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ARTICLE *in* MOLECULAR IMMUNOLOGY · APRIL 2012

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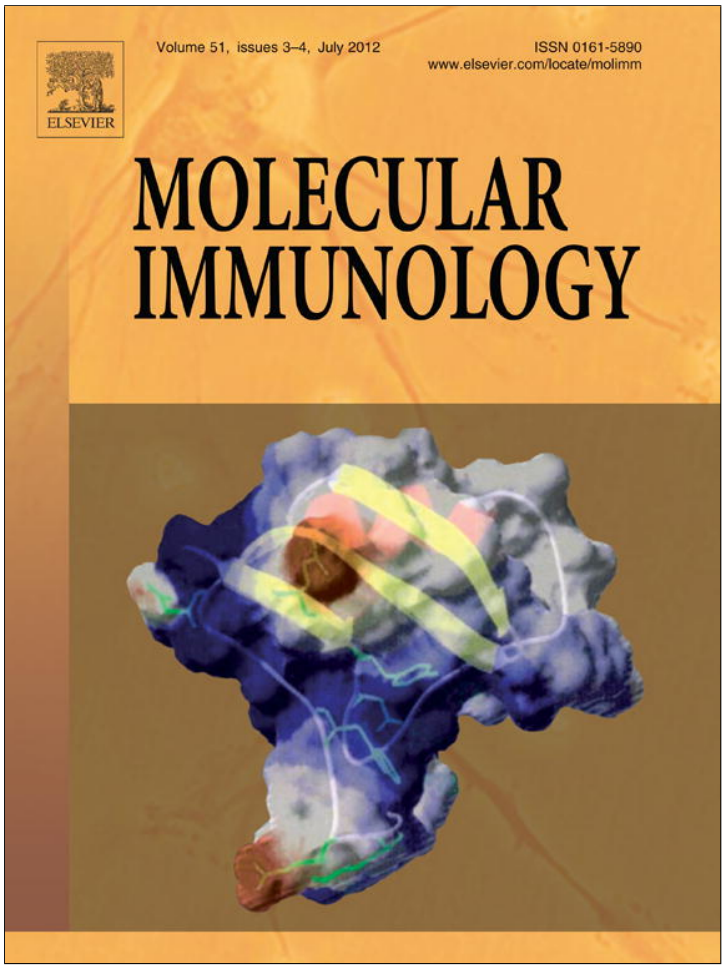

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Molecular Immunology 51 (2012) 310–315

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

mGluR1 interacts with cystic fibrosis transmembrane conductance regulator and modulates the secretion of IL-10 in cystic fibrosis peripheral lymphocytes

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ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form 23 March 2012
Accepted 26 March 2012
Available online 18 April 2012

Keywords:
Cystic fibrosis
Glutamate
IL-10
CFTR

ABSTRACT

Cystic fibrosis (CF) is caused by the mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. CFTR dysfunction in T cells could lead directly to aberrant immune responses. The action of glutamate on the secretion of IL-8 and IL-10 by lymphocytes derived from healthy subjects and cystic CF patients, as well as the expression of metabotropic glutamate receptor subtype 1 (mGluR1) in the membrane fractions of lymphocytes was investigated. Our results have shown that CF-derived T-cells in the presence of IL-2 produce more IL-8 and IL-10, than T-cell from healthy control. However, only in normal lymphocytes a significant increase (144%) in the IL-10 secretion during exposure to high concentration of glutamate (10^{-4} M) was detected. Glutamate-dependent secretion of IL-10 was not inhibited either by NMDA-receptor (NMDAR), or by AMPA-receptor (AMPAR) antagonist. Only mGluR1 antagonist, LY367385, strongly decreases the production of IL-10. Furthermore, the content of mGluR1, as well as cystic fibrosis transmembrane conductance regulator-associated ligand (CAL), Na^+/H^+ exchanger regulatory factor 1 (NHERF-1), was analyzed in plasma membrane of lymphocytes after immunoprecipitation of CFTR. We have found that normal, non-mutated CFTR, as well as mutated forms of CFTR were associated with metabotropic mGluR1, but the level of surface exposed mGluR1 in CF-lymphocytes was much lower than in normal cells. Besides, our results have shown that normal, non-mutated CFTR, as well as mutated forms of CFTR were associated with NHERF-1 and CAL; however in lymphocytes with CFTR mutation the amount of cell-surface expressed CFTR-CAL complex was greatly decreased. We have concluded that CFTR and mGluR1 could compete for binding to CAL, which in turn downregulates the post-synthetic trafficking of mGluR1 and decreases the synthesis of IL-10.
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1. Introduction

