

## Antibodies for CFTR studies

Filipa Mendes<sup>a</sup>, Carlos M. Farinha<sup>a,b</sup>, Mónica Roxo-Rosa<sup>a,b</sup>, Pascale Fanen<sup>c</sup>, Aleksander Edelman<sup>d</sup>, Robert Dormer<sup>e</sup>, Margaret McPherson<sup>e</sup>, Heather Davidson<sup>f</sup>, Edith Puchelle<sup>g</sup>, Hugo De Jonge<sup>h</sup>, Ghanshyam D. Heda<sup>i</sup>, Martina Gentsch<sup>j</sup>, Gergely L. Lukacs<sup>k</sup>, Deborah Penque<sup>a</sup>, Margarida D. Amaral<sup>a,b,\*</sup>

<sup>a</sup>Center of Human Genetics, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal

<sup>b</sup>Department of Chemistry and Biochemistry, University of Lisboa, Portugal

<sup>c</sup>INSERM U468, Créteil, France

<sup>d</sup>INSERM U467, Paris, France

<sup>e</sup>Department of Medical Biochemistry and Immunology, College of Medicine, University of Wales, Cardiff, UK

<sup>f</sup>Department of Medical Sciences, Western General Hospital, The University of Edinburgh, Edinburgh, UK

<sup>g</sup>INSERM U514, Reims, France

<sup>h</sup>Department of Biochemistry, Medical Faculty, Erasmus University Medical Centre, Rotterdam, The Netherlands

<sup>i</sup>The Veterans Affairs Medical Center and The Department of Medicine, University of Tennessee Health Sciences Center, Memphis, TN, USA

<sup>j</sup>Mayo Clinic Scottsdale, S.C. Johnson Medical Research Center, Scottsdale, AZ, USA

<sup>k</sup>Hospital for Sick Children Research Institute, Toronto, Canada

Available online 10 July 2004

### Abstract

For most expression studies focusing on the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, sensitive and specific antibodies (Abs) are critically needed. Several Abs have been produced commercially or by research laboratories for CFTR detection in both cell lines with heterologous or endogenous expression and native cells/tissues. Here, we review the applicability of most Abs currently in use in CF research for the biochemical and/or immunocytochemical detection of CFTR.

© 2004 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

**Keywords:** Anti-CFTR; Antibodies; Immunotechniques; Immunocytochemistry; Western; Expression

### 1. Introduction

There are a variety of factors that influence the success of an immunochemical technique. According to some authors, these include (1) the avidity of the Ab for the antigen (Ag), (2) the concentration of the Ab and its specificity for the Ag, (3) possible alteration of the Ag epitope during the experimental procedure, (4) accessibility of the Ab to the Ag during the technique,

and (5) type and quality of secondary reagents when used [1].

When CFTR is being detected, at least two additional factors should be considered, namely, the polarity status of cells under analysis and the levels of CFTR endogenous expression in cells or tissues, or copy number of transgene, in case of transfected cell lines [2].

Here, we review most Abs currently in use in CF research both polyclonal and monoclonal from commercial sources or produced by research laboratories, and summarize their applicability for the biochemical and/or immunocytochemical detection of CFTR, based on several comparative studies previously published [2–7]. Usage of anti-CFTR Abs described here includes detection in different types of samples, namely, (1) heterologous expression systems, (2) cell lines constitutively synthesizing the protein, and (3) native tissues.

*Abbreviations:* Ab, antibody; Ag, antigen; ELISA, enzyme-linked immunosorbent assay.

\* Corresponding author. Department of Chemistry and Biochemistry, University of Lisbon, Lisboa, Portugal. Tel.: +351-21-751-64-40/+351-21-750-08-61; fax: +351-21-752-64-10/+351-21-750-00-88.

*E-mail address:* mdamaral@fc.ul.pt (M.D. Amaral).

