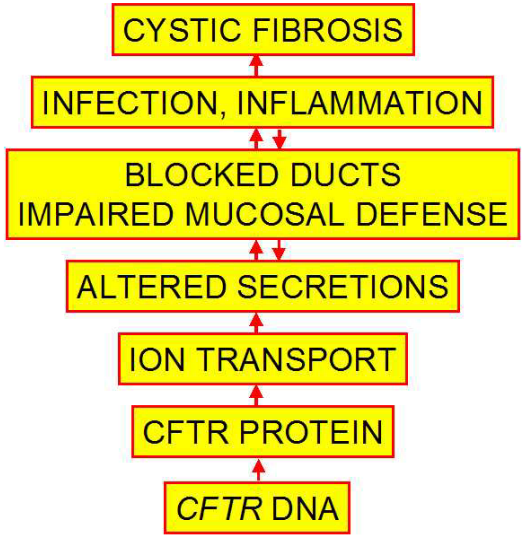
Why and when aspergillosis first appears in cystic fibrosis patients?

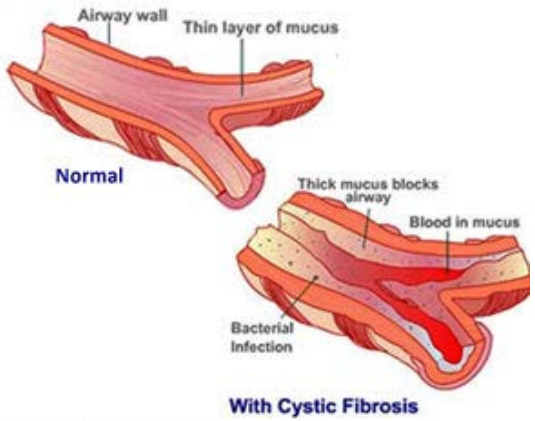
Prof dr Adilia Warris

Paediatric Infectious Diseases Specialist

Co-Director MRC Centre for Medical Mycology
Principal Investigator Aberdeen Fungal Group
Honorary Consultant Royal Aberdeen Children's Hospital
Honorary Consultant Great Ormond Street Hospital, London

Cystic Fibrosis

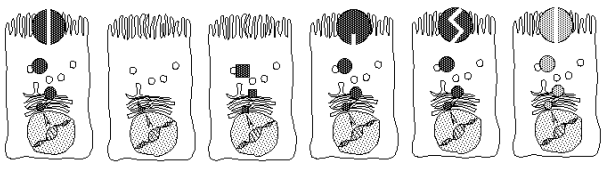




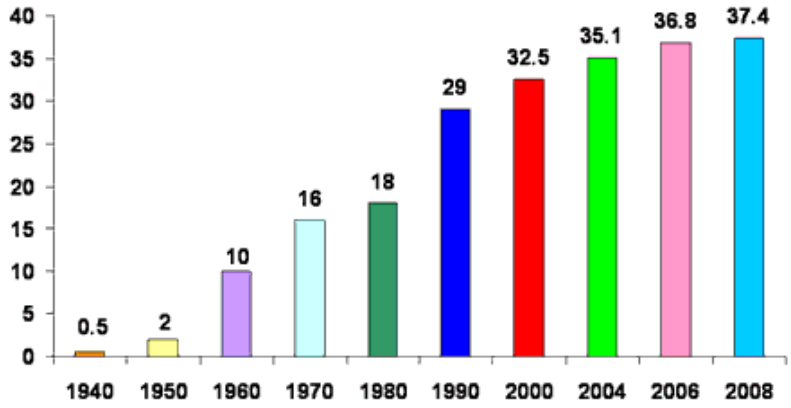
Progressive lung disease due to defective epithelial cells and impaired mechanical clearance of pathogens