Cystic fibrosis (CF), the most common inherited lethal disorder among Caucasians, is caused by the mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel (Riordan et al., 1989; Guggino and Banks-Schlegel, 2004). Although CF manifests as a multiorgan disease, chronic airway dysfunction and inflammation are the main cause of morbidity and mortality among patients (Sheppard and Welsh, 1999; Pamela and Davis, 2006). Airways inflammation in CF is characterized by elevated number and proportion of neutrophils, lymphocytes and their products, high level of the proinflammatory cytokines, especially IL-8 and low concentration of immunosuppressive cytokines, like IL-10, suggesting that an intrinsic cytokine dysregulation might exist in immunoregulatory cells functionally expressing mutant CFTR (Moss et al., 2000; Hubeau et al., 2001; Soltys et al., 2002; Lyczak et al., 2002). An elevated T-cell number in the CF bronchial epithelium has been reported, suggesting an important role of these cells in the disease (Hubeau et al., 2001). Since infiltrated human T cells transcribe the CFTR gene and exhibit CF-defective cAMP-regulated chloride current (McDonald et al., 1992), further study of CF derived lymphocytes would be necessary to define a functional significance of primary immune defect.

Elevated glutamate concentrations could impair lymphocyte functions and cause secondary immunopathological consequences (Eck et al., 1989). This effect of high glutamate produces the suppression of mitogen-induced proliferation and is mediated by glutamate receptors (Droge et al., 1988). Human T lymphocytes express various types of glutamate receptors which control immune responses, cell activation, maturation and death (Lombardi et al., 2001; Ganor et al., 2003; Boldyrev et al., 2004; Miglio et al., 2005; Chiocchetti et al., 2006; Kvaratskhelia et al.,

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http://dx.doi.org/10.1016/j.molimm.2012.03.029

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2009). By direct interaction with the AMPAR of lymphocytes, glutamate triggers the integrin mediated adhesion of laminin and fibronectin (Ganor et al., 2003). Besides, glutamate changes the activity of voltage-gated potassium channels and impairs T lymphocyte proliferation through mGluR (Poulopoulou et al., 2005). Activation of lymphocytes by N-methyl-D-aspartate through NMDAR increases the content of intracellular calcium and reactive oxygen species (Miglio et al., 2005; Boldyrev et al., 2005) and changes the secretion of both, IL-8 and IL-10 (Kvaratskhelia et al., 2009). These findings suggest that lymphocytes express several types of glutamate receptors modulation of which might be beneficial for the treatment of various neuroinflammatory diseases.

Large amounts of endogenous glutamate were released from the lungs after various types of airway inflammation (Dickman et al., 2004; Wang et al., 2009). Since injured neutrophils release glutamate in high (millimolar) concentration (Collard et al., 2002), it could be proposed that elevated amount of neutrophils during airway inflammation causes further activation of lymphocytes. In addition, during the maturation process, dendritic cells (DC) release physiologically relevant amounts of glutamate, which are high enough to activate GluRs (Pacheco et al., 2006). DC-derived glutamate, after productive Ag presentation, stimulates the expression of metabotropic glutamate receptor subtype 1 and enhances T cell proliferation and secretion of Th1 and proinflammatory cytokines. Altogether suggests that mGluR1 could play an important role in lymphocyte regulation during various lung inflammatory diseases. In this study we have analyzed the levels of mGluR1 in the lymphocytes derived from the patients with CF and found that expression of mGluR1a/b in the plasma membranes of CF lymphocytes was greatly decreased during this lung inflammatory disease. Besides, we have found that lymphocytes from CF patients were unable to produce IL-10 in response to high concentration of glutamate and IL-2. We have assumed that the reduced sensitivity to glutamate may be the result of low expression of mGluR1a/b in plasma membrane of CF-lymphocytes, which in turn, decreases the secretion of IL-10.

