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Interleukin-1β regulates CFTR expression in human intestinal T84 cells

Eduardo G. Cafferata [a,b,1], Anatilde M. González-Guerrico [a,1], Luciana Giordano [a], Omar H. Pivetta [b], Tomás A. Santa-Coloma [a,*]

[a] *Instituto de Investigaciones Bioquímicas-Fundación Campomar (IIB, UBA; IIBBA, CONICET), Patricias Argentinas 435, 1405 Buenos Aires, Argentina*

[b] *Centro Nacional de Genética Médica, ANLIS, Buenos Aires, Argentina*

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Abstract

Cystic fibrosis is an autosomal recessive genetic disease, produced by a mutation in the CFTR gene that impairs its function as a chloride channel. In this work, we have examined the effects of interleukin-1β (IL-1β) on the expression of CFTR in human colonic T84 cells. Treatment of T84 cells with IL-1β (0.25 ng/ml) for 4 h resulted in an increased CFTR expression (mRNA and protein). However, higher doses of IL-1β (1 ng/ml and over) produced inhibition of CFTR mRNA and protein expression. The protein kinase C (PKC) inhibitors H7 (50 μM) and GF109203X (1 μM) inhibited the stimulatory effect of IL-1β. Similar effects were seen in the presence of the protein tyrosine kinase (PTK) inhibitors genistein (60 μM) and herbymicin A (2 μM). These results suggest that some PKC isoform(s) and at least a PTK might be involved in the CFTR up-regulation induced by IL-1β. The repression of CFTR up-regulation by cycloheximide (35.5 μM) suggests the participation of a de novo synthesized protein. Results obtained by using the RNA polymerase II inhibitor DRB (78 μM), suggest that the increased mRNA levels seen after IL-1β treatment are not due to an increased stability of the message. We conclude that the CFTR mRNA and protein levels are modulated by IL-1β, this cytokine being the first extracellular protein known to up-regulate CFTR gene expression.

Keywords: Interleukin-1β; CFTR; Cystic fibrosis

1. Introduction

Cystic fibrosis is the most common life-threatening autosomal recessive genetic disorder in Caucasian populations. The disease affects primarily epithelial tissues of airway, intestine, pancreatic duct, genital tract, and sweat glands [1]. A failure in the cystic fibrosis transmembrane conductance regulator (CFTR) gene product is responsible for the disease [2]. The product of the gene is a chloride channel, which is regulated by protein kinase A phosphorylation [3,4] and belongs to a family of ATPase-dependent transporters [5]. More than 900 mutations have been reported [6]. The most important for the CFTR function is a deletion of a phenylalanine at position 508 (ΔF508) [7].

Airway obstruction and chronic endobronchial infection have been known for a long time to be important factors in the pathogenesis of lung disease in cystic fibrosis (CF). However, it was only recently recognized that the inflammatory process may play

* Corresponding author. Fax: +54-1-865-2246; E-mail: tasc@lab107.iib.uba.ar

[1] E.G.C. and A.M.G.-G. contributed equally to this work.

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an important role in lung damage [8]. In the respiratory airway, lymphoid cells are found adjacent to the secretory epithelium and produce cytokines in response to acute and chronic inflammation or infection [9]. Among the different cytokines produced by these cells, interleukin-1β (IL-1β) is particularly interesting since it has been found elevated in sputum samples from CF patients [10]. IL-1β can stimulate a wide range of cell types besides those with roles in immunity and inflammation [11]; however, its effects on CFTR mRNA or protein levels are unknown.

Studies done by other researchers using HT29 and T84 colon epithelial-derived tumor cells lines, known to express the CFTR gene, have demonstrated that IFN-γ [12] and TNF-α [13] down-regulate CFTR mRNA transcript levels, in a dose- and time-dependent manner. We have also used T84 cells as a model system to study the effects of IL-1β on CFTR gene expression, since in these cells the CFTR gene is highly expressed and the IL-1 receptor is also present [14]. Using this model system, we have obtained evidence that IL-1β up-regulates the steady-state levels of the CFTR mRNA and protein, being the IL-1β first extracellular up-regulator described for CFTR.