courtesy of Dr. Rick Hallick

Molecular consequences of CFTR mutations

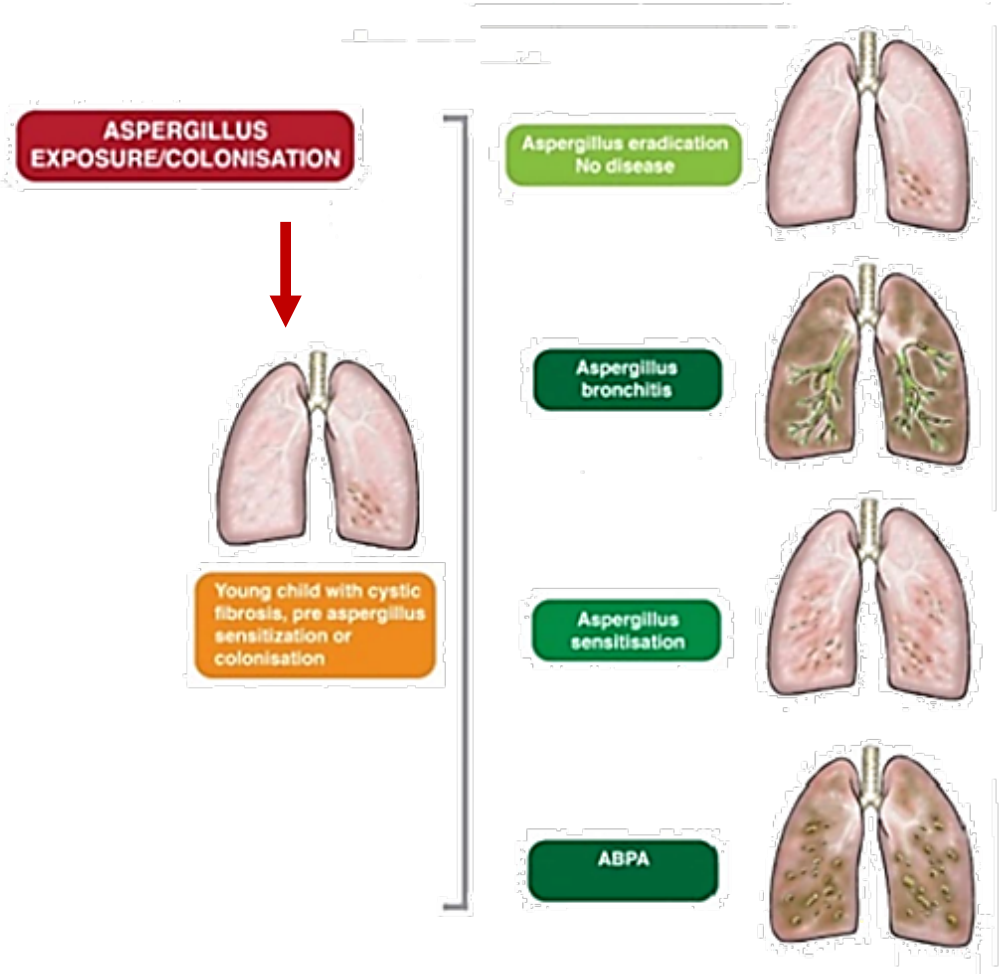


Normal	I	II	III	IV	V
	No synthesis	Block in processing	Block in regulation	Altered conductance	Reduced synthesis
	Nonsense G542X Frameshift 394delTT Splice junction 1717-1G→A	Missense N1303K AA deletion ΔF508	Missense G551D	Missense R117H R347P	Missense A455E Alternative splicing 3849+10kbC→T



Source: Cystic Fibrosis Foundation

Aspergillus infections and disease in CF



From Armstead et al, PLoS One 2014

Aspergillus colonisation data

References	Country	Patient populations	Colonisation
Nelson, Am Rev Resp Dis 1979	USA	46 pts	56.7%
Laufer, JACI 1984	USA	100 pts aged 2 – 34 yrs	9%
Bauernfeind, Infection 1987	Germany	102 pts	5.9%
Mroueh, Chest 1994	USA	236 pts aged 6 – 41 yrs	25.4%
Flume, AJRCCM 1994	USA	27 pts prior to lung transplantation	63%
Becker, Chest 1996	USA	49 adult patients	16%
Milla, Ped Pulm 1996	USA	212 paediatric patients	21%
Cimon, J Med Mycol 1995	France	210 pts	21.4%
Burns, JID 1999	USA	520 pts ≥ 6 yrs of age, FEV1 25%-75% predicted	25%
Cimon, J Med Mycol 2000	France	128 pts	46.1%
Bakare, Mycoses 2003	Germany	94 pts, median age 28 yrs	45.7%
Skov, Res Med 2005	Australia	270 paediatric and adult patients	18.8%
Chotirmall, Resp Care 2008	Irish	50 patients	30%
Valenza, J Cyst Fibrosis 2008	Germany	60 adult and paediatric patients	58.3%
Wainwright, JAMA 2011	Australia	153 pts, BAL fluid from children < 5 yrs	9%
Heltshe, CID 2015	USA	151 pts ≥18 yrs	15%

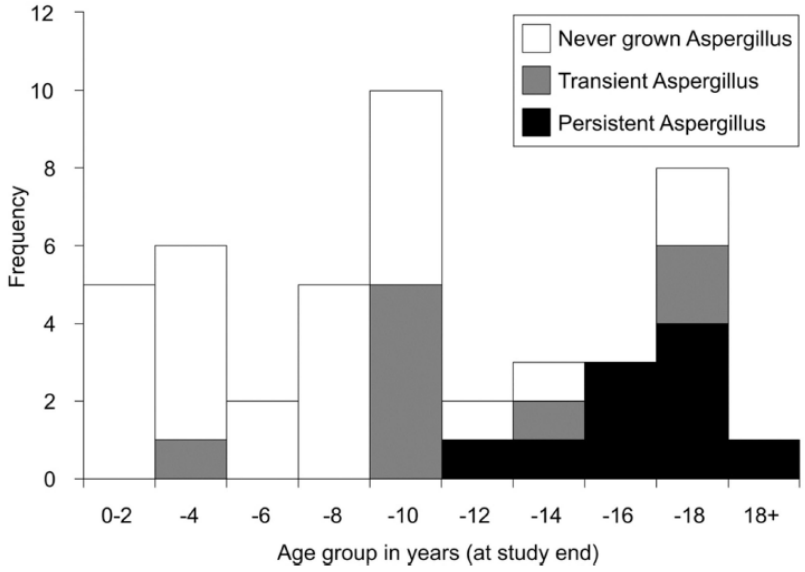
Aspergillus colonisation data
time of acquisition

- In an epidemiological study conducted in two CF centers in France, mean age of the patients at date of first isolation of *A. fumigatus* was 12.3 yrs

Pihet et al, Med Mycol 2009

Aspergillus colonisation data
time of acquisition

- 8 yrs retrospective cohort study; 1024 respiratory specimens; 45 children
- 42% (n=19) at least one positive sputum culture
- 20% (n=9) transiently colonised
- 22% (n=10) persistently colonised
  - 29% of BAL fluids (n=48) positive (median age 6.6 yrs)
  - 14% of sputum samples (n=976) positive (median age 11.5 yrs)



7 out of 33 children severe lung disease, 4 of whom showed persistent colonisation