2. Materials and methods

2.1. Materials

All the reagents were purchased from Sigma–Aldrich (Sigma–Aldrich Inc., USA) unless otherwise specified. Polyclonal antibodies against the CFTR, CAL, NHERF1, mGluR1a/b were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Subjects

Peripheral blood (10–15 ml) was obtained by venipuncture from adult subjects. The study subjects included 12 healthy adult laboratory personnel (six males, six females, ages 4–15 years) and 18 patients with CF (7 males, 11 females, age 4–14 years). All patients with CF had diagnosis confirmed by pilocarpine iontophoresis sweat test and were pancreatic insufficient. The CF patients were in stable clinical condition, were not suffering from pulmonary exacerbation, and were not receiving systemic corticosteroids. All CF patients were on chronic suppressive antibiotic therapy for chronic bacterial endobronchitis. All patients were genotyped for 31 CFTR mutations. Six patients (50%) were homozygous for the ΔF508 mutation. Except for one patient who was heterozygous for G542x, the remainders (45%) were heterozygous for ΔF508. The study was approved by Human Subjects Committee of the Institute of Medical Biotechnology and informed consent was obtained from all participants.

2.3. PBL (peripheral blood lymphocyte) preparation

Venous blood from healthy subjects and CF patients was diluted with an equal volume of Hepes-buffered saline (pH 7.4; 10 mM Hepes, 0.1% BSA, 5 mM D-glucose, 145 mM NaCl and 5 mM KCl). Mononuclear cells were separated by density gradient centrifugation on Ficoll-Paque and then washed twice; monocytes were depleted by adhesion to plastic. Preparations have showed that >98% of the cells were small mature lymphocytes.

Cells were washed 3 times with glutamate-free RPMI-1640, plated at $2–3 \times 10^5$ cells/well, prestimulated by IL-2 (10 ng/ml, 37 °C, 4 h) and incubated in RPMI-1640 supplemented with 1×10^{-5} M of glutamate in a humidified atmosphere containing 5% CO_2 for 48 h at 37 °C. No serum was added to the cultures. After incubation, the cells were separated from medium by centrifugation at $800 \times g$ for 20 min. Viability as assessed by 0.4% trypan blue exclusion was 70–80%.

2.4. Preparation of cytosolic and plasma membrane-enriched fractions

Preparation of plasma membrane-enriched fraction for immunoprecipitation and blotting was performed by Cuadra et al. (2004), with small modifications. Briefly, lymphocytes were washed with ice-cold PBS and resuspended in 0.5 ml of hypotonic lysis buffer containing 20 mM Tris–HCl, pH 7.5, 5 mM EDTA, 1 mM DTT, 0.5 mM PMSF and protease inhibitors' cocktail. After 30 min incubation at 4 °C temperature, cells were homogenized, nuclei and cells' debris were removed by centrifugation for 10 min at $1000 \times g$. Membranes were obtained by centrifugation of the post-nuclear homogenate for 40 min at $14{,}000 \times g$, supernatants were lyophilized and used as cytosolic preparations. Obtained pellets were resuspended in 1% sodium cholate in 50 mM Tris–HCl, pH 7.5, incubated overnight at 4 °C temperature and re-centrifuged for 40 min at $14{,}000 \times g$. Supernatants were used as solubilized preparations.

2.5. Immunoprecipitation and Western blotting

Cytosolic and plasma membrane (PM) solubilized proteins were incubated with anti-CFTR or anti-mGluR1a/b antibodies for 60 min at 4 °C. In immunoprecipitation experiments protein A/G-agarose (20 μl) was added and the incubation continued for 2 h. Samples were centrifuged at $2500 \times g$ and the pellets were washed four times with TEE buffer (50 mM Tris–HCl, pH 7.4, 1 mM EDTA, and 1 mM EGTA). The combined supernatant obtained after immunoprecipitation, as well as pellets were concentrated by lyophilization, boiled with SDS-PAGE sample buffer, and resolved by SDS-PAGE.

For immunoblotting experiments 50 μg of protein was separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose sheets. After blocking with blocking buffer (5% bovine serum albumin, 0.05% Tween 20 in Tris–HCl-buffered saline), the sheets were incubated with primary antibodies in the blocking solution. Labeled bands were visualized using enhanced chemiluminescence (Amersham, California, USA) and analyzed by densitometric scanning. The content of proteins was quantified from the intensity of the bands, which is linear to the quantity of samples applied to the gel.