2. Materials and methods

2.1. Cell culture and treatments

T84 human colon carcinoma cells (ATCC cell line CCL248, Rockville, MD) were grown in DMEM-F12 1:1 mixture supplemented with 10 U/ml penicillin, 10 mg/ml streptomycin, 5% fetal bovine serum (Life Technology, Gaithersburg, MD). Sub-confluent cells (70–75%) were incubated in serum-free medium (10 ml in 100-mm Petri dishes) for 48 h and then IL-1β was added to the medium at different concentrations, as indicated in the legend to the figures. For inhibition experiments, the cells were preincubated with different inhibitors for 30 min before adding IL-1β. To inhibit protein kinase C (PKC), we used H7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine HCl) (Sigma, St. Louis, MO), at a final concentration of 50 μM [15] and the highly selective PKC inhibitor GF109203X (bisindolyl-maleimide I) (Sigma, St. Louis, MO), at a final concentration of 1 μM [16]. To inhibit protein tyrosine kinases, we used genistein (4,5,7-trihydroxysoflavone) (Sigma, St. Louis, MO), at a final concentration of 60 μM [17] and herbymicin A (Sigma, St. Louis, MO), at a final concentration of 2 μM [18]. Cycloheximide was used as inhibitor of protein synthesis (Sigma, St. Louis, MO), at a final concentration 35.5 μM [19]. DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) (Sigma, St. Louis, MO), which inhibits RNA polymerase II causing premature termination of transcription, was used at a final concentration of 78 μM [20]. TPA (12-*o*-tetradecanoylphorbol-13-acetate), (Sigma, St. Louis, MO) was used at a final concentration of 100 ng/ml (0.16 μM) as a control for down-modulation of the CFTR gene [21].

2.2. Preparation of total RNA

A guanidine-isothiocyanate denaturation procedure with subsequent phenol-chloroform extraction of the proteins was used as reported before [22]. The mRNA was precipitated from 50% isopropanol and the ratios at A_{260}/A_{230} (greater than 2.0) and A_{260}/A_{280} (from 1.7 to 2.0) were determined to verify RNA purity.

2.3. Northern blots

For Northern blot analysis, equal amounts of total RNA (30 μg) were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde and transferred to Nytran membranes [22]. After transference, RNA was stained using a solution of 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2), scanned and quantified (NIH Image program) to control sample loading [22]. The membranes were hybridized at 65°C with a 3.3-kb CFTR cDNA probe (ATCC 61136), labeled with ^{32}P ([α-^{32}P]dCTP, 3000 Ci/mmol, New England Nuclear, Boston, MA) by random priming (Prime a gene labeling system, Promega, Madison, WI), washed at 65°C and exposed for various times at −70°C using an intensifying screen as indicated previously [22].

2.4. Immunoblot analysis of CFTR expression

Subconfluent monolayers of T84 cells (2 days in serum free DMEM-F12), were incubated for 4 h in

the presence/absence of IL-1β or TPA. The monolayers were washed with ice-cold PBS and lysated in Ripa buffer (25 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP40, 2 mM EDTA, pH 8). Nuclei and unbroken cells were removed by centrifugation (15 000 × *g* for 15 min at 4°C). Soluble proteins in the supernatant were denatured with Laemmli sample buffer, fractionated on 7% PAGE gels and transferred to a nitrocellulose membrane. Transferred proteins were probed with the monoclonal anti-CFTR antibody M3A7 [23], generously provided by Dr. J.R. Riordan. The primary antibody was visualized by using horseradish peroxidase-conjugated anti-mouse immunoglobulin and the enhanced chemiluminescence (ECL) Western blot kit (Amersham, Little Chalfont, Buckinghamshire, UK).

2.5. Quantification and statistical analysis

Northern an Western blots were scanned in an HP4C scanner and quantified by using the NIH Image program PC compatible (http://www.scioncorp.com). Sample loading in Northern blots was quantified by using methylene blue staining. A lineal response to methylene blue staining was obtained up to 40 μg of total RNA, therefore, a maximum of 30 μg was used in each assay. Statistical analysis was done by ANOVA and Tukey HSD test.

