

Mechanisms behind the CF - ABPA link



Dr. Dominik Hartl
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1. CF – ABPA links

2. The role of chemokines

1. CF – ABPA links

ABPA

ABPA is a Th2 disease

Is CF a Th2 disease?

1. CF – ABPA links

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Pulmonary hypersensitivity disease mediated by an allergic response to *Aspergillus fumigatus*.



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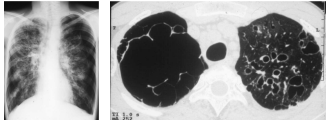
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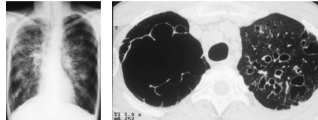
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Clinical need for reliable serum markers of ABPA (Stevens et al., 2003)

1. CF – ABPA links

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PBMCs from ABPA patients: **increased sensitivity to IL-4** with upregulation of the low-affinity IgE receptor on B cells
(Knutsen 2003, Khan 2000, McClellan 1999)

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ABPA in CF / ABPA in asthma

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TCR activation: **IL-4 production \downarrow** , **NFAT \uparrow** compared to WT
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IL-4, IL-10 and IFN-γ secretion => "low responders" and „high responders“;
***Pseudomonas* infection => higher IL-4 levels**
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***Pseudomonas* infection results in pulmonary Th2 response**
BALF: CCR4⁺CD4⁺ Th2 cells; IL-4, IL-13 and TARC ↑
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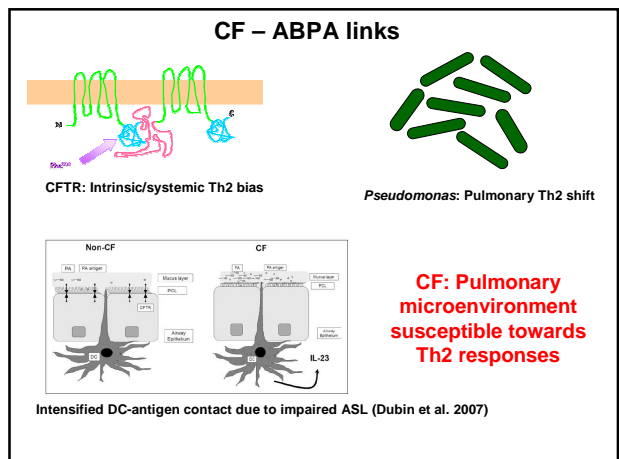
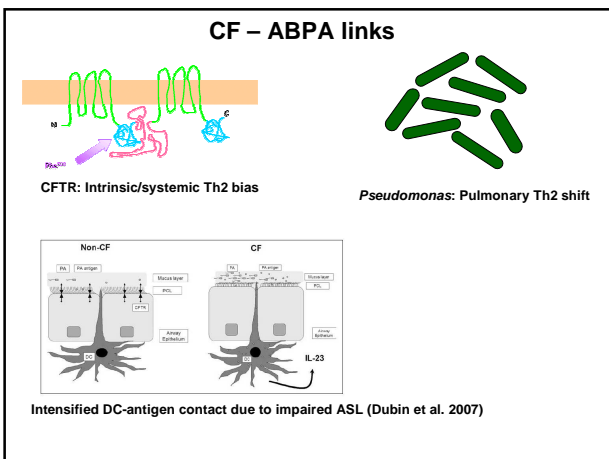
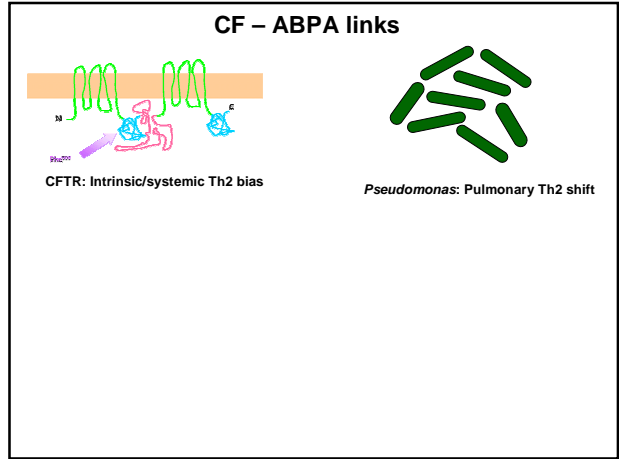
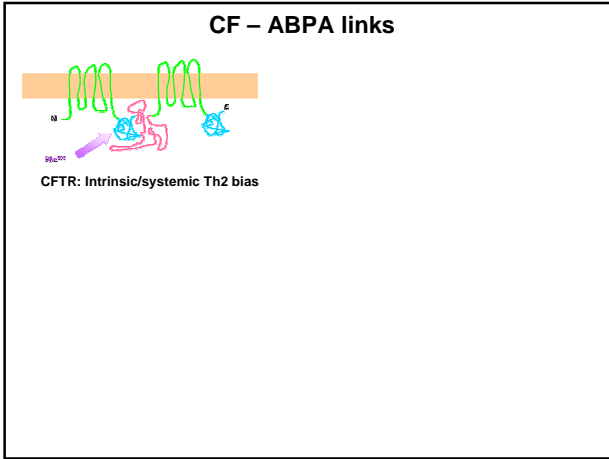
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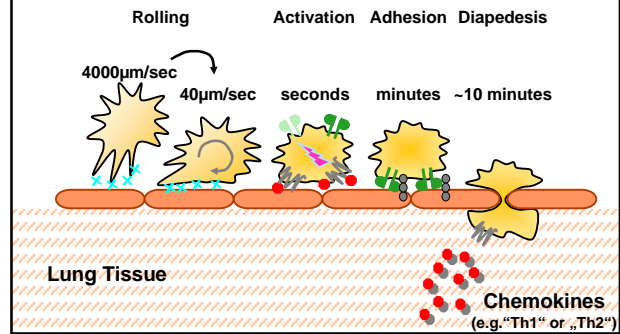
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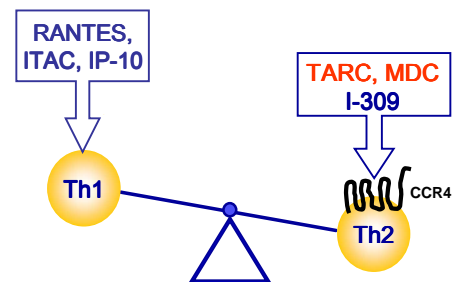