Protein concentration was determined using a dye-binding method (Bio-Rad).

2.6. ELISA for IL-8 and IL-10

The ELISAs for IL-8 and IL-10 detection, which were sensitive down to a level of 5 pg/ml and 2 pg/ml, respectively, were performed in the culture supernatants by following the manufacturer's

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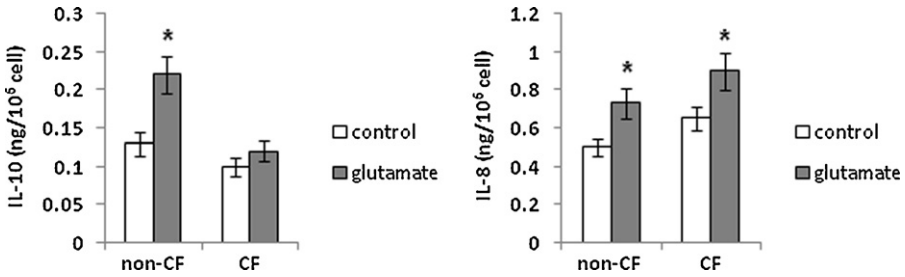

Fig. 1. Levels of IL-8 and IL-10 secreted in cultured human non-CF and CF T-lymphocytes after their exposure for 48 h to glutamate (10^{-4} M) in the presence of IL-2 (20 ng/ml). Note different *y* axis scale for each cytokine panel. Values in the ELISAs (means ± SD) are representative of three experiments and are reported as ng of IL-8 or IL-10/ml/10^6 cells. Each assay was performed at least in duplicate. *$P < 0.05$ compared with the control (in the absence of glutamate).

instructions in commercially available ELISA kits (Biosource International, Camarillo, CA). Data were expressed as pg/ml/1×10^6 viable cells.

2.7. Statistical analysis

Differences between CF patients and healthy subjects were compared using a two-way analysis of variance (ANOVA) and data from each experiment were analyzed separately. Data are expressed as means ± SD. A *P* value < 0.05 was considered significant.

3. Results

Proinflammatory cytokine dysregulation and neutrophil-dominated inflammation play major roles in the pathogenesis of lung disease in CF (Lyczak et al., 2002). IL-8 is markedly increased in CF bronchial samples, where it contributes to the transendothelial migration of neutrophils into the CF airways (Muhlebach and Noah, 2002). On the other hand, deficiency of anti-inflammatory molecules such as IL-10 was found in airways of cystic fibrosis patients (Chmiel et al., 2002). Thus, imbalance of pro-inflammatory and anti-inflammatory cytokines results in an excessive inflammation in the CF lung tissue. Considering that glutamate could be a modulator of T-cell activation (Pacheco et al., 2006), we have studied the effect of glutamate (10^{-4} M) on the secretion of IL-8 and IL-10 by lymphocytes derived from CF patients. Quantification of cytokines by ELISA revealed elevated levels of IL-8 in CF lymphocytes, whereas the secretion of IL-10 was not changed significantly (Fig. 1). Additions of glutamate markedly increase the production of IL-8 in both, CF and non-CF lymphocytes; however, elevated secretion of IL-10 under the action of glutamate was detected only in non-CF lymphocytes. These data show that lymphocytes derived from healthy subjects are characterized by a higher capability to produce IL-10 level in response to glutamate, than CF lymphocytes.

To identify the type of glutamate receptors associated with the production of interleukins in CF and non-CF lymphocytes, the effects of glutamate receptors antagonists on the production of interleukins were analyzed. Neither NMDA-R antagonist (MK-801), nor AMPAR antagonist (NBQX) changes glutamate-dependent production of interleukins (Fig. 2). Only LY-367385, an antagonist of group 1 mGluR significantly decreases the production of IL-10 in normal lymphocytes. Thus, these data suggest that the effect of glutamate on the IL-10 production in normal lymphocytes is mediated by mGluR1.