Table 1  
Review of anti-CFTR antibodies applicability

Antibody	Type <sup>a</sup>	Epitope	Source	Applicability <sup>b</sup>				Specificity	References <sup>c</sup>
				Cell lines		Native tissues/cells			
				WB	IP	ICC	IHC		
N-term-Birm <sup>d</sup>	PC	N-term (2–79)	D Cyr (Birmingham, AL, USA)	ND	T (+++)	ND	ND	H	[13]
MM13-4	MC (IgG1)	N-term (25–35)	Chemicon (Temecula, CA, USA)	T (+++)/E (+)	T (+++)/E (+)	N (+/-)	ND	H	[14]
MA1-935	MC (IgM)	1st EL (103-117)	Affinity Bioreagents (Golden, CO, USA)	ND	ND	ND	ND	H, M	[15,16]
PA1-935	PC (IgM)	1st EL (103-117)	Affinity Bioreagents	ND	ND	ND	ND	H	[15,16]
MATG 1031	MC (IgG1)	1st EL (107-117)	Transgène (Strasbourg, France)	ND	ND	ND	SG (-)	H	[17]
L12B4	MC (IgG2a)	Pre-NBD (386–412)	Chemicon	T (+++)/E (+)	T (+++)/E (-)	N (+/-)	ND	H, M, Ra	[14]
181	PC	Pre-NBD (415–427)	W Guggino (Baltimore, MD, USA)	Unsp	ND	ND	ND	H	[18,19]
MATG 1061	MC (IgG2a)	NBD1 (503–507/509–515)	Transgène	ND	ND	N (+++)	A (+++)/I (++)/SG (+)	H	[17,20]
NBD1-Birm	PC	NBD1-R	D Cyr	T (+++)/E (+)	T (+++)/E (+)	ND	ND	H	[2]
13-1	MC (IgG1)	R (590–830)	R&D Systems <sup>e</sup> (Abrington, UK)	ND	ND	N (+/-)	A (-)/I (++)	H	[21,22]
G449	PC	R (653–716)	H De Jonge	T (++)/E (+)	T (++)/E (+)	N (+++) <sup>f</sup>	I (++)	H	[23,24]
CC24-R	PC	R (693–716)	H De Jonge	T (+++)/E (++)	T (+)/E (-)	N (++)	A (-)/I/SG (++)	H	[23]
MATG 1104	MC (IgG1)	R (722–734)	Transgène	T (+++)/E (+/-)	T (-)/E (-)	N (++) <sup>g</sup>	A (++)/I (++)/SG (++)	H	[3,25]
169	PC	R (724–746)	W Guggino	ND	T (+++)/E (+)	N (++)	A (-)	H	[18,19]
M3A7	MC (IgG1)	NBD2-C-term (1197–1480)	Chemicon	T (+++)/E (+)	T (+++)/E (++)	N (+/-)	A (+/-)/I (+/-)/SG (++)	H, M, Ra	[3,14]
24-1	MC (IgG2a)	end NBD2-C-term (1377–1480)	R & D Systems <sup>e</sup>	T (+++)/E (++)	T (+++)/E (++)	N (+++)	A (++)/I (+/-)	H	[26–28]
GA-1	MC	end NBD2-C-term (1382–1480)	K Kirk (Birmingham, AL, USA)	-	T (+++)/E (+/-)	ND	ND	H	[29,30]
C1468	PC	C-term (1468–1480)	R Kopito (Stanford, CA, USA)	-	T (-)/E (ND)	ND	ND	H	[31,32]
Lis-1	PC	C-term (1468–1480)	MD Amaral	T (+++)	T (+++)	N (+++)	ND	H, M	[2,7]
R3195	PC	C-term (1468–1480)	C Marino (Memphis, TN, USA)	T (+++)/E (+)	T (+++)	ND	I (++)	H, M, Ra	[33–35]
MP-CT1	PC	C-term	R Dorner	T (+++)/E (++)	T (+++)/E (++)	N (+++)	A (++)/I (++)	H, M	[36–38]

<sup>a</sup> Polyclonal (PC); monoclonal (MC).