Saunders et al, Med Mycol 2016

Aspergillus colonisation data

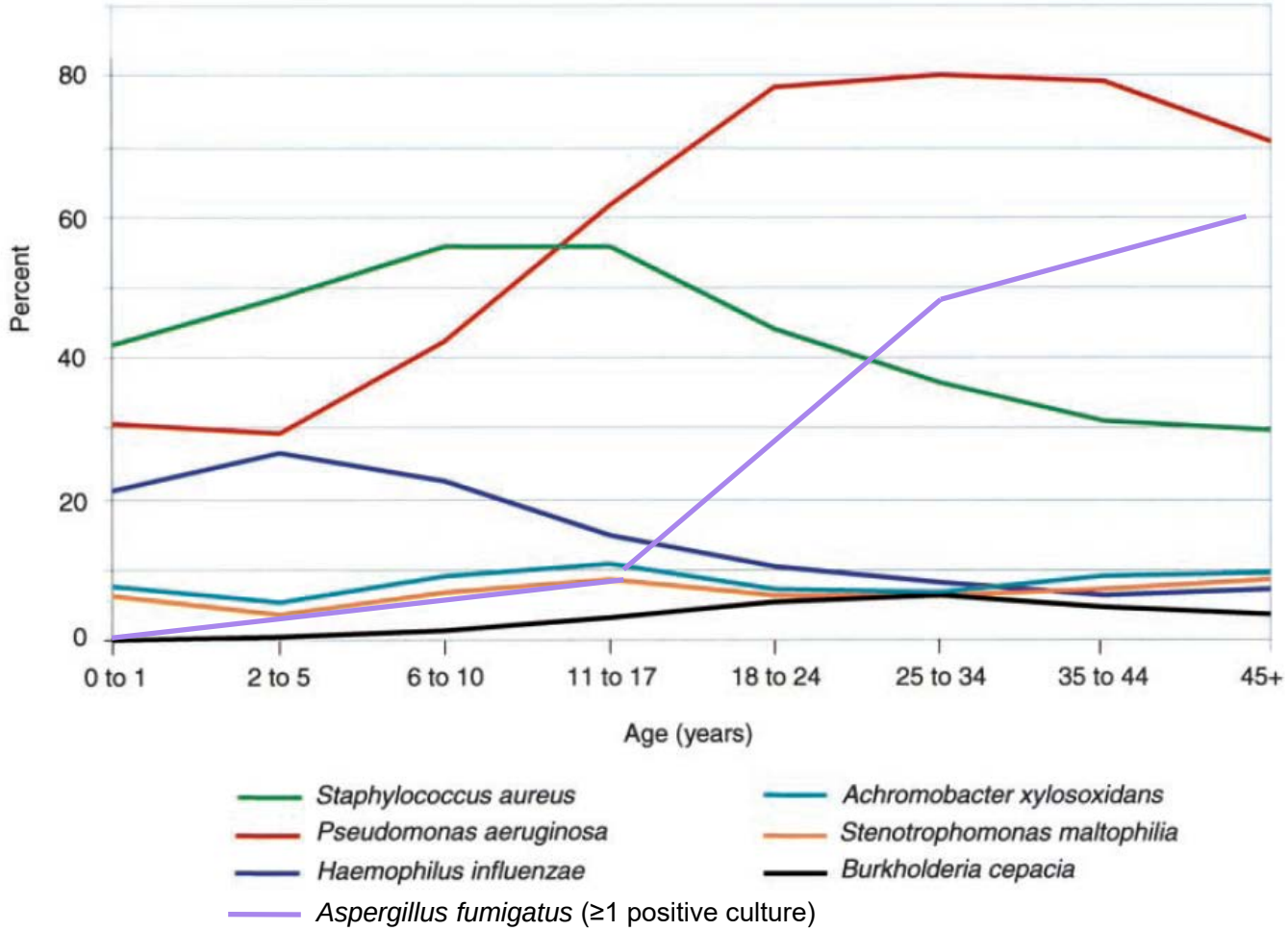
In children with *A. fumigatus* in BAL-fluid, only 19.5% of sputum samples and 2.7% of cough swabs obtained within three months after a positive BAL showed growth of *A. fumigatus*.

Immunology	n = 24	n = 9	n = 10
Total IgE > 500 IU/ml n (%)	0 (0)	1 (11)	3 (30)*
Aspergillus fumigatus-specific IgE > 0.35 kU/l n (%)	4 (17)	7 (78)*	7 (70)
IgG precipitins > 90 mg/l n (%)	0 (0)	3 (33)*	5 (50)*