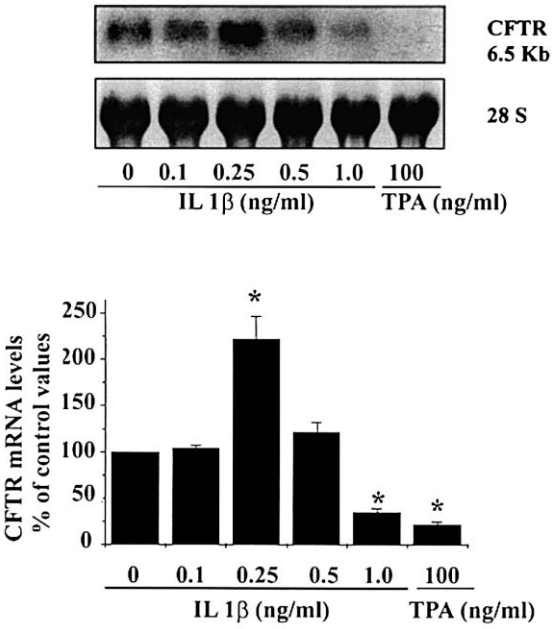

Fig. 1. Northern blot analysis of CFTR expression in T84 cells treated with IL-1β. T84 human carcinoma cells were treated with varying concentrations of IL-1β for 4 h. Northern blot analysis was then performed using total RNA (30 μg) and a 3.3-kb CFTR cDNA probe. The image was quantified and normalized for RNA loading using the rRNAs of the same membrane, stained with methylene blue (indicated as 28S). In different experiments, the maximum response varied between 0.25–0.5 ng/ml. Quantification of CFTR mRNA expression is shown in the bottom panel. Values are mean ± S.E.M.($n = 6$) expressed as percentage of control. Significant differences compared to 0.1 ng/ml IL-1β: $^*P < 0.01$.

3. Results and discussion

We first studied the possible effects of IL-1β on the CFTR mRNA levels. As shown in Fig. 1, a biphasic effect was observed after treatment of T84 colonic carcinoma cells with IL-1β for 4 h. Maximal IL-1β stimulation was obtained at 0.25 ng/ml and inhibition at 1.00 ng/ml. The concentration of IL-1β to obtain maximum response exhibited a variation in the range of 0.25–0.50 ng/ml for separate experiments (not shown). At concentrations of IL-1β of 1.00 ng/ml (Fig. 1) and higher (up to 5.0 ng/ml were tested, results not shown), a strong inhibitory effect was observed, comparable to the inhibition seen with TPA [21]. Although this is the first time that a biphasic effect for IL-1β is reported, similar behavior has been observed for TNF-α [24], which shares some intracellular pathways with IL-1β. Similar biphasic effects were seen by using Caco-2 and LS180 cells (results not shown).

To determine whether the levels of the CFTR protein correlated with the modulation of its mRNA by IL-1β, a Western blot analysis was performed. For the analysis, we used the monoclonal antibody M3A7, which recognizes the CFTR protein [23]. As shown in Fig. 4, the CFTR protein was elevated in the presence of IL 1β (0.5 ng/ml) and decreased in the presence of IL 1β at 2.5 ng/ml. The protein levels were also diminished in the presence of TPA (100 ng/ml). These results show a good correlation with those obtained by Northern blot analysis, indicating that IL-1β affects both the CFTR mRNA and protein. Although IL-1β up-regulates the CFTR gene and protein, this is done in a very narrow range (around 0.25–0.5 ng/ml). At higher concentrations (1 ng/ml and over), a very strong inhibition of the message