<sup>b</sup> Based in Refs. [2–7]. For further details, please refer to original articles. Scale is from (+++) good detection, (++) reasonable detection, (+) poor detection, (-) no detection to unspecific (Unsp) or not determined (ND).

<sup>c</sup> Original reference, when applicable, in boldface.

<sup>d</sup> Abbreviations: C-terminus (C-term), extracellular loop (EL), Regulatory domain (R), nucleotide binding domain (NBD), N-terminus (N-term), Western blot (WB), immunoprecipitation (IP), immunocytochemistry (ICC), immunohistochemistry (IHC), endogenous (E), transfected (T), nasal (N), airways (A), intestine (I), sweat gland (SG), human (H), rat (Ra), mouse (M).

<sup>e</sup> These Abs were formerly available from Genzyme.

<sup>f</sup> H. Davidson and H. De Jonge, unpublished results.

<sup>g</sup> D. Penque and H. Davidson, unpublished results.

## 2. Materials and methods

### 2.1. Antibodies

All anti-CFTR Abs described here, as well as their original sources and/or references are presented in Table 1.

### 2.2. Affinity purification

The affinity purification of the antiserum usually improves the performance of an Ab. A good protocol can be found at the European Working Group on CFTR Expression website [8]. An alternative protocol is described elsewhere [2]. Briefly, antisera purification involves coupling of the epitope peptide to activated Sepharose beads, which are then used to fill up a column. The CFTR antiserum is then passed through this column and acid-eluted in 0.5-ml fractions, followed by a neutralization step. An additional step for desalting and concentration can be performed in columns designed for this purpose, with 10,000 MW cutoff limits [2]. The eluate fractions should then be assessed for efficiency in detecting the CFTR peptide epitope [e.g., on enzyme-linked immunosorbent assay (ELISA) plates] or CFTR directly (e.g., by Western blot). Affinity-purified Ab can be stored at 4 °C in the presence of sodium azide.

### 2.3. Immunodetection techniques

Consensus protocols for immunodetection of CFTR are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and [<sup>35</sup>S]-labeling followed by immunoprecipitation of CFTR) are described by Farinha et al. [9]. Detection of CFTR protein by [<sup>32</sup>P]-phosphorylation assays is described elsewhere [10]. The detection of CFTR by immunocytochemistry in native cells is described by Harris et al. [11] and by immunohistochemistry in tissues elsewhere [12].

## 3. Conclusion

CFTR protein is difficult to study and analysis based on detection of its presence is critically dependent on the use of robust Abs. We provide here a review of most anti-CFTR Abs available either from commercial or research sources that evidenced good results for the detection of wt- and F508del-CFTR by at least one immunochemical technique. The end-user researcher can thus save time and effort by choosing the Ab that best applies to the desired purpose.

## Acknowledgements

European Thematic Network around Cystic Fibrosis and Related Diseases (EU-QLK3-CT-1999-00241).