Only 4 out of 19 transiently/persistently colonised developed ABPA

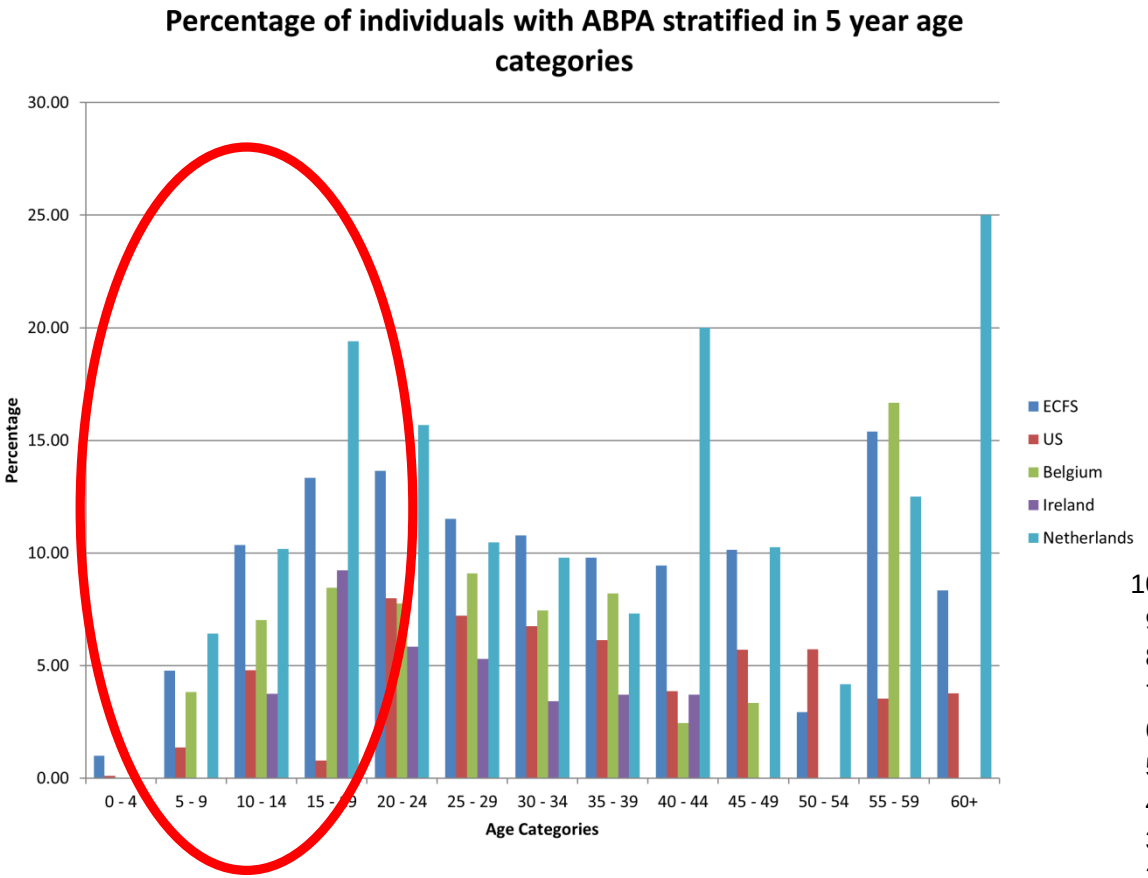
Saunders et al, Med Mycol 2016

Cystic Fibrosis
colonisation dynamics



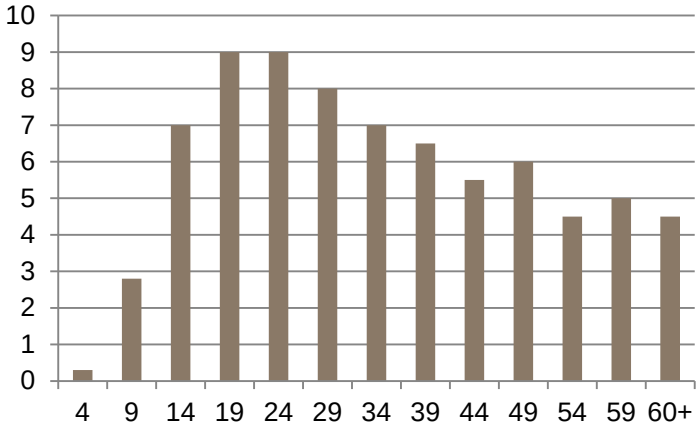
adapted from Gibson 2003 & de Vrankrijker 2010

ABPA in CF



Armstead, PLoS ONE 2014

Combined data:



ABPA in paediatric CF

Country	Incidence < 18 years	Incidence ≥ 18 years
US	6.0% (n=824)	6.4% (n=869)
UK	8.2% (n=324)	11.8% (n=582)
France	9.1% (n=277)*	17.9% (n=463)*
Germany	4.8% (n=117)	7.3% (n=184)
Belgium	6.0% (n=32)	8.3% (n=48)
Israel	5.4% (n=14)	7% (n=19)
Austria	2.2% (n=6)	5.3% (n=9)
Switzerland	18.6% (n=32)	11% (n=2)
Ireland	3.2% (n=18)	4.4% (n=22)

Adapted from Armstead et al, PLoS ONE 2014

Aspergillus infection and disease in CF

TABLE II. Average values of latent class variables for 130 triazole-naive patients

	Class 1 (n = 49)	Class 2 (n = 23)	Class 3 (n = 19)	Class 4 (n = 39)
Average class probability	0.981	0.996	0.921	0.992
Median sIgG (mg/L) (SD)	36 (20)	112 (132)	47 (24)	98 (29)
Median sIgE (kUa/L) (SD)	0 (0.6)	13.2 (19.0)	5.2 (3.9)	0 (1.0)
Median tIgE (kUI/L) (SD)	28 (28)	690 (1230)	170 (98)	35 (157)
Mean RT-PCR C_t value $(38 - C_t)^*$ ± SD	2.6 ± 3.3	8.2 ± 4.0	3.7 ± 3.3	7.1 ± 4.0
Probability of positive PCR	0.497	1.000	0.778	1.000
Mean GM index ± SD	0.161 ± 0.19	2.544 ± 2.04	0.135 ± 0.17	2.960 ± 2.11
Probability of positive GM	0.004	0.946	0.000	1.000

- Class 1: no disease (37%) — PCR+, GM-, sIgG-, sIgE-, tIgE-
- Class 2: ABPA-S (18%) — PCR+, GM+, sIgG+, sIgE+, tIgE+
- Class 3: IgE sens (15%) — PCR±, GM-, sIgG-, sIgE+, tIgE+
- Class 4: infection (30%) — PCR+, GM+, sIgG+, sIgE-, tIgE-

Suggestion to add:

- Class 5: colonization — Culture+ (AND/OR PCR+ AND/OR GM+?)