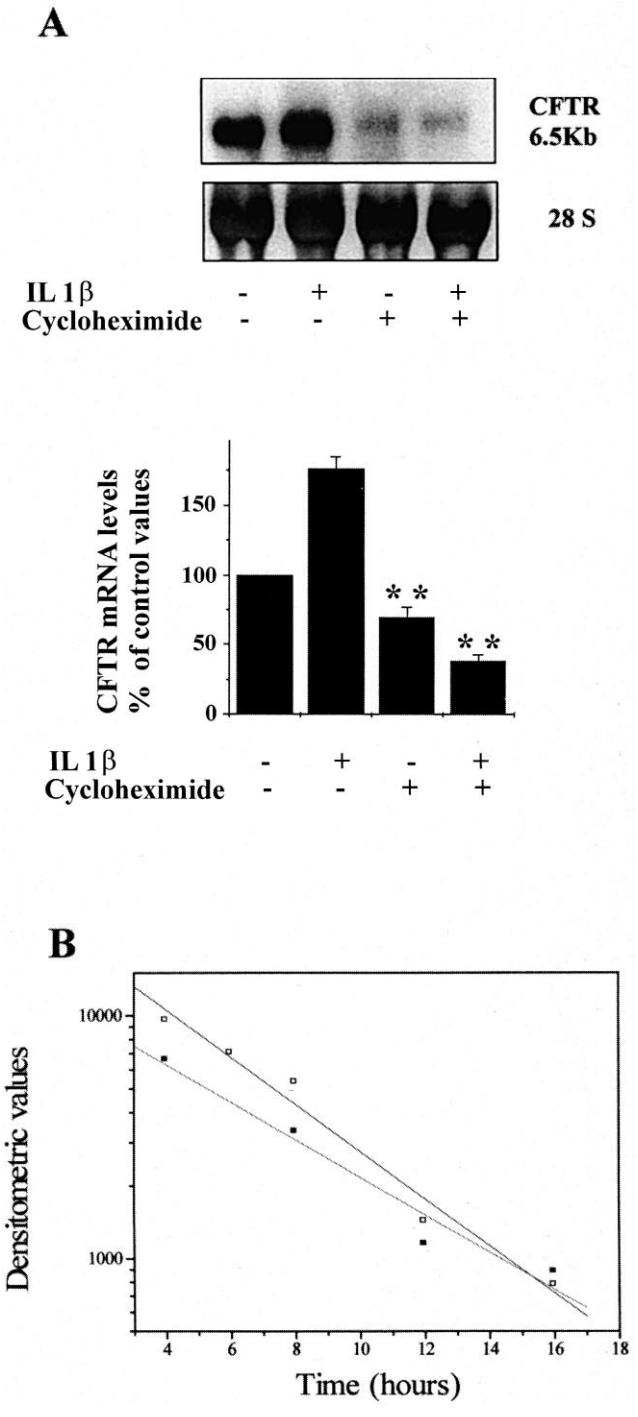

Fig. 2. Northern blot analysis of CFTR in T84 cells treated with IL-1β and cycloheximide (CHX) and DRB. (A) T84 cells were pre-incubated for 30 min in the presence or absence of CHX (35.5 μM) and then incubated for 4 h with IL-1β (0.25 ng/ml). Then, Northern analysis was performed on total RNA (30 mg). The rRNA on the same membrane (indicated as 28S) was stained with methylene blue to control for sample loading. Inhibition of both basal and IL-β stimulated cells was observed. Quantification of CFTR mRNA expression is shown in the bottom panel. Values are mean ± S.E.M.($n = 2$) expressed as percentage of control. Significant differences as compared to 0.25 ng/ml IL-1β: $^{**}P < 0.05$. (B) T84 cells were incubated in the presence (open squares) or in the absence (closed squares) of IL-1β (0.5 ng/ml) for 4 h. Then, DRB (78 μM) was added to block further transcription and the CFTR mRNA levels where determined at different times (4–16 h) by using Northern blot analysis on total RNA (30 μg).
←

and protein was observed. Interestingly, the inhibitory effect of IL-1β on the CFTR protein was even stronger than the effect obtained with TPA at 100 ng/ml. This is noteworthy, since the CFTR protein has normally a long half-life time (longer than 24 h) [25]. Therefore, it seems that IL-1β, at high doses, not only reduces mRNA levels for CFTR, but also reduces the protein half-life.

To determine whether the up-regulation of the CFTR by IL-1β required de novo synthesis of proteins, control and stimulated cells were incubated with cycloheximide (CHX). In the presence of CHX (35.5 μM), both basal and IL-1β (0.50 ng/ml) stimulated cells showed a decrease in the CFTR mRNA levels, suggesting that maintenance of basal and stimulated steady-state levels of the CFTR mRNA requires de novo protein synthesis (Fig. 2A). To study whether the effects of IL-1β were due to increased CFTR mRNA stability, cells were incubated in the presence or absence of IL-1β (0.50 ng/ml for 4 h), and then were exposed to DRB (78