## References

- [1] Harlow D, Lane D. Choosing antibodies. In: Harlow D, Lane D, editors. Using antibodies, a laboratory approach. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1999, p. 39–59.
- [2] Farinha C, Mendes F, Roxo-Rosa M, Penque D, Amaral MD. Applicability of 14 antibodies for the biochemical detection of the cystic fibrosis transmembrane conductance regulator protein. Mol Cell Probes, accepted.
- [3] Kälin N, Claass A, Sommer M, Puchelle E, Tümmler B. DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. J Clin Invest 1999;103:1379–89.
- [4] Claass A, Sommer M, De Jonge H, Kälin N, Tümmler B. Applicability of different antibodies for immunohistochemical localization of CFTR in sweat glands from healthy controls and from patients with cystic fibrosis. J Histochem Cytochem 2000;48:831–7.
- [5] Penque D, Mendes F, Beck S, Farinha C, Pacheco P, Nogueira P, et al. Cystic Fibrosis F508del patients have apically localized CFTR in a reduced number of airway cells. Lab Invest 2000;80:857–68.
- [6] Doucet L, Mendes F, Montier T, Delepine P, Penque D, Ferec C, et al. Applicability of different antibodies for the immunohistochemical localization of CFTR in respiratory and intestinal tissues of human and murine origin. J Histochem Cytochem 2003;51:1191–9.
- [7] Carvalho-Oliveira I, Efthymiadou A, Malho R, Nogueira P, Tzetzis M, Kanavakis E, et al. CFTR localization in native airway cells and cell lines expressing wild-type or F508del-CFTR by a panel of different antibodies. J Histochem Cytochem 2004;52:193–203.
- [8] The Online Virtual Repository of Cystic Fibrosis European Network 2003 (Section C): <http://central.igc.gulbenkian.pt/cftr/vr/biochemistry.html>.
- [9] Farinha C, Penque D, Roxo-Rosa M, Lukacs GL, Dormer R, McPherson M, et al. Biochemical methods to assess CFTR expression and membrane localization. J Cystic Fibros 2004;3:S1.
- [10] Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell 1991;66:1027–36.
- [11] Harris CM, Mendes F, Dragomir A, Doull IJM, Penque D, Amaral MD, et al. Assessment of CFTR localisation in native airway epithelial cells obtained by nasal brushing. J Cystic Fibros 2004;3:S1.
- [12] The Online Virtual Repository of Cystic Fibrosis European Network 2003 (Section B): <http://central.igc.gulbenkian.pt/cftr/vr/histology.html>.
- [13] Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM. The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis. EMBO J 1999;18:1492–505.
- [14] Kartner N, Augustinas O, Jensen TJ, Naismith AL, Riordan JR. Mislocalization of delta F508 CFTR in cystic fibrosis sweat gland. Nat Genet 1992;1:321–7.
- [15] Walker J, Watson J, Holmes C, Edelman A, Banting G. Production and characterisation of monoclonal and polyclonal antibodies to different regions of the cystic fibrosis transmembrane conductance regulator (CFTR): detection of immunologically related proteins. J Cell Sci 1995;108:2433–44.
- [16] Pier GB, Grout M, Zaidi T, Meluleni G, Mueschenborn SS, Banting G, et al. *Salmonella typhi* uses CFTR to enter intestinal epithelial cells. Nature 1998;393:79–82.
- [17] Demolombe S, Baro I, Laurent M, Hongre AS, Pavirani A, Escande D. Abnormal subcellular localization of mutated CFTR protein in a cystic fibrosis epithelial cell line. Eur J Cell Biol 1994;65:214–9.
- [18] Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, et al. Immunocytochemical localization of the cystic fibrosis gene product CFTR. Proc Natl Acad Sci U S A 1991;88:9262–6.
- [19] Zeitlin PL, Crawford I, Lu L, Woel S, Cohen ME, Donowitz M, et al. CFTR protein expression in primary and cultured epithelia. Proc Natl Acad Sci U S A 1992;89:344–7.
- [20] Puchelle E, Gaillard D, Ploton D, Hinnrasky J, Fuchey C, Bouterin MC, et al. Differential localization of the cystic fibrosis transmem-