To evaluate the hypothesis that reduced susceptibility to glutamate in CF lymphocytes was a result of mGluR1 down-regulation, total amount of surface expressed mGluR1 in lymphocytes was determined. For this purpose, the content of mGluR1a/b in the solubilized plasma membrane preparation was analyzed. A robust decrease of the level of mGluR1a/b in CF lymphocytes compared with the control preparation was found, suggesting that surface expression of mGluR1 in the lymphocytes derived from CF patients was significantly lower (Fig. 3). However, any changes in the amount of glutamate receptor in the cytosolic fraction of lymphocytes were not observed.

To find out, how the reduction of mGluR1 is associated with CFTR-mutation, we have performed the immunoprecipitation experiments and quantitative analysis of CFTR-binding partners. Several studies have revealed a complex network of protein–protein interactions that are required for correct CFTR trafficking, including a number of PDZ-containing (PSD-95, disks-large, zonula occludens-1) proteins (Guggino and Stanton, 2006). C terminus of CFTR could bind several PDZ domain-containing proteins, like Na^+/H^+ exchanger regulatory factor (NHERF) or CFTR-associated protein (CAL) (Cheng et al., 2002; Broere et al., 2007). Some of these proteins, for example NHERF1, rescue functions of mutated CFTR (Bossard et al., 2007), suggesting that protein–protein interaction through PDZ domains possibly is very important for surface expression of mutant CFTR. On the other hand, several PDZ-containing proteins also bind to the glutamate receptor subtypes, that is important in forming of the spatial convergence of signaling mechanisms (Kim and Sheng, 2004). To test whether human lymphocytes express glutamate receptors associated with CFTR in the plasma membrane, we have attempted to detect mGluR1a/b, in human blood lymphocytes derived from CF patients and healthy subjects after immunoprecipitation and Western blotting. Thus, firstly, the solubilized membrane proteins derived from lymphocytes were incubated with anti-CFTR antibody to immunoprecipitate CFTR protein, and the immunoprecipitates were probed for mGluR1a/b, as well as for adapter proteins, such as CAL and NHERF1, using corresponding antibodies. A robust co-immunoprecipitation of CFTR with mGluR1a/b was observed in normal lymphocytes, revealing the existence of a physical complex between CFTR and this type of glutamate receptor (Fig. 3). Surprisingly, we have found that in lymphocytes derived from CF

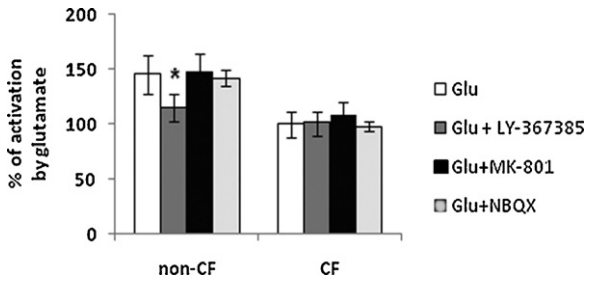

Fig. 2. IL-10 production by human non-CF and CF T-lymphocytes after their exposure for 48 h to glutamate (10^{-4} M) in the presence of IL-2 (20 ng/ml) with and without 10^{-6} M MK-801, NBQX and LY 367385. Values in the ELISAs (means ± SD) are representative of four independent experiments. Each assay was performed at least in duplicate. *$P < 0.05$ compared with the control (in the absence of inhibitors).