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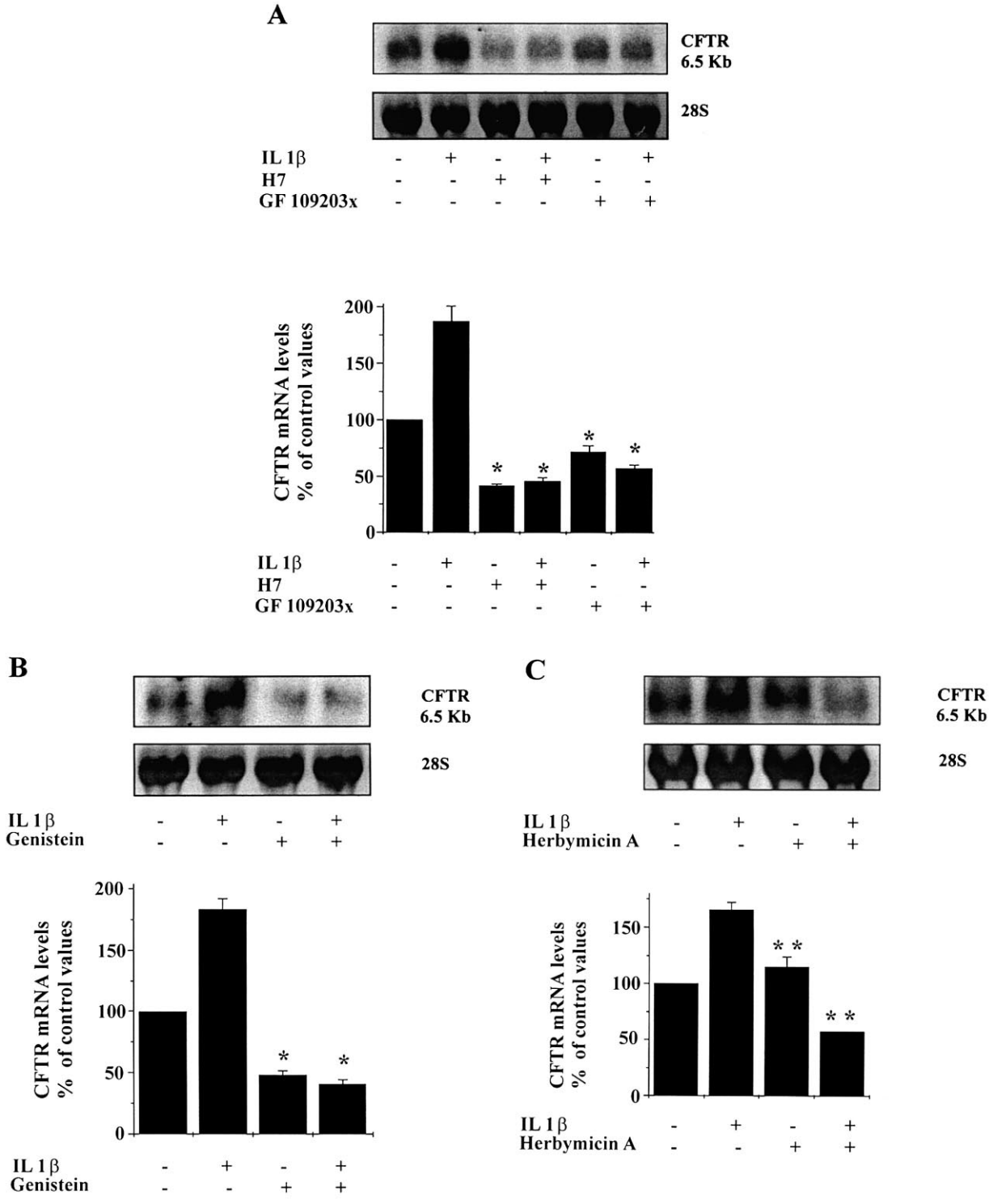
Fig. 3. Effects of PKC and PTK inhibitors on the up-regulation of the CFTR by IL-1β. T84 cells were preincubated with PKC inhibitors (H7 50 μM and GF109203X 1.0 μM) and PTK inhibitors (genistein 60 μM and herbymicin A 2 μM) for 30 min and then incubated for 4 h in the presence or absence of IL-1β at concentrations that elicit maximum stimulation (0.25–0.5 ng/ml depending on the IL-1β batch). Northern blot analysis for CFTR was then performed on total RNA (30 μg). (A) IL-1β 0.25 ng/ml, H7, GF109203X. (B) IL-1β 0.5 ng/ml, genistein. (C) IL-1β 0.5 ng/ml, herbymicin A. Immediately after transference, rRNA (28S) on the same membrane was stained with methylene blue, to control for sample loading. Quantification of CFTR mRNA expression is shown in the bottom panel of each figure. Values are mean ± S.E.M. ($n = 3$) expressed as percentage of control. Significant differences compared to the maximum IL-1β response: $^{*}P < 0.01$; $^{**}P < 0.05$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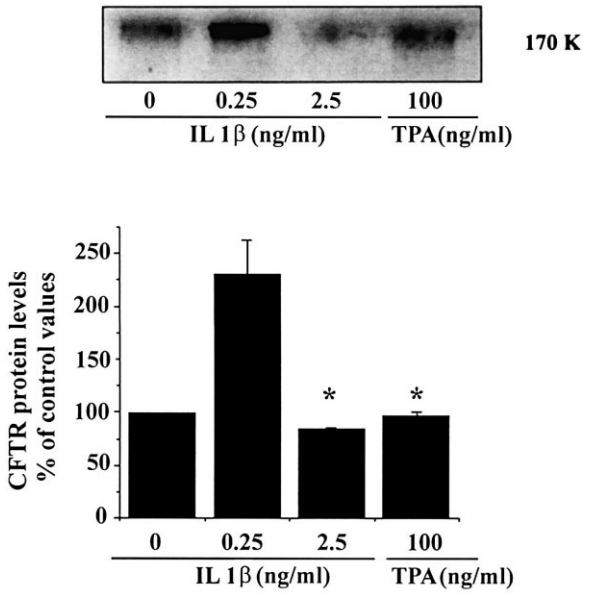