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Th1/Th2 Chemokines = Th1/Th2 Cytokines

Th1/Th2 Chemokines



Sallusto et al., Annu Rev Immunol. 2000

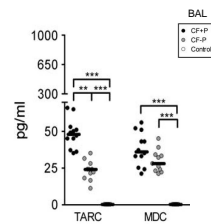
Chemokines in CF lung disease

Predominant chemokine: **IL-8** (KC, MIP-2)

Lack of Th1 chemokine **RANTES** secretion in CFTR^{-/-} epithelial cells (Schwiebert et al., 1999)

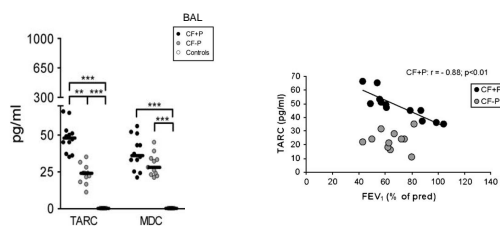
High levels of Th2 chemokines **TARC** and **MDC** and in CF BALF, especially Pseudomonas⁺ (Hartl et al., 2006)

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Hartl et al., JACI, 2006

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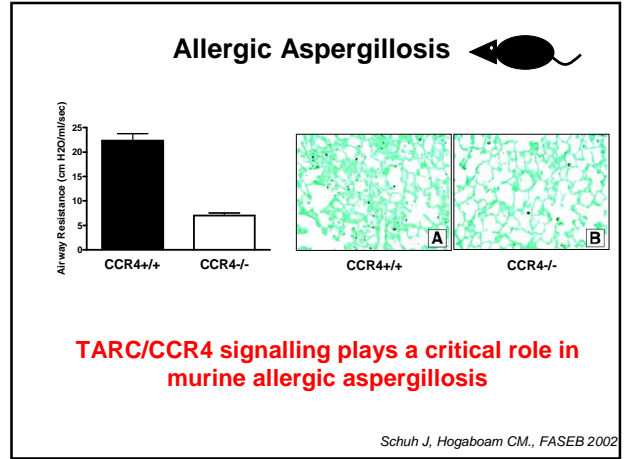
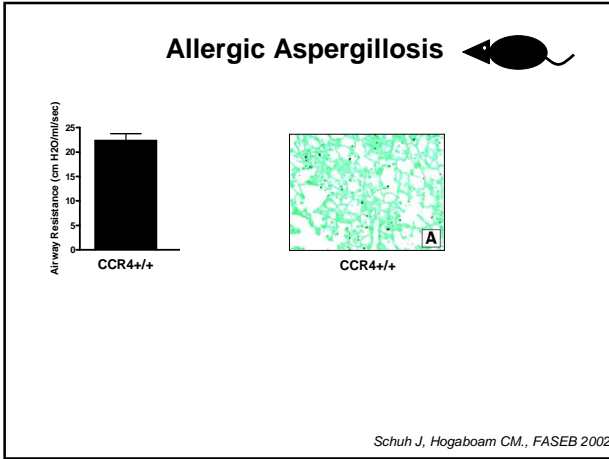
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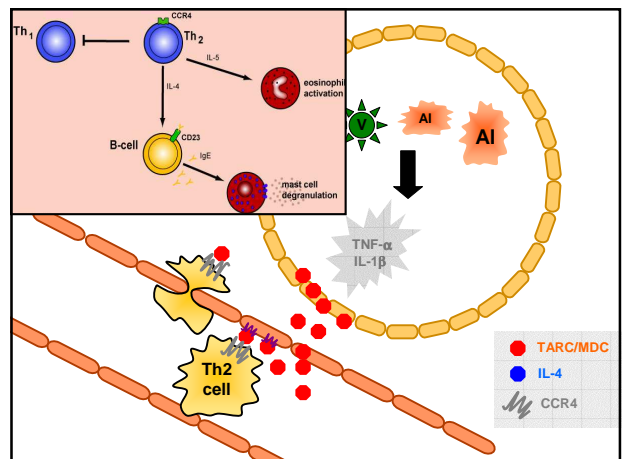
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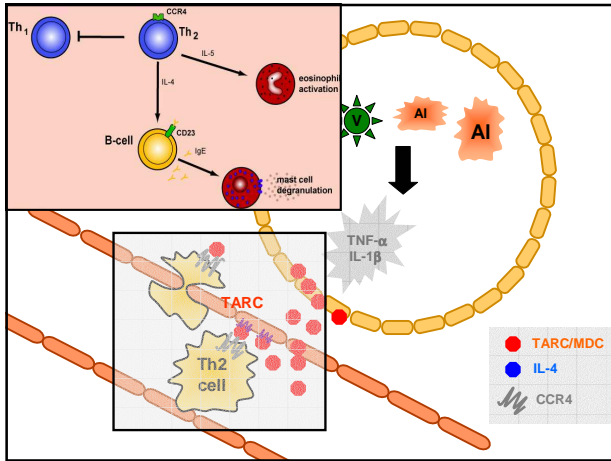
Th1/Th2 dysbalance in CF
A special role for TARC?