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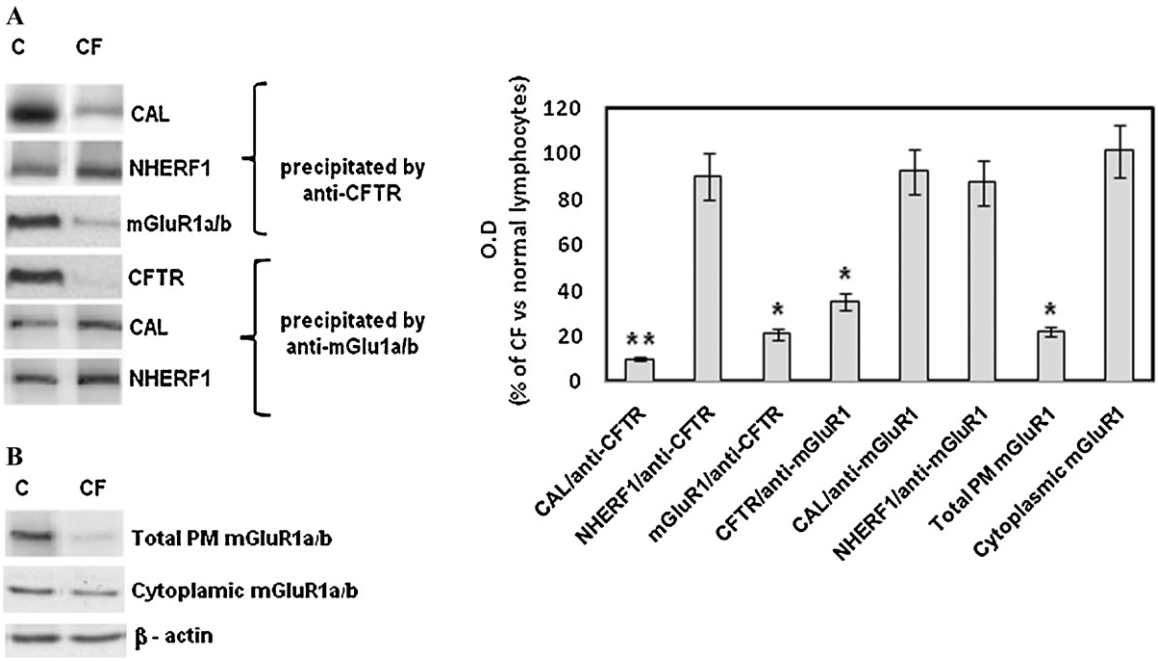

Fig. 3. Western blot of CFTR, CAL, NHERF1, mGluR1 and β-actin in human non-CF and CF T-lymphocytes. (A) Co-immunoprecipitation of CAL, NHERF1 and mGluR1 by anti-CFTR and CFTR, CAL and NHERF1 by anti-mGluR1a/b. Solubilized membrane proteins from healthy subjects lymphocytes (control) and lymphocytes derived from patients with CF were immunoprecipitated by CFTR and mGluR1a/b antibodies, separated on SDS-PAGE, and analyzed using immunoblots probed with CAL, NHERF1, and mGluR1a/b as described in Section 2. (B) Cytosolic and plasma membrane-enriched fractions (PM) from human non-CF and CF-lymphocytes were prepared as described in Section 2. The fractions were separated on SDS-PAGE and blotted with anti-mGluR1a/b. β-Actin was also visualized by Western blotting to confirm equal loading of the fractions. (C) The average densitometric quantification of the results of four independent experiments. Immunoreactive bands were scanned and the results are expressed as % of bands intensity of proteins in CF lymphocytes versus normal lymphocytes. OD is presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.001$. *t*-Test compared with corresponding control groups.

patients, association between CFTR and mGluR1a/b was significantly lower, than in lymphocytes derived from healthy subjects. To verify the association between CFTR and mGluR1a/b, the reverse experiments, immunoprecipitation of solubilized membrane proteins by mGluR1a/b antibody and blotting for CFTR were performed. Only in non-CF lymphocytes the detectable amount of CFTR was found. Any significant interaction between CFTR and mGluR1a/b in CF lymphocytes was not observed, confirming the lack of cellular association between these two proteins in CF patients.

An adaptor protein CAL plays an important role in the intracellular trafficking and localization of both, CFTR and mGluR1. Overexpression of CAL was found in CF that leads to a dramatic decrease in the plasma-membrane levels of both wild type and mutant CFTR (Cheng et al., 2002; Wolde et al., 2007). On the other hand, mGluR1a/b subunit via its C terminus interacts with the PDZ domain of CAL (Zhang et al., 2008). Thus, CAL is a protein, which participates in the trafficking of both type of molecules, CFTR and mGluR1. We have found that significantly large amounts of CAL immunoprecipitates with expressed CFTR in plasma membranes of normal lymphocytes, whereas no differences in the binding of CAL with mGluR1 between normal and CF-derived lymphocytes were found (Fig. 3). We conclude that CFTR and mGluR1 possibly compete for binding to CAL and overexpression of CAL in CF could decrease the trafficking of mGluR1.