Fig. 4. Immunoblot analysis of CFTR expression in T84 cells in response to IL-1β. T84 cells were incubated for 4 h in the presence/absence of IL-1β or TPA. Solubilized T84 proteins (100 μg) were separated on a 7% SDS-polyacrylamide gel and probed with the monoclonal antibody M3A7 that recognizes the CFTR protein. The spots were detected by using chemiluminescence. Quantification of CFTR protein expression is shown in the bottom panel. Values are mean ± S.E.M. ($n = 4$) expressed as percentage of control. Significant differences compared to maximum response to IL-1β: $^*P < 0.01$.

μM) for 4–16 h. CFTR mRNA levels decreased similarly in both cases (control and IL-1β-treated cells) (Fig. 2B), with a slight increase in the slope of the IL-1β-treated cells. These results suggest that the up-regulation in the CFTR mRNA steady-state levels induced by IL-1β (at low doses) does not reflect an increased stability of the CFTR transcripts. Interestingly, IL-1β behaves differently from TNF-α, that produces down-modulation of CFTR mRNA due to changes in message stability [13].

Different protein-kinase inhibitors were then used to obtain preliminary evidence about possible mechanisms involved in the up-regulation of the CFTR mRNA induced by IL-1β. First, we studied the effects of the PKC inhibitors H7 and GF109203X. As shown in Fig. 3A, both PKC inhibitors were able to inhibit IL-1β induction of CFTR mRNA, suggesting that a PKC isoform might be involved in the mechanism of up-regulation of CFTR induced by IL-1β. Similar results have been reported in osteoblast-like cells, in which induction of IL-6 by IL-1 seems to be mediated by PKC [26]. Both inhibitors also affected basal levels of CFTR expression, although a stronger effect was obtained with H7.

Then, we decided to test whether protein tyrosine kinases (PTKs) also might be involved in the up-regulation induced by IL-1β. Cells were preincubated for 30 min with genistein (60 μM) and herbymicin A (2 μM) as selective PTK inhibitors. Under these conditions, inhibition of the IL-1β-stimulated CFTR expression was observed with both inhibitors (Fig. 3B,C). Inhibition of basal CFTR levels was observed only in the presence of genistein. These results suggest that a PTK-dependent pathway might be also involved in the up-regulation of the CFTR mRNA induced by IL-1β. Other researchers obtained similar results with the bradykinin B1 receptor gene, which is up-regulated by IL-1β [27] by a PTK. However, the regulation of the bradykinin receptor by IL-1β does not requires protein synthesis, it is achieved through a PTK and not PKC, and showed both transcriptional activation and post-transcriptional mRNA stabilization. On the other hand, the synthesis of IL-8 is stimulated in HT-29 cells (also a colon cell line) by IL-1β in a way that is independent of PKC, but depends on protein tyrosine-phosphorylation [28]. Therefore, in T84 cells, the response of the CFTR gene to IL-1β appears to be different, since the up-regulation of the CFTR by IL-1β requires both a PKC and a PTK.

