with untreated sample (time 0) in each case (p < 0.05). The dose used in A was 10 ng/ml, and in B treatment was for 8 h. Western blot analysis of CFTR, STAT, and actin expression in RCMC and T84 after a time course (hours) of 10 ng/ml IFNγ treatment (C and D) (n = 3).](image-url)
not SB202190 and U0126. Densitometry analysis of three independent blotting experiments confirmed that AG-490 did not affect IFNγ-mediated up-regulation of CFTR protein expression in both LAD2 and RCMC (Fig. 4B; p < 0.05).

To confirm the actions of AG-490, SB202190, and U0126, RCMC were treated with IFNγ (10 ng/ml) in the presence of these inhibitors, and STAT1, p38, and ERK1/2 phosphorylation was assessed by Western blotting (Fig. 5). As expected, AG-490 but not SB202190 or U0126 inhibited STAT1 constitutive and IFNγ-induced phosphorylation (Fig. 5A). U0126 inhibited ERK1/2 constitutive and IFNγ-induced phosphorylation (Fig. 5B). SB202190 inhibited IFNγ-induced p38 phosphorylation (Fig. 5C).

IFNγ Inhibits Both Constitutive and Antigen-Induced Cl− Flux in Mast Cells. To determine the effect of IFNγ on Cl− flux in resting and antigen-IgE-activated mast cells, we used two methods: measurement of 36Cl− uptake and assessment of Cl− sensitive fluorescence using MQAE. Studies with 36Cl− have shown that IFNγ treatment decreases Cl− uptake of PMC (Fig. 6A; p < 0.01). A time course of PMC Cl− uptake shows that IFNγ did not have an effect at the earlier treatment points (less than 2 h) but decreased Cl− uptake at 20 and 24 h (Fig. 6B). Similar results were obtained with 36Cl− uptake measurements in RCMC (data not shown).

Fluorescence measurements were performed with mast
cells loaded with MQAE in HTB solution and chloride efflux was measured after placing cells in gluconate buffer. Figure 6C shows that IFNγ treatment significantly reduced Cl− flux in sensitized PMC not challenged with antigen \((p < 0.05; n = 8 \text{ and } 11, \text{control} \text{and} \text{IFNγ-treated cells, respectively})\). After antigen challenge \((10 \text{ worm equivalents/ml})\) the magnitude of the IFNγ-mediated depression in Cl− efflux was reduced (Fig. 6D). Although antigen challenge in the absence of IFNγ treatment showed a trend toward reduced Cl− efflux, this was not statistically significant \((p > 0.5; n = 3)\). Identical results were obtained in both PMC and RCMC under conditions when the cells were loaded in gluconate buffer and placed in HTB to measure Cl− influx (data not shown).

Measurements of halide permeabilities indicated that Br− was more permeable that Cl− and I− in PMC cells \([\text{Br}− (1.34) > \text{Cl}− (1.00) > \text{I}− (0.68); n = 3 \text{ in each set}]\). Similar results were obtained with RCMC \([\text{Br}− (1.19) > \text{Cl}− (1.00) > \text{I}− (0.61); n = 3 \text{ in each set}]\). The halide permeability sequence, \(\text{Br}− > \text{Cl}− > \text{I}−\), is characteristic of CFTR Cl− channels (Illek et al., 1999) and suggests that CFTR channels are an important component of Cl− flux in mast cells.

**Discussion**

This is the first study demonstrating that CFTR expression is regulated differently in epithelial and nonepithelial cells. Moreover, we show that IFNγ-induced up-regulation of CFTR in MC involved MAPK signaling pathways, whereas IFNγ-induced down-regulation of CFTR in epithelial cells involved JAK/STAT pathways. Paradoxically, we also show that despite IFNγ up-regulation of CFTR in mast cells, IFNγ treatment depressed mast cell Cl− flux in multiple assay systems.

It is now well established that CFTR gene expression is regulated in a complex, cell- and stimulus-specific manner that may involve both transcriptional and posttranscriptional mechanisms. For example, TNF decreases CFTR mRNA in human colonic epithelial cells but not in airway epithelial cells, whereas IL-1β increases it only in airway epithelial cells (Baudouin-Legros et al., 2005). Although stimulation of CFTR gene expression by IL-1β involves activation of the CFTR promoter (Brouillard et al., 2001), down-regulation of CFTR by TNF and IFNγ involves mainly posttranscriptional mechanisms (Baudouin-Legros et al., 2005). The results of this study show that in mast cells both TNF and IFNγ increase CFTR mRNA, but whether this process affects CFTR gene transcription and/or mRNA stability is presently unknown. However, the fact that IFNγ increased CFTR protein levels to a greater extent than the mRNA suggests that IFNγ treatment may increase mRNA stability rather than CFTR gene transcription.