The interaction between CFTR and mGluR1 could be mediated by the scaffolding protein NHERF1. This protein contains two PDZ-domains, which is able to bind to the COOH terminus of CFTR through its first domain (Wang et al., 1998), whereas the second domain remains available to interact with other proteins (Mohler et al., 1999). Thus, in the next series of experiments, the associations between NHERF1 and CFTR, also between NHERF1 and mGluR1 were determined. No differences in the association of these proteins between normal and CF-derived lymphocytes were found (Fig. 3). These data suggest that NHERF1 does not play a significant role in the down-regulation of complex containing CFTR and mGluR1.

4. Discussion

Chronic *Pseudomonas aeruginosa* airway infection and the accompanying inflammatory response are the major clinical problems for CF patients. Bacterial factors appear to contribute to the inadequacy of the innate immune system in eliminating *P. aeruginosa* from the CF respiratory tract. In addition to the innate system, the acquired immunity is also ineffective at eliminating this pathogen, because disease is characterized by an abnormal T helper cell type 2-biased immune responses including affected cytokine production (Hartl et al., 2006; Brazova et al., 2005). Recent investigations have suggested that these aberrations are primarily due to CFTR deficiency in lymphocytes rather than in the epithelium and this dysfunction in T cells could lead directly to inappropriate immune responses (Mueller et al., 2011).

Large amounts of endogenous glutamate were released from the lungs in airway inflammation (Said, 1999; Dickman et al., 2004; Wang et al., 2009) and injured neutrophils could be one of the major sources of this amino acid (Collard et al., 2002). Another source of glutamate may be DCs. DCs are the most potent antigen presenting cells specialized in the initiation of immune responses by directing of the activation and differentiation of naive T lymphocytes (Lanzavecchia and Sallusto, 2001). DCs are distributed throughout the lungs in the conducting airways of the tracheobronchial tree and in the parenchymal lung, and play a pivotal role in controlling the immune response to inhaled antigens (Von Garnier et al., 2005). These cells could stimulate the polarized outgrowth of distinct T cell subsets (including Th1 and Th2) inducing appropriate immune responses against pathogens. In the pioneering works,

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Pacheco et al. (2004, 2006) have shown that during DC maturation and T cell–DC contact, DC releases physiologically relevant glutamate amounts, which are high enough to activate glutamate receptors. They have found that DC-released glutamate acts on the constitutively expressed mGluR5, impairing T cell activation. However, after productive Ag presentation, mGluR1 is expressed in T cells to mediate enhanced T cell proliferation and secretion of Th1 and proinflammatory cytokines, including IL-10. Thus, elevated concentration of glutamate could induce polarization of T lymphocytes in the direction of Th1 cell immunity. All together suggests that glutamate may be one of the major players in the pathogenesis of CF.

We have found that in the presence of IL-2 an elevated level of IL-8 and IL-10 was secreted by CF lymphocytes compared with non-CF lymphocytes; however, additions of glutamate markedly increased the production of IL-10 only in non-CF lymphocytes, whereas CF-lymphocytes were insensitive to glutamate. These data show that lymphocytes derived from healthy subjects are characterized by higher sensitivity to glutamate and produce more IL-10, than CF lymphocytes.

Our results have shown that mGluR1 participates in the enhancement of secretion of IL-10 in normal lymphocytes, because neither NMDAR antagonist (MK-801), nor AMPAR antagonist (NBQX) decreased the effect of glutamate. Only LY-367385, an antagonist of group 1 mGluR significantly reduces the production of IL-10 to the basal levels. These data completely agree with the observation of Pacheco et al. (2004), showing that group 1 mGluR antagonists impaired IL-10 production in lymphocytes. All together suggest that glutamate, through activation of mGluR1 increases the production of IL-10 in normal lymphocytes, and does not affect the lymphocytes derived from CF patients.