It is interesting that IL-1β is elevated in the sputum from CF patients [10], possibly as a result of the chronic infection with *Pseudomonas aeruginosa*. Therefore, IL-1β cytokine might reach enough concentration in situ to produce a strong inhibition of the CFTR gene and protein expression. In CF patients, such a strong inhibitory effect could potentially deteriorate even more the already affected accessibility of the CFTR mutated protein to the plasma membrane [29]. Therefore, it would be very important to further elucidate the mechanisms involved in the biphasic response obtained with IL-1β on CFTR, which might facilitate the identification of new targets for possible therapy.

In summary, we present here the first evidence for a regulatory role of IL-1β on CFTR expression. Using specific inhibitors, we have obtained preliminary evidence suggesting that a PKC isoform and at least one PTK might be involved in the up-regulation of

CFTR by IL-1β. The possible mechanisms involved in the down-modulation of CFTR by IL-1β at doses of 1.00 ng/ml and higher are unknown, although they might be similar to those involved in the down-modulation induced by treatments with TNF-α and TPA.

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References

[1] S.J. Tebbutt, Animal studies of cystic fibrosis, Mol. Med. Today 1 (1995) 336–342.
[2] J.R. Riordan, J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.L. Chou, Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, Science 245 (1989) 1066–1073 [Erratum, Science 245 (1989) 1437].
[3] R.A. McDonald, R.P. Matthews, R.L. Idzerda, G.S. McKnight, Basal expression of the cystic fibrosis transmembrane conductance regulator gene is dependent on protein kinase A activity, Proc. Natl. Acad. Sci. USA 92 (1995) 7560–7564.
[4] J. Luo, M.D. Pato, J.R. Riordan, J.W. Hanrahan, Differential regulation of single CFTR channels by PP2C, PP2A, and other phosphatases, Am. J. Physiol. 274 (1998) C1397–1410.
[5] J.R. Riordan, The cystic fibrosis transmembrane conductance regulator, Annu. Rev. Physiol. 55 (1993) 609–630.
[6] L.C. Tsui, CF Genetic Analysis Consortium http:/www.genet.sickkids.on.ca/cftr (1999).
[7] B. Kerem, J.M. Rommens, J.A. Buchanan, D. Markiewicz, T.K. Cox, A. Chakravarti, M. Buchwald, L.C. Tsui, Identification of the cystic fibrosis gene: genetic analysis, Science 245 (1989) 1073–1080.
[8] M.W. Konstan, M. Berger, Current understanding of the inflammatory process in cystic fibrosis: onset and etiology, Pediatr. Pulmonol. 24 (1997) 137–142; discussion 159–156.
[9] R.G. Crystal, Oxidants and respiratory tract epithelial injury: pathogenesis and strategies for therapeutic intervention, Am. J. Med. 91 (1991) 39S–44S.
[10] A. Schuster, A. Haarmann, V. Wahn, Cytokines in neutrophil-dominated airway inflammation in patients with cystic fibrosis, Eur. Arch. Otorhinolaryngol. 1 (Suppl.) (1995) S59–60.
[11] A.J. Cunningham, C.A. Murray, L.A. O'Neill, M.A. Lynch, J.J. O'Connor, Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro, Neurosci. Lett. 203 (1996) 17–20.
[12] F. Besancon, G. Przewlocki, I. Baro, A.S. Hongre, D. Escande, A. Edelman, Interferon-gamma downregulates CFTR gene expression in epithelial cells, Am. J. Physiol. 267 (1994) C1398–1404.
[13] H. Nakamura, K. Yoshimura, G. Bajocchi, B.C. Trapnell, A. Pavirani, R.G. Crystal, Tumor necrosis factor modulation of expression of the cystic fibrosis transmembrane conductance regulator gene, FEBS. Lett. 314 (1992) 366–370.
[14] E.P. Molmenti, T. Ziambaras, D.H. Perlmutter, Evidence for an acute phase response in human intestinal epithelial cells, J. Biol. Chem. 268 (1993) 14116–14124.
[15] G. Forstner, Y. Zhang, D. McCool, J. Forstner, Regulation of mucin secretion in T84 adenocarcinoma cells by forskolin: relationship to Ca2+ and PKC, Am. J. Physiol. 266 (1994) G606–612.
[16] E. Batlle, J. Verdu, D. Dominguez, M. del Mont Llosas, V. Diaz, N. Loukili, R. Paciucci, F. Alameda, A.G. de Herreros, Protein kinase C-alpha activity inversely modulates invasion and growth of intestinal cells, J. Biol. Chem. 273 (1998) 15091–15098.
[17] R.K. Rao, R.D. Baker, S.S. Baker, A. Gupta, M. Holycross, Oxidant-induced disruption of intestinal epithelial barrier function: role of protein tyrosine phosphorylation, Am. J. Physiol. 273 (1997) G812–823.
[18] C.T. Taylor, A. Murphy, D. Kelleher, A.W. Baird, Changes in barrier function of a model intestinal epithelium by intraepithelial lymphocytes require new protein synthesis by epithelial cells, Gut 40 (1997) 634–640.
[19] C. Carrillo, E.G. Cafferata, J. Genovese, M. O'Reilly, A.B. Roberts, T.A. Santa-Coloma, TGF-beta1 up-regulates the mRNA for the Na+/Ca2+ exchanger in neonatal rat cardiac myocytes, Cell. Mol. Biol. 44 (1998) 543–551.
[20] R.O. Zandomeni, Kinetics of inhibition by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole on calf thymus casein kinase II, Biochem. J. 262 (1989) 469–473.
[21] B.C. Trapnell, P.L. Zeitlin, C.S. Chu, K. Yoshimura, H. Nakamura, W.B. Guggino, J. Bargon, T.C. Banks, W. Dalemans, A. Pavirani, Down-regulation of cystic fibrosis gene mRNA transcript levels and induction of the cystic fibrosis chloride secretory phenotype in epithelial cells by phorbol ester, J. Biol. Chem. 266 (1991) 10319–10323.
[22] E.G. Cafferata, A.M. Gonzalez-Guerrico, O.H. Pivetta, T.A. Santa-Coloma, Identification by differential display of a mRNA specifically induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in T84 human colon carcinoma cells, Cell. Mol. Biol. 42 (1996) 797–804.
[23] A. Mohamed, D. Ferguson, F.S. Seibert, H.M. Cai, N. Kartner, S. Grinstein, J.R. Riordan, G.L. Lukacs, Functional expression and apical localization of the cystic fibrosis

