

SUPPLEMENTAL MATERIAL

Kisand et al., <http://www.jem.org/cgi/content/full/jem.20091669/DC1>

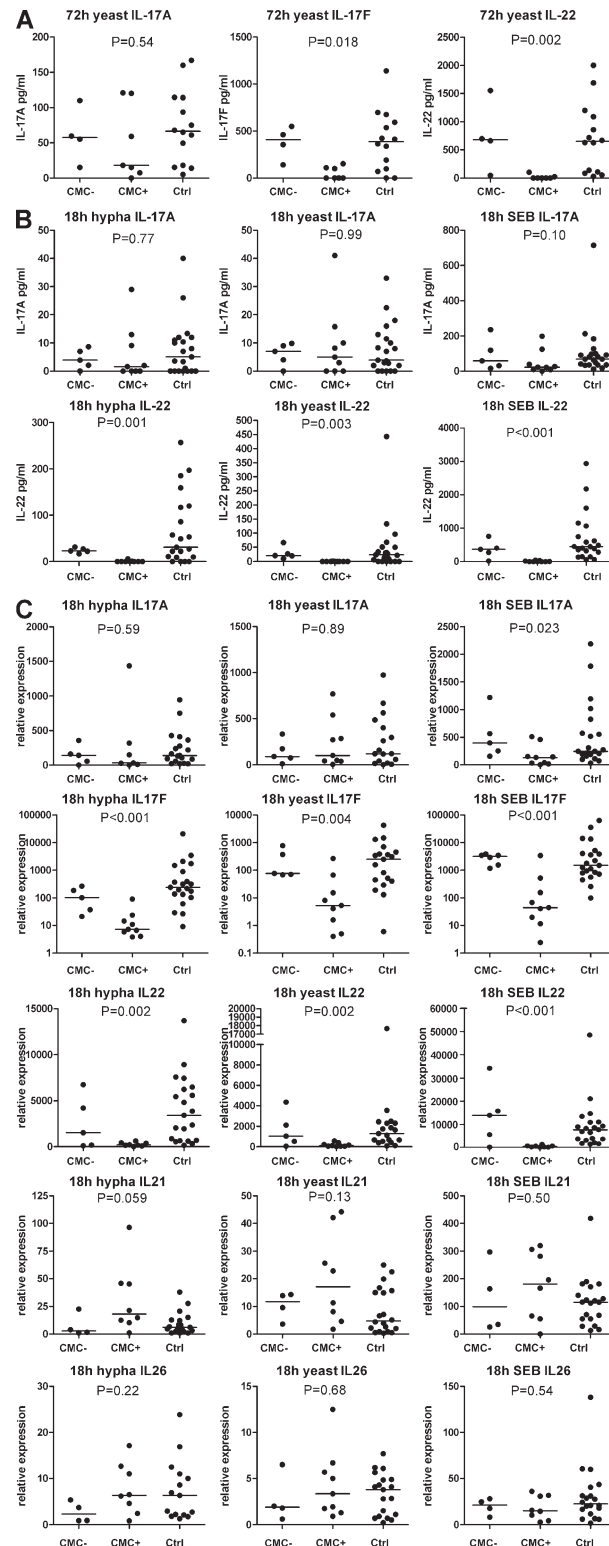


Figure S1. Additional cytokine responses not shown in Fig. 1. Cytokine production by PBMC from APECED patients with CMC (CMC+), without CMC (CMC-), and healthy controls (Ctrl) in response to *C. albicans* yeasts or hyphae or SEB, measured by ELISA (A and B) and by quantitative RT-PCR in relation to β -actin (C). Horizontal bars represent median values. Group median values were compared with the Kruskal-Wallis test. The reductions in responses from patients with CMC are similar to those against hyphae and SEB shown in main Fig. 1. They are consistently significant for IL-17F and almost complete for IL-22 but negligible for IL-17A, IL-26, or IL-21.