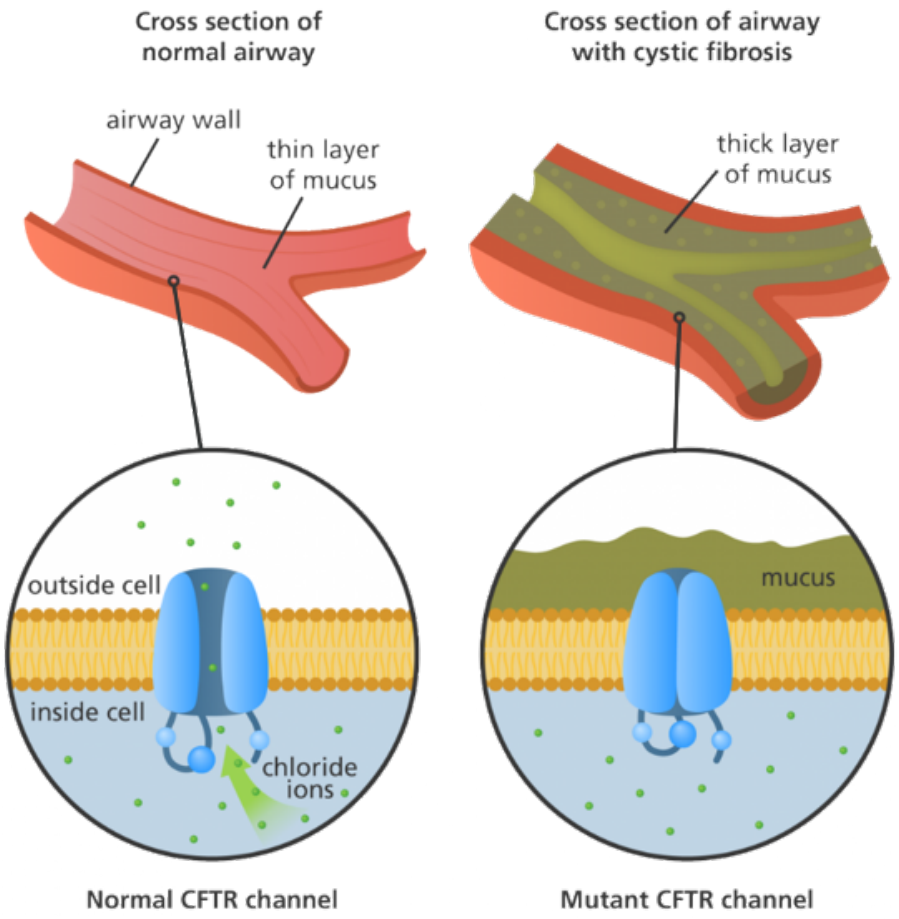
Baxter et al, JACI 2013

The clinical phenotype

- *Aspergillus* colonization associated with progressive decrease in lung function, more severe bronchiectasis, and increased number of hospital admissions
- Higher risk of *Aspergillus* and *Pseudomonas* infections post-lung transplantation in CF-host compared to other hosts receiving lung transplants

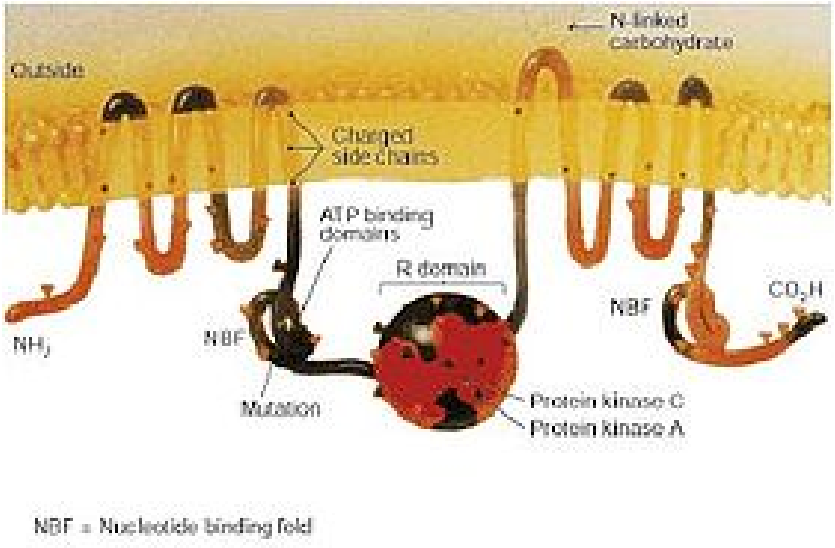
Luong, Transplantation 2013
McMahon, Eur J Radiol 2011
Amin, Chest 2010
Ratner, Am J Resp Cell Mol Biol 2012
Bonvillain, J Heart Lung Transplant 2007
Pihet, Med Mycol 2009

Understanding *Aspergillus* infections in CF
the sticky mucus



Immune responses in the CF-host
intrinsically defective?

CFTR protein expression described on macrophage and neutrophil membranes, secretary vesicles, and phagocytic vacuoles



Di, Nat Cell Biol 2006; Del Porto, PlosOne 2011
Painter, Biochem 2006; Zhou, J Innate Imm 2013

nature medicine **REVIEW**

Cystic fibrosis: a mucosal immunodeficiency syndrome

Taylor Sitarik Cohen & Alice Prince

NATURE MEDICINE VOLUME 18 | NUMBER 4 | APRIL 2012

ELSEVIER

Review

Innate immunity in cystic fibrosis lung disease

Journal of Cystic Fibrosis www.elsevier.com/locate/jcf

D. Hartl [a,*], A. Gaggar [b,c], E. Bruscia [d], A. Hector [a], V. Marcos [e], A. Jung [f], C. Greene [g], G. McElvaney [g], M. Mall [h], G. Döring [i]

CHEST Translating Basic Research Into Clinical Practice

CHEST 2012; 141(4):1055–1062

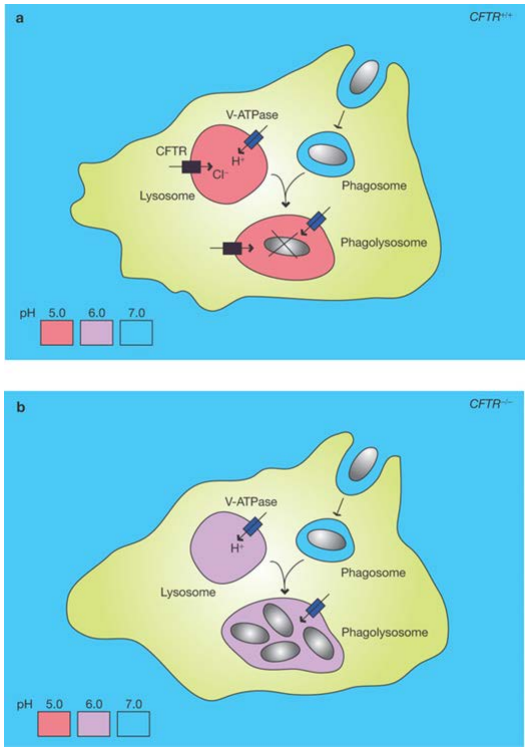
Defective Phagocytosis in Airways Disease

Louise E. Donnelly, PhD; and Peter J. Barnes, DM, FCCP

Immune responses in the CF-host
intrinsically defective?