transmembrane conductance regulator in MDCK I cells, Biochem. J. 322 (1997) 259–265.
[24] G. van der Pluijm, W. Most, L. van der Wee-Pals, H. de Groot, S. Papapoulos, C. Lowik, Two distinct effects of recombinant human tumor necrosis factor-alpha on osteoclast development and subsequent resorption of mineralized matrix, Endocrinology 129 (1991) 1596–1604.
[25] G.L. Lukacs, X.B. Chang, C. Bear, N. Kartner, A. Mohamed, J.R. Riordan, S. Grinstein, The delta F508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells, J. Biol. Chem. 268 (1993) 21592–21598.
[26] O. Kozawa, A. Suzuki, H. Tokuda, T. Kaida, T. Uematsu, Protein kinase C activation by interleukin (IL)-1 limits IL-1-induced IL-6 synthesis in osteoblast-like cells: involvement of phosphatidylcholine-specific phospholipase C, J. Cell. Biochem. 67 (1997) 103–111.
[27] X. Zhou, P. Polgar, L. Taylor, Roles for interleukin-1beta, phorbol ester and a post-transcriptional regulator in the control of bradykinin B1 receptor gene expression, Biochem. J. 330 (1998) 361–366.
[28] V. Gross, T. Andus, R. Daig, E. Aschenbrenner, J. Scholmerich, W. Falk, Regulation of interleukin-8 production in a human colon epithelial cell line (HT-29), Gastroenterology 108 (1995) 653–661.
[29] A.E. Smith, Treatment of cystic fibrosis based on understanding CFTR, J. Inherit. Metab. Dis. 18 (1995) 508–516.