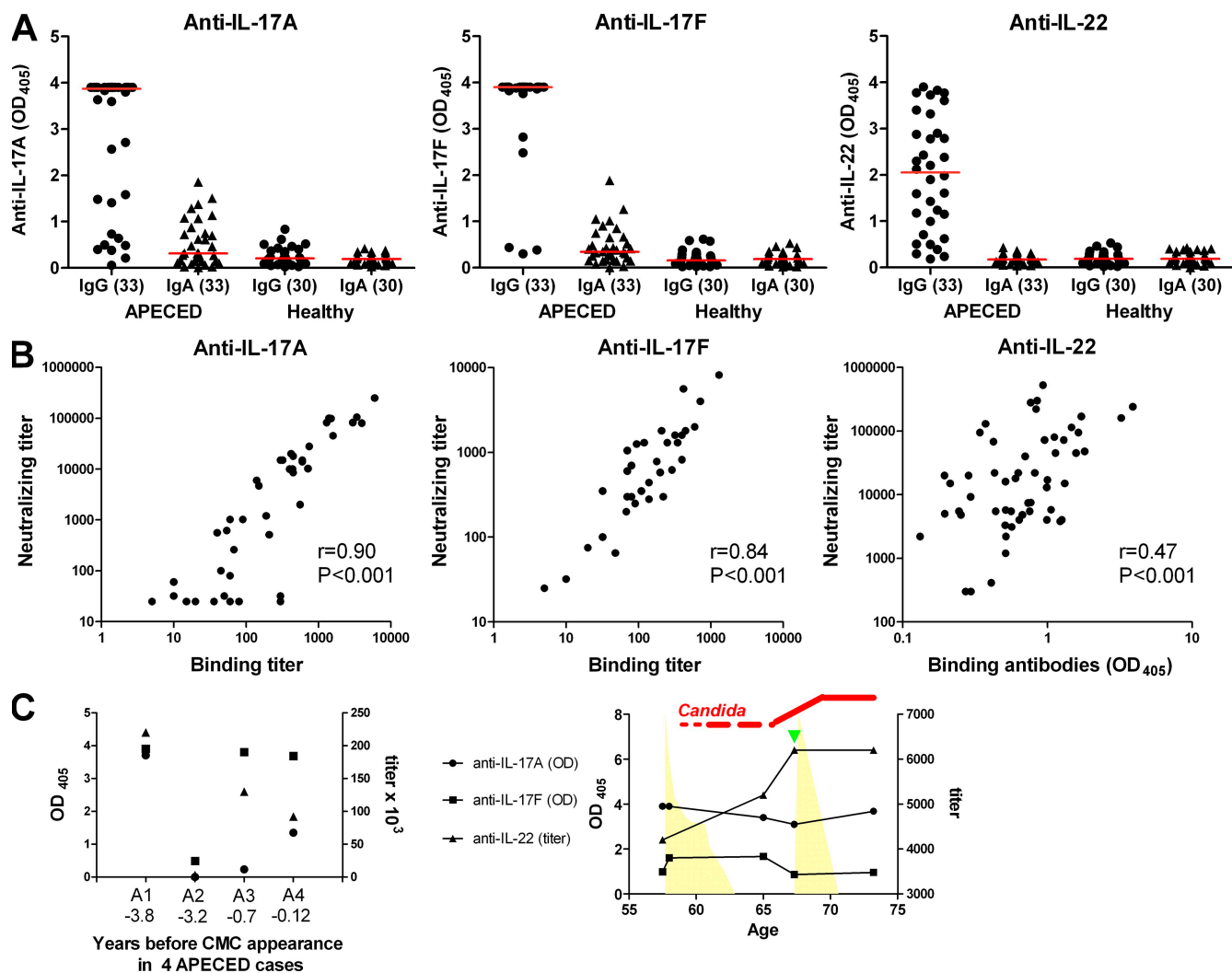


Figure S2. Features of anti-cytokine autoantibodies. (A) Binding IgG and IgA autoantibodies assayed by ELISA in sets of APECED (33) and control (30) sera. Red bars indicate group medians. (B) Correlations of neutralizing and binding titers and signals of APECED sera against IL-17s and IL-22. The apparent failure of a few sera with moderate binding signals to neutralize IL-17A in in vitro functional assays may reflect enhancement of growth-related oncogene α production by the responder cell line in response to human sera at 1/10–1/120. Against IL-22, neutralization titers are plotted against direct absorbances, as these rarely reached saturation. It is possible that IL-22 either binds more weakly to plastic or then loses certain conformations. (C) Anti-Th17 cytokine autoantibody levels in four APECED cases sampled before onset of CMC (left) and antibody levels, clinical evolution, and treatments in thymoma patient T1 (right). She already had high levels of autoantibodies against Th17 cytokines ~ 15 mo before onset of *C. albicans* infection. She was given corticosteroids and azathioprine for her myasthenia gravis (their tapering dosages are symbolized by the colored areas), which was soon followed by radiotherapy for the thymoma. Her CMC began in the nail beds, and it gradually became her major and continuous problem (thickening red line). Since the age of ~ 67 , she has lost >20 kg in weight because of persistent severe oropharyngeal candidiasis. ▼ indicates MG relapse.

