Antibodies for CFTR studies

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

\textsuperscript{a}Center of Human Genetics, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal
\textsuperscript{b}Department of Chemistry and Biochemistry, University of Lisboa, Portugal
\textsuperscript{c}INSERM U468, Créteil, France
\textsuperscript{d}INSERM U467, Paris, France
\textsuperscript{e}INSERM U468, Creteil, France
\textsuperscript{f}Department of Medical Biochemistry and Immunology, College of Medicine, University of Wales, Cardiff, UK
\textsuperscript{g}Department of Medical Sciences, Western General Hospital, The University of Edinburgh, Edinburgh, UK
\textsuperscript{h}INSERM U514, Reims, France
\textsuperscript{i}Department of Biochemistry, Medical Faculty, Erasmus University Medical Centre, Rotterdam, The Netherlands
\textsuperscript{j}The Veterans Affairs Medical Center and The Department of Medicine, University of Tennessee Health Sciences Center, Memphis, TN, USA
\textsuperscript{k}Mayo Clinic Scottsdale, S.C. Johnson Medical Research Center, Scottsdale, AZ, USA
\textsuperscript{l}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.
Table 1
Review of anti-CFTR antibodies applicability

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Epitope Source</th>
<th>Applicability</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Type</td>
<td>Source</td>
<td>Applicability</td>
<td>Specificity</td>
</tr>
<tr>
<td>N-term-Birm</td>
<td>D Cyr (Birmingham, AL, USA)</td>
<td>ND</td>
<td>T (+++)</td>
</tr>
<tr>
<td>MM13-4</td>
<td>Chemicon (Temecula, CA, USA)</td>
<td>T (+++)/E (+)</td>
<td>T (+++)/E (+)</td>
</tr>
<tr>
<td>MA1-935</td>
<td>Affinity Bioreagents (Golden, CO, USA)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PA1-935</td>
<td>Affinity Bioreagents (Golden, CO, USA)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MATG 1031</td>
<td>Transgène (Strasbourg, France)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L12B4</td>
<td>Chemicon (Baltimore, MD, USA)</td>
<td>T (+++)/E (+)</td>
<td>T (+++)/E (+)</td>
</tr>
<tr>
<td>13-1</td>
<td>R &amp; D Systems (Arlington, UK)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>G449</td>
<td>H De Jonge (Baltimore, MD, USA)</td>
<td>T (+)/E (+)</td>
<td>T (+)/E (+)</td>
</tr>
<tr>
<td>CC24-R</td>
<td>H De Jonge (Baltimore, MD, USA)</td>
<td>T (+)/E (+)</td>
<td>T (+)/E (+)</td>
</tr>
<tr>
<td>MATG 1104</td>
<td>Transgène (Baltimore, MD, USA)</td>
<td>T (++)</td>
<td>T (-)</td>
</tr>
<tr>
<td>24-1</td>
<td>R &amp; D Systems (Arlington, UK)</td>
<td>T (++)</td>
<td>T (++)</td>
</tr>
<tr>
<td>GA-1</td>
<td>Kirk (Birmingham, AL, USA)</td>
<td>T (++)</td>
<td>T (++)</td>
</tr>
<tr>
<td>C1468</td>
<td>R Kopito (Stanford, CA, USA)</td>
<td>T (--)</td>
<td>ND</td>
</tr>
<tr>
<td>Lis-1</td>
<td>MD Amaral (Memphis, TN, USA)</td>
<td>T (++)</td>
<td>T (++)</td>
</tr>
<tr>
<td>R3195</td>
<td>C Marino (Memphis, TN, USA)</td>
<td>T (++)</td>
<td>T (++)</td>
</tr>
<tr>
<td>MP-CT1</td>
<td>R Dormer (Memphis, TN, USA)</td>
<td>T (++)</td>
<td>T (++)</td>
</tr>
</tbody>
</table>

a Polyclonal (PC); monoclonal (MC).

b 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).

c Original reference, when applicable, in boldface.

d 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).

e These Abs were formerly available from Genzyme.

f H. Davidson and H. De Jonge, unpublished results.

g 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 \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement. Biochemical detection techniques (namely Western blot and \(^{35}\)S-labeling followed by immunoprecipitation of CFTR) are described elsewhere in this supplement.

The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10]. The detection of CFTR by immunocytochemistry in tissues elsewhere [10].

2.4. Consensus protocols


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 biochemical 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