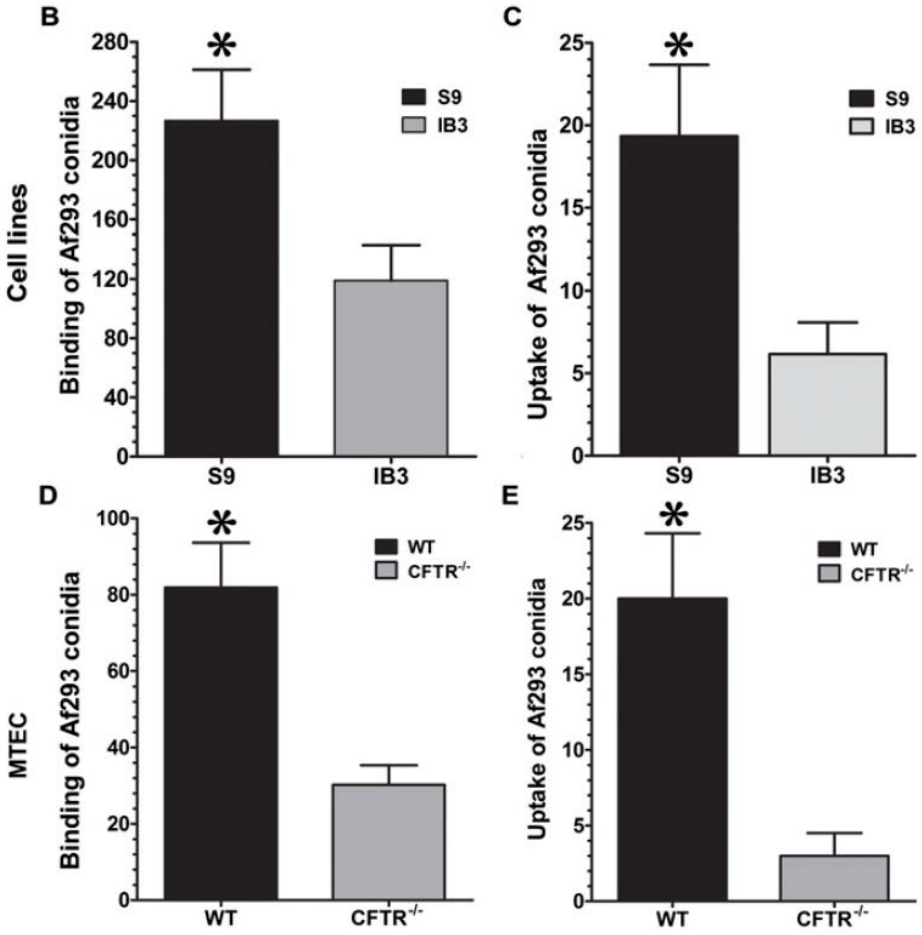
functional studies (with gram- bacteria) implicate that the absence of a functional CFTR-protein in phagocytes leads to:

- inadequate intraphagosomal Cl transport
- resulting in intracellular alkalization
- alteration in degranulation
- diminished production of HOCl
- an overall impaired superoxide production
- increased release of pro-inflammatory cytokines



Ratner et al, Am J Resp Cell Biol 2011

Understanding *Aspergillus* infections in CF
the epithelial cells

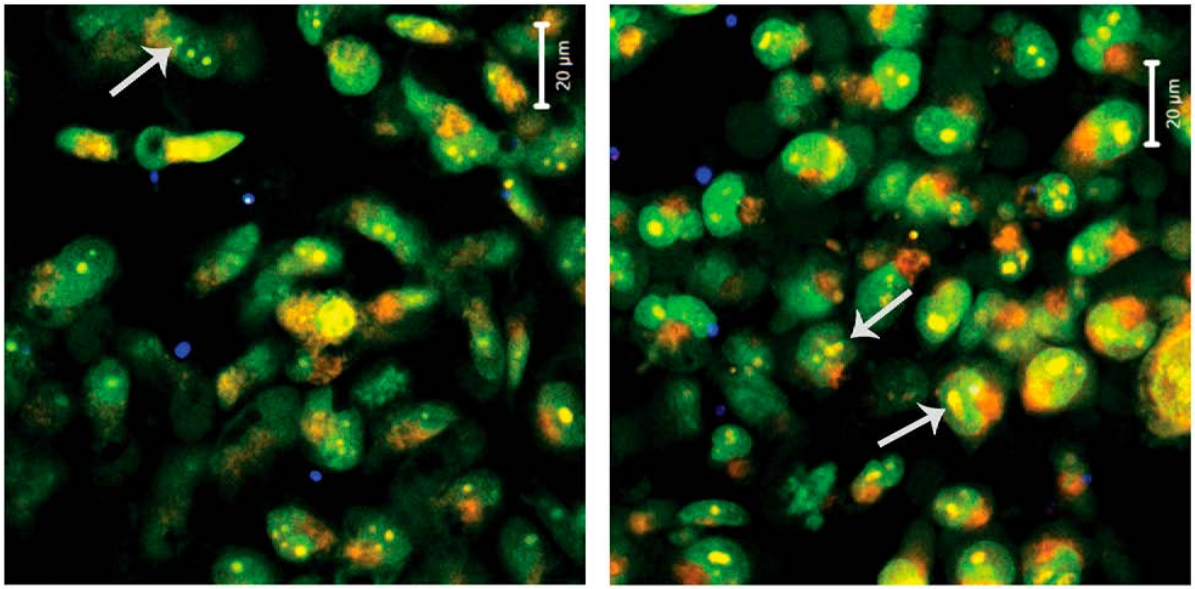


ΔF508 epithelial cell line IB3 and mouse tracheal epithelial cells demonstrate reduced binding and uptake of *A. fumigatus* conidia