Table S1. Associations between CMC and autoantibodies against Th17 cytokines

Autoantibody	CMC ^a		With CMC	Significance ^b
	Negative	Positive		
			%	
Anti-IL-17A				
Negative	12	73	85	NS
Positive	3	64	96	
Anti-IL-17F				
Negative	12	20	63	P < 0.001
Positive	3	116	97	
Anti-IL-22				
Negative	6	8	57	P < 0.001
Positive	8	123	94	

^aFour patients are missing because of incomplete clinical records. Some sera were exhausted before the anti-IL-22 neutralization assay was developed, hence the slightly different total numbers tested. Patients with dominant-negative p.G228W are not included here.

^bFisher's exact test.

Table S2. Candidiasis and anti-Th17 cytokine antibodies in thymoma patients

Patient ^{a,d}	MG onset age	Clinical evolution: other features (at ages)	Age bled	CMC ^b duration	Autoantibodies against: ^c					
					IFN- α 2	IFN- ω	IL-12	IL-17A	IL-17F	IL-22
	yr	(yr)	yr	yr						
Thymoma with CMC										
T1 (B3)	46	Thymoma invading; radioRx (57)	57	−1.3	167	17	0.92	3.90	0.98	4.2
		Oropharyngeal CMC (~66)	73	+14	350	250	3.85	3.64	0.96	6.2
T2 (B2)	27	Thymoma recurrence; radioRx (49)								
		Neuromyotonia (50); <i>Herpes zoster</i> (58)	50	+6	>320	21	–	3.90	0.91	1.8
T6	20	alopecia (43)	45	+7	>10	10	3.85	0.15	0.09	3.4
T7 (B2)	34	Thymoma recurrence; chemoRx (38)	39	+0.5	>51	51	3.85	0.76	0.77	–
T8 (B2)	45	Thymoma + AI hepatitis (53)	67	+0.5	6	0.6	3.85	0.28	0.33	–
Thymoma with intercurrent <i>C. albicans</i> infections										
T9 ^e (B3)	38.5	thymoma recurrence (45)	46	−8	150	512	3.85	0.22	–	–
			48	−5.4	150	96	3.85	0.24	0.17	20.0
		RBC aplasia ^e + oral candidiasis (54)	54	0	200	512	3.85	0.07	0.09	4.5
		<i>H. zoster</i> (56), candidiasis (58, 62)	60	+6.5	200	512	3.85	0.04	0.07	3.5
T11 (B3)	36.7 ^f	Recurrence; radioRx (46)								
		Candidiasis at esophagoscopy (48)								
		<i>Listeria monocytogenes</i> meningitis (49.8)	49	0	2.6	1	1.28	0.04	0.04	–
T10 ^e (B2)	25.7	Septicemia + neutropenia ^e (37)	37	−5	8	0.5	0.65	0.04	0.04	–
		Recurrence + polyarthritides (42)	39	−3	320	10	1.39	3.44	0.08	–
	42 ^f	Oropharyngeal candidiasis (42–44)	45	0	100	2	0.32	0.08	0.11	–
Thymoma patients unusually positive for antibodies without known candidiasis										
T3 (B3)	56 ^f	Respiratory infections (57)	56	–	512	24	2.8	0.62	0.75	8
T4 (AB)	80.2	<i>H. zoster</i> (83.2), septicemia (84.1)	81	–	0.5	ND	2.3	0.65	0.05	24
T5 (A)		COPD; no MG	61	–	–	–	–	0.45	0.06	–

–, negative; radioRx, radiotherapy; chemoRx, chemotherapy; AI, autoimmune; COPD, chronic obstructive pulmonary disease.

^aThis Table details patients who either tested positive for anti-IL-17s and/or –IL-22 (T1–T5) from the random screen (Fig. 2) or were selected because of known episodes of *C. albicans* infection. The many who screened negative are shown in Fig. 2 (A–C and F).

^bCMC duration in years at the time of sampling

^cAnti-IFN and anti-IL-22 antibodies are given as neutralization titers $\times 10^{-3}$ (the reciprocal of the serum dilution that reduces 10 U/ml of IFN to 1 U/ml; for anti-IL-22 the reciprocal of the serum dilution yielding an ELISA absorbance value halfway between the positive and negative controls); anti-IL-12 and anti-IL-17s are given as ELISA values (absorbance at 405 nm, which saturate at ~3.8).

^dThymoma WHO histological types are shown in parentheses.

^eThe RBC aplasia in T9 and the neutropenia in T10 both responded well to immunosuppressive drug treatment. For further details on the thymoma patients, contact nick.willcox@imm.ox.ac.uk.

^fThymoma invasive.