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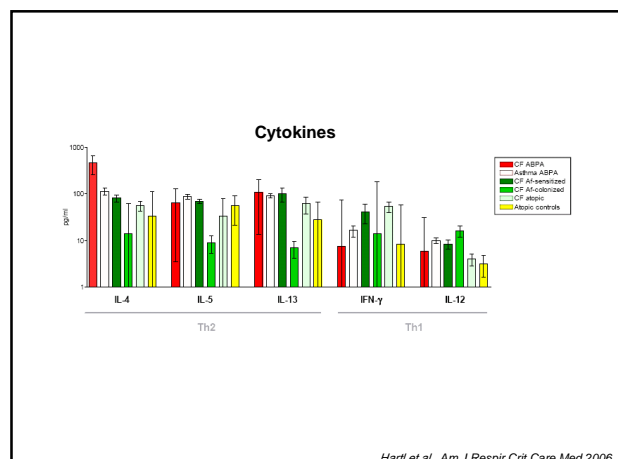
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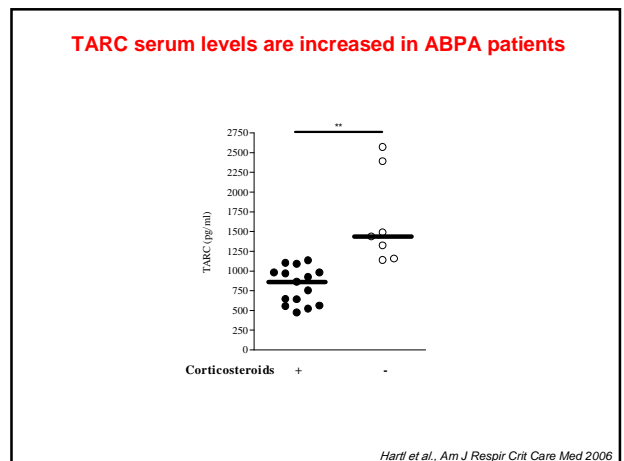
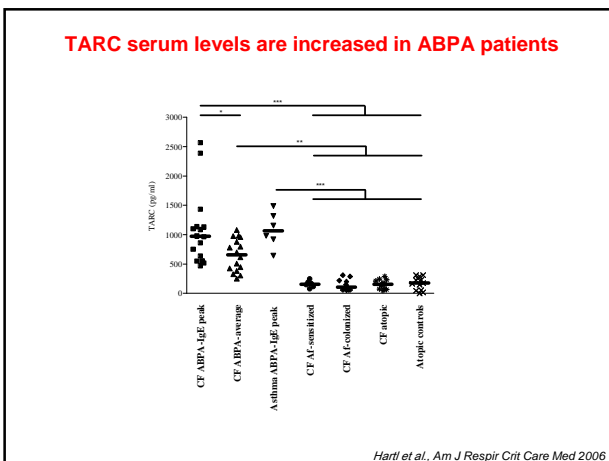
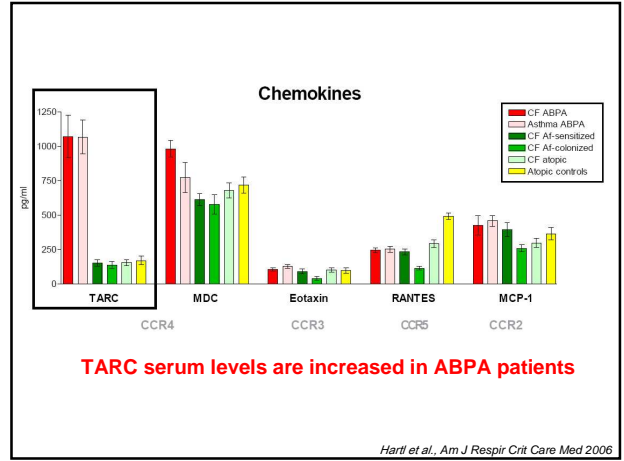
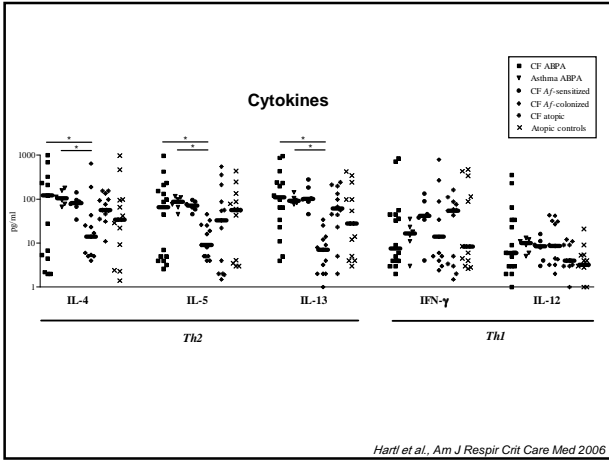
TARC may be a serum marker for ABPA