Chaudhary et al, Am J Resp Crit Care Med 2012

Understanding *Aspergillus* infections in CF
the epithelial cells

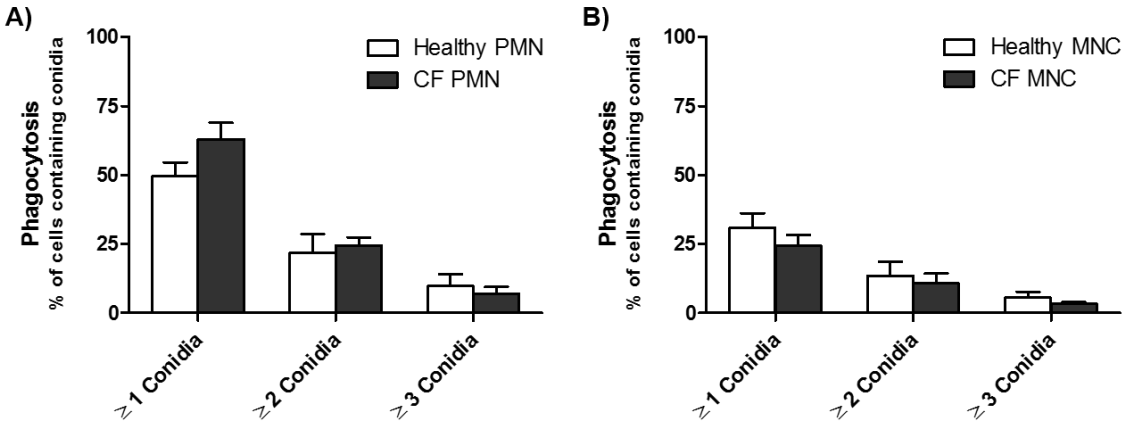
and a defect in killing of internalized conidia