- brane conductance regulator in normal and cystic fibrosis airway epithelium. *Am J Respir Cell Mol Biol* 1992;7:485–91.
- [21] Gregory RJ, Cheng SH, Rich DP, Marshall J, Paul S, Hehir K, et al. Expression and characterization of the cystic fibrosis transmembrane conductance regulator. *Nature* 1990;347:382–6.
- [22] Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, et al. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990;63:827–34.
- [23] Piccioletto MR, Cohn JA, Bertuzzi G, Greengard P, Naim AC. Phosphorylation of the cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 1992;267:12742–52.
- [24] French PJ, Bijman J, Edixhoven M, Vaandrager AB, Scholte BJ, Lohmann SM, et al. Isotype-specific activation of cystic fibrosis transmembrane conductance regulator-chloride channels by cGMP-dependent protein kinase II. *J Biol Chem* 1995;270:26626–31.
- [25] Dupuit F, Kalin N, Brezillon S, Hinnrasky J, Tummler B, Puchelle E. CFTR and differentiation markers expression in non-CF and delta F508 homozygous CF nasal epithelium. *J Clin Invest* 1995;96:1601–11.
- [26] Denning GM, Anderson MP, Amara JF, Marshall J, Smith AE, Welsh MJ. Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. *Nature* 1992;358:761–4.
- [27] Haardt M, Benharouga M, Lechardeur D, Kartner N, Lukacs GL. C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. *J Biol Chem* 1999;274:21873–7.
- [28] Taouil K, Hinnrasky J, Hologne C, Corlieu P, Klossek JM, Puchelle E. Stimulation of beta 2-adrenergic receptor increases cystic fibrosis transmembrane conductance regulator expression in human airway epithelial cells through a cAMP/protein kinase A-independent pathway. *J Biol Chem* 2003;278:17320–7.
- [29] Cormet-Boyaka E, Di A, Chang SY, Naren AP, Tousson A, Nelson DJ, et al. CFTR chloride channels are regulated by a SNAP-23/syntaxin 1A complex. *Proc Natl Acad Sci U S A* 2002;99:12477–82.
- [30] Naren AP, Cobb B, Li C, Roy K, Nelson D, Heda GD, et al. A macromolecular complex of beta 2 adrenergic receptor, CFTR, and ezrin/radixin/moesin-binding phosphoprotein 50 is regulated by PKA. *Proc Natl Acad Sci U S A* 2003;100:342–6.
- [31] Ward CL, Kopito RR. Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. *J Biol Chem* 1994;269:25710–8.
- [32] Johnston JA, Ward CL, Kopito RR. Aggregosomes: a cellular response to misfolded proteins. *J Cell Biol* 1998;143:1883–98.
- [33] French PJ, van Doorninck JH, Peters RH, Verbeek E, Ameen NA, Marino CR, et al. A delta F508 mutation in mouse cystic fibrosis transmembrane conductance regulator results in a temperature-sensitive processing defect in vivo. *J Clin Invest* 1996;98:1304–12.
- [34] Ameen NA, van Donselaar E, Posthuma G, De Jonge H, McLaughlin G, Geuze HJ, et al. Subcellular distribution of CFTR in rat intestine supports a physiologic role for CFTR regulation by vesicle traffic. *Histochem Cell Biol* 2000;114:219–28.
- [35] Ameen NA, Marino C, Salas PJ. cAMP-dependent exocytosis and vesicle traffic regulate CFTR and fluid transport in rat jejunum in vivo. *Am J Physiol Cell Physiol* 2003;284:C429–38.
- [36] Llyod Mills C, Pereira MMC, Dormer RL, McPherson MA. An antibody against a CFTR-derived synthetic peptide, incorporated into living submandibular cells, inhibits beta-adrenergic stimulation of mucin secretion. *Biochem Biophys Res Commun* 1992;188:1146–52.
- [37] Bulteau L, Derand R, Mettey Y, Metaye T, Morris MR, McNeilly CM, et al. Properties of CFTR activated by the xanthine derivative X-33 in human airway Calu-3 cells. *Am J Physiol Cell Physiol* 2000;279:C1925–37.
- [38] Dormer RL, Derand R, McNeilly CM, Mettey Y, Bulteau-Pignoux L, Metaye T, et al. Correction of deltaF508-CFTR activity with benzo(c)-quinolizinium compounds through facilitation of its processing in cystic fibrosis airway cells. *J Cell Sci* 2001;114:4073–81.