Is TARC useful to differentiate CF ABPA patients from

- CF patients colonized with *Aspergillus fumigatus*
- CF patients sensitized to *Aspergillus fumigatus*
- Atopic CF patients
- Atopic healthy control subjects

	CF ABPA	Asthma ABPA	CF <i>A. fumigatus</i> sensitization	CF <i>A. fumigatus</i> colonization	CF atopic	Non-CF atopic
Number	16	6	6	13	12	13
Age (years)	18 (11-31) ^a	24 (21-30)	21 (15-28)	24 (11-42)	21 (14-37)	17 (7-19)
Sex (MF)	10/6	3/3	3/3	6/7	7/5	6/7
CRP (mg/dl)	0.6 (0-6.5)	0.3 (0-1.2)	0.4 (0-1.6)	0.6 (0-1.7)	0.8 (0-2)	0
IgE (IU/ml)	3254 (445-6606)**	1899 (1321-2242)*	643 (395-988)	47 (1-120)	627 (338-2395)*	838 (446-4165)*
Specific IgE to <i>A. fumigatus</i>	4 (0-6)**	4 (0-5)**	3 (0-4)**	0 (0-0)	0 (0-0)	0 (0-0)
rAsp f4 (kUI)	13.8 (0.2-53.4)	10.5 (1.2-34.5)	0.38 (0-4.9)	0 (0-0)	n.d.	n.d.
rAsp f6 (kUI)	2.4 (0.1-8.3)	1.7 (0-7.1)	0.36 (0-5.2)	0 (0-0)	n.d.	n.d.
<i>A. fumigatus</i> in sputum culture	16/16**	6/6**	6/6**	13/13**	0/12	0/13
Blood Eosinophils (%)	10 (5-20)*	9 (3-18)*	5 (0-8)	2 (0-5)	7 (4-11)*	8 (2-10)*
FEV ₁ (% of pred)	46 (32-81)	62 (41-79)	60 (24-93)	57 (15-104)	61 (37-92)	n.d.
FVC (% of pred)	61 (34-89)	83 (65-95)	71 (45-84)	65 (36-91)	72 (41-93)	n.d.
MEF ₂₅₋₇₅ (% pred)	22 (12-39)	31 (17-35)	29 (19-37)	24 (15-41)	26 (17-40)	n.d.
Inhaled steroids	13/16	6/6	3/6	7/13	7/12	5/13
Systemic steroids	11/16**	4/6*	0/6	1/13	1/12	0/13
Itraconazole	10/16**	4/6*	0/6	3/13*	0/12	0/13
<i>P. aeruginosa</i>	10/16	0/6	2/6	9/13	6/12	0/13
<i>S. maltophilia</i>	7/16	0/6	0/6	3/13	3/12	0/13
dFS08	9/6/1	n.d.	4/2	3/6/4	5/5/2	n.d.
homo/hetero/other						





Conclusions Study I

1. TARC serum levels **indicate** clinically active ABPA in CF and asthma patients vs *Aspergillus* colonization and sensitization

2. TARC serum levels associated with **corticosteroid** treatment

Hartl et al., Am J Respir Crit Care Med 2006

Open Questions:

1. Can these results be **reproduced** in a different CF population? CF populations and centers show a great variability in their microbiological colonization, their atopic status and their genetic background

2. Is the elevation of TARC levels just an epiphenomenon of the **allergy** in ABPA patients?

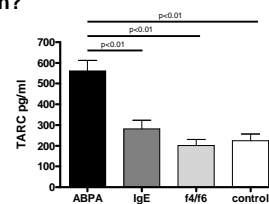
3. What is the **diagnostic accuracy** of TARC compared to other serological markers for ABPA?