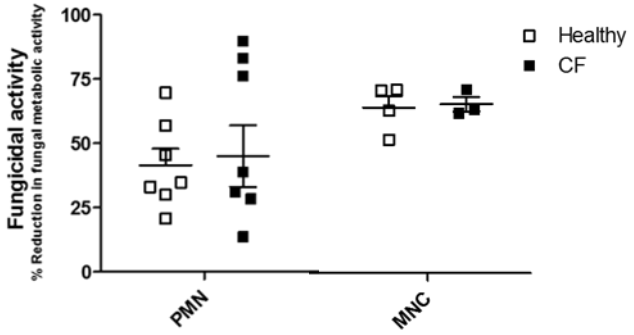


Chaudhary et al, Am J Resp Crit Care Med 2012

Understanding *Aspergillus* infections in CF *the neutrophils*

Phagocytosis and fungicidal activity in tact

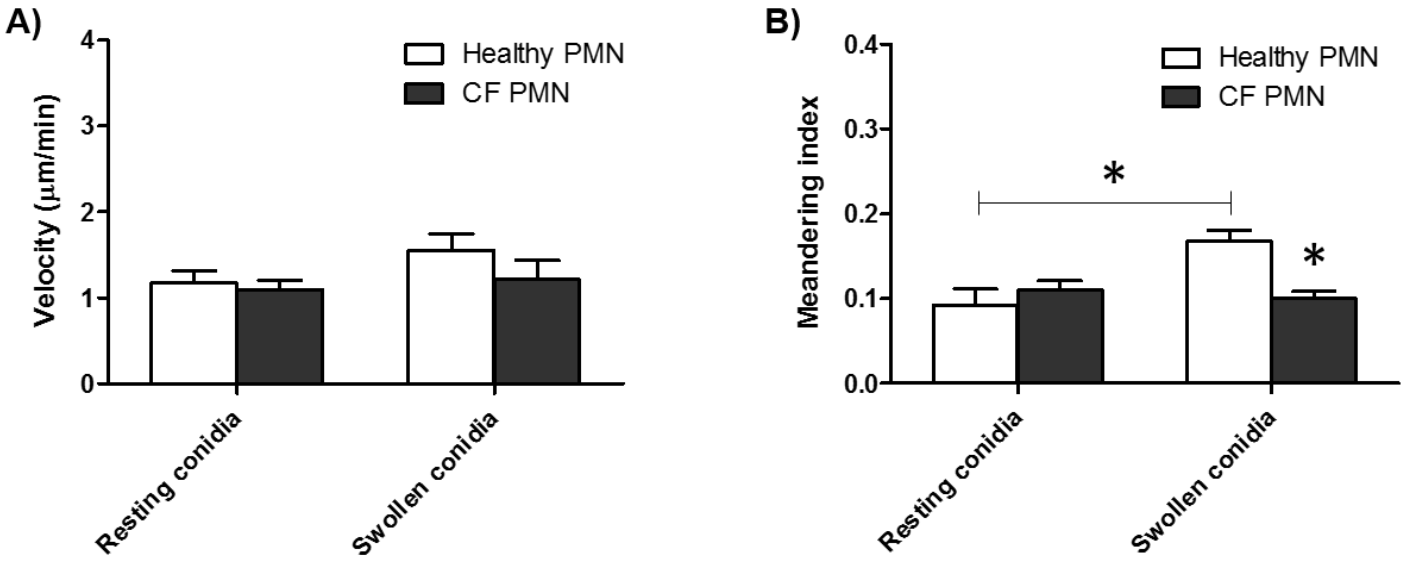




Brunel et al, ERJ Open Research *in press*

Understanding *Aspergillus* infections in CF
the neutrophils

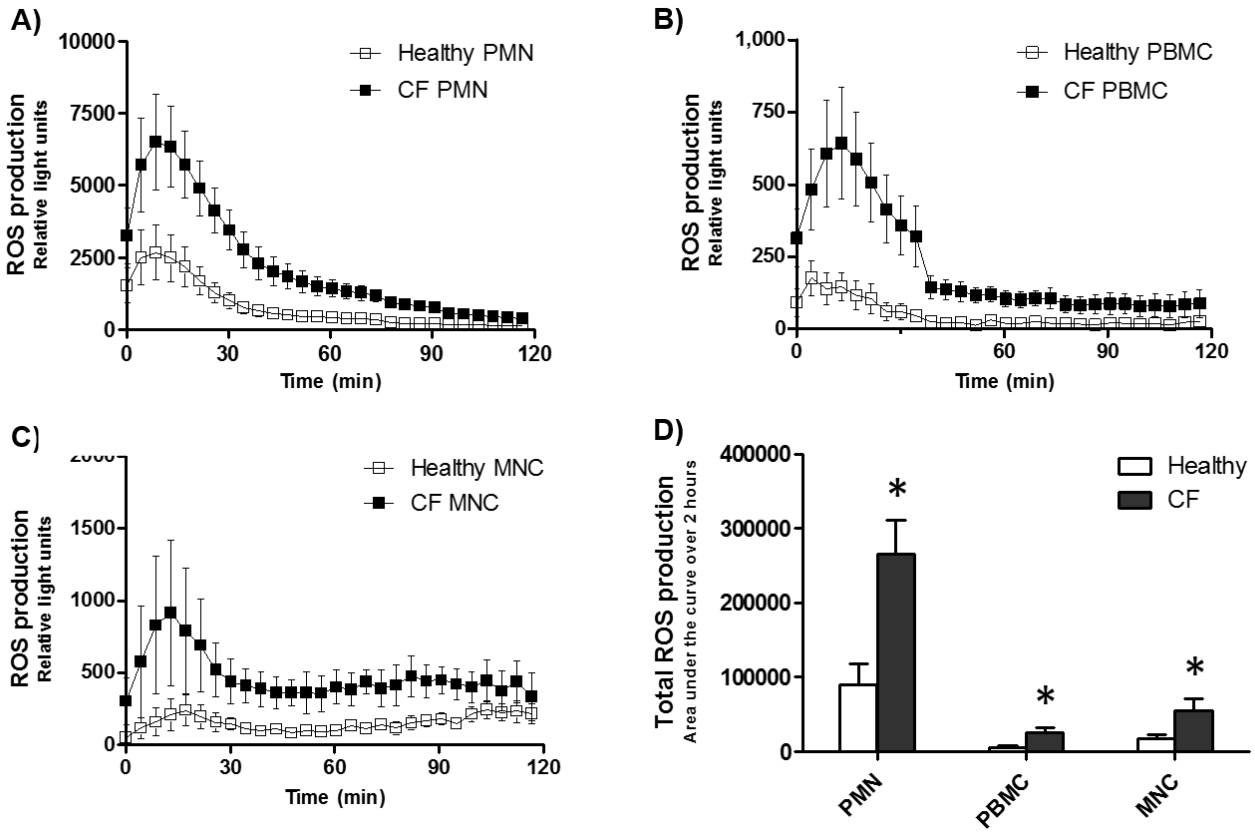
Intact chemotaxis, but impaired meandering



Brunel et al, ERJ Open Research *in press*

Understanding *Aspergillus* infections in CF *the neutrophils*

Excessive reactive oxygen species production



Brunel et al, ERJ Open Research *in press*

Understanding *Aspergillus* infections in CF
clinical correlates

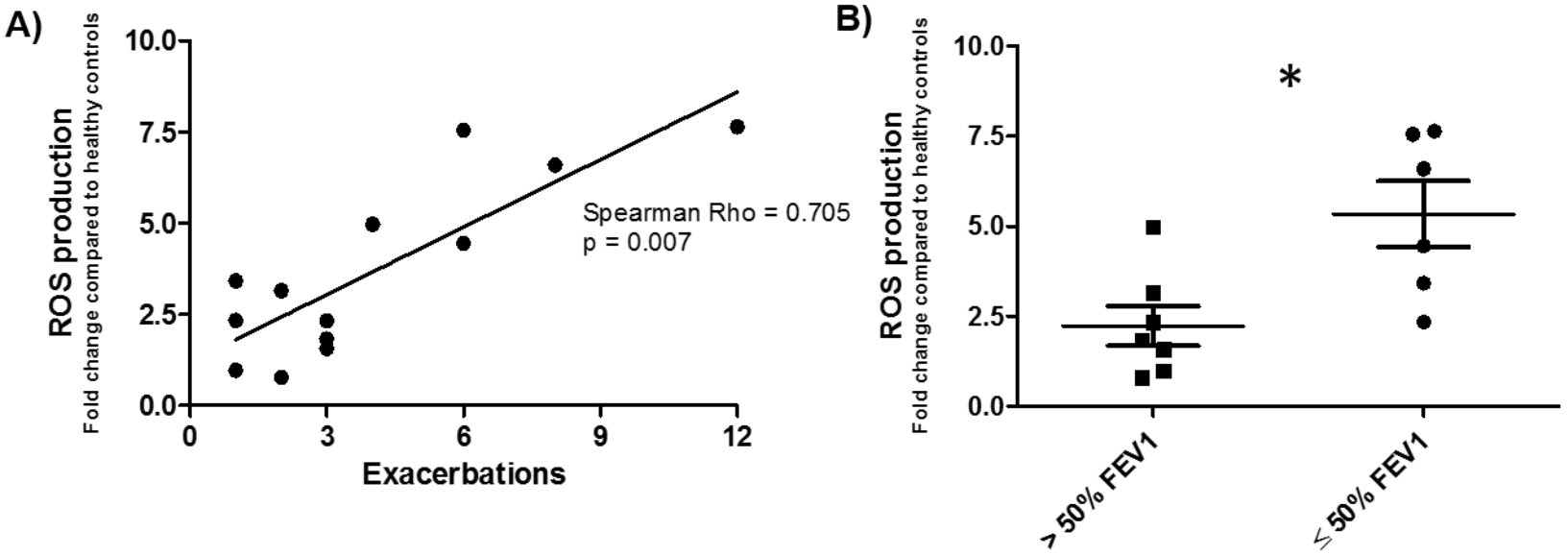
Patient population

24 adult CF patients, at least one deltaF508 mutation

Body mass index: mean 22.3 (18.3-29)

Forced expiratory volume: mean 50% (26-92)

Exacerbations/yr: mean 3 (1-12)



Brunel et al, ERJ Open Research *in press*

Why and when aspergillosis first appears in cystic fibrosis patients?
summary

- Data incomplete with respect to *A. fumigatus* colonization dynamics and time of acquisition
- Under-reporting highly likely due to huge variety in diagnostic measurement and inadequate tools to stratify clinical phenotypes of *Aspergillus* infection and disease in CF
- CFTR-deficient epithelial and innate immune cells are clearly impaired in antifungal immune responses
- *A. fumigatus* provokes increased inflammation and plays a role in progressive lung disease as observed in CF
- Mechanistic insight warranted to identify new targets for therapy

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