Various types of ionotropic and metabotropic receptors are expressed in lymphocytes (Lombardi et al., 2001; Ganor et al., 2003; Boldyrev et al., 2004; Miglio et al., 2005; Chiocchetti et al., 2006; Kvaratskhelia et al., 2009). We have analyzed mGluR1 content in the complex with CFTR, because this receptor could be associated with CFTR through intermediary PDZ-containing scaffolding proteins. Our results have shown that in non-CF lymphocytes, activated by IL-2, mGluR1a/b is expressed on cell surface in association with CFTR. However, in contrast to normal lymphocytes, T-cells derived from CF patients, are unable to express supramolecular complex containing CFTR and mGluR1a/b. Besides, we have found that total amount of surface exposed mGluR1a/b is lower in CF-lymphocytes. These data demonstrate that mGluR1a/b possibly participates in the formation of supramolecular complex of CFTR, which is significantly impaired in CF-lymphocytes. It could be supposed that reduced expression of mGluR1 in CF patients evokes in lymphocytes the loss of sensitivity to glutamate, decreases the secretion of IL-10 and causes inappropriate immune response to antigen.

There are two possible explanations for the reduction of mGluR1 in the plasma membranes of CF-lymphocytes: mGluR1 either is not synthesized after T-cells activation by IL-2 and glutamate, or post-synthetic trafficking and scaffolding of mGluR1 in CF lymphocytes are limited. Further study is needed to clarify this issue. Nevertheless, because in the cytosolic fraction the content of mGluR1 did not change in CF-lymphocytes, it may be supposed that post-synthetic trafficking is disrupted in CF. For identification of the scaffold protein participating in the formation of complexes between CFTR and mGluR1 we have performed immunoprecipitation experiments with NHERF1. The multivalent properties of NHERF1 allow the formation of macromolecular complexes that anchor various membrane proteins. COOH terminus of mGluR1 bound to NHERF1 through PDZ2 domain (Paquet et al., 2006), whereas COOH terminus of CFTR is able to bind to the NHERF-11 through its PDZ1 domain (Wang et al., 1998). Thus, it would be wise to assume that this multivalent protein can assemble both, CFTR and mGluR1. However, we have not found any evidence that mGluR1, CFTR and NHERF1 could assemble in supramolecular complex, because no differences in the association between NHERF1 and CFTR or between NHERF1 and mGluR1 in normal and CF-derived lymphocytes were found. It would be possible to conclude that other PDZ proteins, like Shank proteins (Lee et al., 2007) could be recruited to this complex.

A Golgi-associated PDZ protein CAL has been found to modulate post-synthetic trafficking of both, CFTR (Cheng et al., 2002) and mGluR1a/b (Zhang et al., 2008). CAL is a scaffolding protein with two predicted coiled–coiled domains and one PDZ domain which binds to the COOH terminus of several membrane proteins, including CFTR and mGluR1a/b. Overexpression of CAL in cells leads to a dramatic decrease in the plasma-membrane levels of both wild type and ΔF508-CFTR mutant (Cheng et al., 2002; Wolde et al., 2007). We have found that the amount of surface expressed CFTR-CAL complex was greatly decreased in CF lymphocytes suggesting that overexpression of CAL interferes with the trafficking of CFTR. It is likely that CFTR and mGluR1a/b compete for binding to CAL, which in turn could regulate the expression of these two proteins. Similar competition for CAL between CFTR and mucin MUC3 was found in enterocytes (Pelaseyed and Hansson, 2011).

In conclusion, we have shown here that glutamate, in the presence of IL-2 increases the secretion of IL-10 in normal lymphocytes, whereas the lymphocytes from CF patients were unable to produce high amount of this cytokine under the action of glutamate. mGluR1, integrated in the supramolecular complex with CFTR could participate in the activation of *IL-10* gene. It seems that competition for CAL between CFTR and mGluR1a/b in CF mutant lymphocytes down-regulates the trafficking of mGluR1a/b, which occurs in normal cells after T-cell activation. Thus, it is possible to conclude that scaffolding and trafficking of mGluR1a/b in CF lymphocytes are limited by CAL, where overexpression in CF directs CFTR for degradation. Since mGluR1a/b, besides IL-10, also causes enhancement of Th1-responses and induces proinflammatory cytokine secretion, down-regulation of this receptor could impair adequate immune response in CF. Our findings reveal a novel binding partner to the CFTR, thus opening a new window of understanding of regulation of T-cell activity through mGluR1a/b in CF.

Acknowledgments

This work was supported by N5048 research grant from the Science and Technology Center in Ukraine (STCU) and Shota Rustaveli National Science Foundation (SRNSF).

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