ABPA study II: Switzerland

	CF			
	ABPA	Non-ABPA		
		IgE	rAsp f4/f6	control
Number of patients	12	12	8	16
Age at first serum sample in years	12 (5-20)	12 (7-42)	17 (9-40)	13 (6-29)
Number of serum samples:	87	62	31	85
Duration of continuous serum samples (months)	40 (25-80)	54 (0-61)	13 (0-72)	37 (24-78)
Sex (M/F)	7/5	6/6	4/4	9/7
IgE (IU/ml)	1050	978	450	254

Latzin et al., ERJ 2007

1. Can these results be **confirmed** in a different CF population?



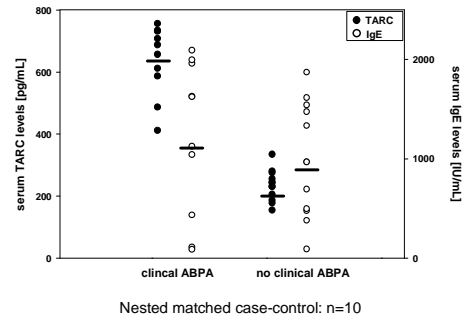
	ABPA group	Non-ABPA groups		
		IgE	f4/f6	Control
Number of patients	12	12	8	16
Number of measurements	85	54	23	36
TARC level median [pg/ml]	589	256	200	207
TARC level IQR [pg/ml]	465-673	213-342	167-245	177-281
TARC level range [pg/ml]	243-956	154-531	106-317	132-389

Latzin et al., ERJ 2007

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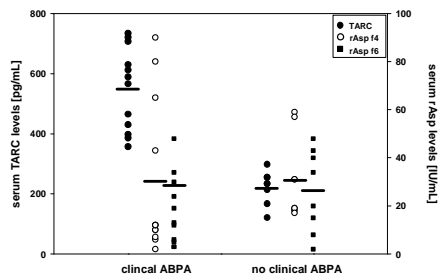
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Nested matched case-control: n=10

Latzin et al., ERJ 2007

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Latzin et al., ERJ 2007

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	TARC	IgE	rAsp f1	rAsp f3	rAsp f4	rAsp f6	IgG
Sensitivity [%]	89	64.7	68.3	65.9	81.7	64.6	53.7
Specificity [%]	91.2	81.0	83.3	85.9	71.2	86.5	83.7
Diagnostic accuracy [%]	93.4	74.3	78.1	79.0	74.8	79.0	73.2
Positive Likelihood ratio	17.3	3.4	4.1	4.7	2.8	4.8	3.3
Negative Likelihood ratio	0.09	0.44	0.38	0.40	0.26	0.41	0.55
Area under the ROC	0.98	0.84	0.83	0.82	0.79	0.80	0.73

Cut-off levels of 386 pg/ml for TARC (a cut-off level of 388 pg/ml yields to the same diagnostic accuracy with a sensitivity of 89.4% and a specificity of 96.5%, respectively), 514 IU/ml for IgE, 75 IU/ml for rAsp f1, 140 IU/ml for rAsp f3, 10 IU/ml for rAsp f4, 16 IU/ml for rAsp f6 and 140 EU/ml for IgG.