IFNγ modulation of gene expression is mediated by both STAT1-dependent and independent pathways (Gil et al., 2001). Our results show that IFNγ activates STAT1, ERK1/2, and p38 but not JNK, suggesting that these pathways are also induced in mast cells. Using inhibitors to JAK/STAT, ERK, and p38, we determined that in both rat and human mast cells, CFTR up-regulation is JAK/STAT independent but requires activation of the MAPK pathways mediated by ERK and p38. In T84 cells, IFNγ-mediated down-regulation of CFTR is inhibited by AG-490 but is unaffected by the p38 and ERK inhibitors. STAT1 expression, by comparison, is up-regulated by IFNγ in both mast cells and T84 epithelial cells and is inhibited by AG-490, suggesting that STAT1 up-regulation is a positive feedback mechanism that sensitizes mast cells to IFNγ as previously observed in human macrophages (Duffy et al., 2001). In human LAD2 cells, IFNγ-mediated up-regulation of STAT1 is also sensitive to ERK and p38 inhibitors, perhaps indicating the importance of the STAT1-ERK axis in these cells.
of MAPK pathways in IFNγ signaling. Therefore, IFNγ activates at least two pathways in mast cells—the JAK/STAT pathway responsible for up-regulation of STAT1 and the p38/ERK pathway(s) that is responsible for up-regulation of CFTR. Although ERK activation is involved in CFTR up-regulation, PMA activates ERK but does not up-regulate CFTR (Fig. 1). This suggests that ERK activation requires activation of other molecules, perhaps p38, for up-regulation of CFTR mRNA.

The exact role of CFTR in mast cell function is unknown and is the subject of other work in our laboratory. To date, we have established that diphenylamine-2-carboxylate, a drug known to not only inhibit CFTR but also to have other activities, blocks FceRI-stimulated degranulation of PMC (Kulka et al., 2002a). Moreover, knockdown of CFTR expression by antisense oligonucleotides in the human mast cell line HMC-1 reduces Cl− flux, adhesion to fibronectin and calcium ionophore A23187-induced degranulation and IL-6 production (A. Schwingshackl and R. Dery, unpublished data). Our working hypothesis is that CFTR in mast cells is an important component of Cl− flux and perhaps of other activities, as recognized for epithelial cells (Rowe et al., 2005).

In turn, the role of mast cells in cystic fibrosis is unclear. Recently, mast cells have been recognized as important players in innate and acquired immune responses (Marshall, 2004). Moreover, there are increased numbers of mast cells in nasal polyps from cystic fibrosis patients compared with non-cystic fibrosis patients and many show signs of activation in cystic fibrosis (Henderson and Chi, 1992). Differences have also been found in mast cell numbers in human fetal trachea between cystic fibrosis and noncystic fibrosis specimens (Hubeau et al., 2001). Interestingly, mast cell numbers and mast cell-specific genes and others genes associated with innate immunity are up-regulated in the intestine in CFTR null mice that show a severe intestinal phenotype (Norkina et al., 2004). Thus, the role of mast cells in cystic fibrosis warrants further investigation.

The finding of IFNγ-mediated increase in CFTR expression and decrease in Cl− flux could be explained in several ways. For example, if IFNγ treatment leads to cell depolarization, this would tend to reduce Cl− flux under our experimental conditions, perhaps by channels other than CFTR. Alternatively, IFNγ may modulate expression of other proteins involved in Cl− flux, e.g., soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins, which inhibit CFTR activity by decreasing channel open probability (Cormet-Boyaka et al., 2002). It is also possible that although IFNγ increases CFTR expression in mast cells, this may not involve maturation of CFTR and its translocation to the plasma membrane, where it could be fully functional. Indeed, our confocal studies of CFTR expression support the hypothesis that the increase in CFTR expression in MC is mainly observed intracellularly, most likely in association with granules (Fig. 1D). In addition, studies of the biosynthetic processing and intracellular trafficking of CFTR indicate that CFTR undergoes constitutive endocytosis and recycling (Picciano et al., 2003). Thus, IFNγ treatment could affect the balance between CFTR degradation and recycling back to the plasma membrane, reducing the effective amount of CFTR in the plasma membrane.

The role of IFNγ-mediated up-regulation of CFTR in mast cell physiology is difficult to determine. Further studies are required to characterize the functional effects of increased CFTR on mast cell functions such as Cl− transport, degranulation, and mediator release in response to stimuli such as allergens. Furthermore, the transcription factors involved in CFTR up-regulation in mast cells also must be examined to provide insight into regulation of the CFTR promoter. The mechanisms that modulate CFTR gene expression through extracellular and intracellular signals may ultimately provide targets for therapy in cystic fibrosis where CFTR expression is abnormal.

Acknowledgments

We thank Lynelle Watt for help in the preparation of this manuscript and Dr. Dean D. Metcalfe for helpful advice. James Dooley provided skilled technical support for the confocal analyses.

References


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