Latzin et al., ERJ 2007

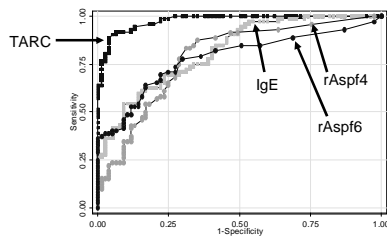
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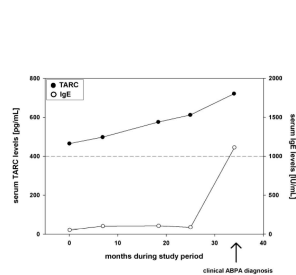


ROC curves for TARC, total IgE, rAsp f4 and rAsp f6 including all serum samples.

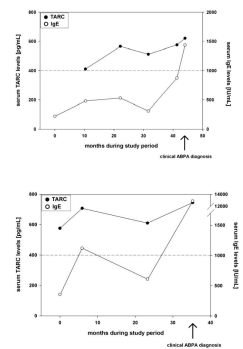
- Cut-off levels of 386 pg/ml for TARC
- including all available time points (n=265)

Latzin et al., ERJ 2007

Longitudinal analysis



In CF patients who develop ABPA
TARC serum levels are early elevated (>400pg/ml)



Latzin et al., ERJ 2007

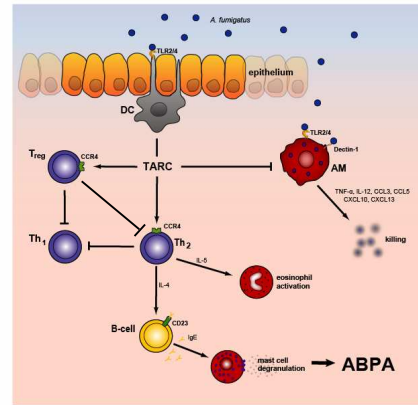
Methodological issues

Immuno- sandwich ELISA (R&D Systems, MN, USA)

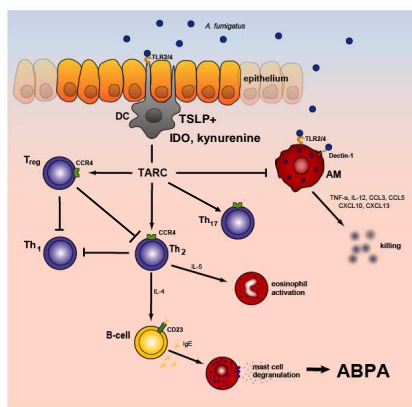
Pitfalls:

- >> sensitive to pre-analytical handling (freezing, thawing, blood in serum)
- >> reliable standard curves
- >> detection limits (~7 pg/ml - 3000 pg/ml)
- >> Intra-assay variability (5 serum samples 10x same assay): CV = 6 - 9.3%
- >> Inter-assay variability (5 serum samples 5 consecutive assay): CV = 8.4 - 17%

=> Multicenter study => One center, one technician, one thawing



Hartl D and Hogaboam CM, Allergy & Inflammation 2006



Hartl D and Hogaboam CM, Allergy & Inflammation 2006

Open questions: CF – ABPA link

Th2 imbalance in CF

- Basic mechanism: CFTR => Th2 (NFAT, Ca?)
- Subgroup of patients (high/low responders), SNPs?
- Age, disease severity, CFTR mutation, *Pseudomonas* associated?

Immunological key players

- TARC (predictive value? SNPs? effects on pulmonary cells?)
- Role of IL-17/IL-23: interface between T cells and PMNs (Dubin and Kolls, 2007)
- Role of DCs / regulatory T cells (Romani L)

Acknowledgements

Munich

Dietrich Reinhardt
Mathias Giese
Markus Krane
DFG Grant

Bern

Philipp Lätzin
Carmen Casaulta
Martin Schöni
Urs Frey

Cory Hogabaam (Aan Arbor)
Dirk Roos (Amsterdam)
Taco Kuijpers (Amsterdam)
Gernot Zissel (Freiburg)
Richard Moss (Stanford)