Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I

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Abbreviations used: APS-I, autoimmune polyendocrine syndrome type I; auto-Abs, autoantibodies; CMC, chronic mucocutaneous candidiasis; FI, fluorescence intensity.

Most patients with autoimmune polyendocrine syndrome type I (APS-I) display chronic mucocutaneous candidiasis (CMC). We hypothesized that this CMC might result from autoimmunity to interleukin (IL)-17 cytokines. We found high titers of autoantibodies (auto-Abs) against IL-17A, IL-17F, and/or IL-22 in the sera of all 33 patients tested, as detected by multiplex particle-based flow cytometry. The auto-Abs against IL-17A, IL-17F, and IL-22 were specific in the five patients tested, as shown by Western blotting. The auto-Abs against IL-17A were neutralizing in the only patient tested, as shown by bioassays of IL-17A activity. None of the 37 healthy controls and none of the 103 patients with other autoimmune disorders tested had such auto-Abs. None of the patients with APS-I had auto-Abs against cytokines previously shown to cause other well-defined clinical syndromes in other patients (IL-6, interferon [IFN]-γ, or granulocyte/macrophage colony-stimulating factor) or against other cytokines (IL-1β, IL-10, IL-12, IL-18, IL-21, IL-23, IL-26, IFN-β, tumor necrosis factor [α], or transforming growth factor β). These findings suggest that auto-Abs against IL-17A, IL-17F, and IL-22 may cause CMC in patients with APS-I.

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Autoimmune polyendocrine syndrome type I (APS-I), also known as autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (Online Mendelian Inheritance in Man no. 240300), is a rare, autosomal recessive primary immunodeficiency first described clinically in 1929 (Notarangelo et al., 2006; Husebye et al., 2009). APS-I is characterized principally by multiple autoimmune endocrinopathies, hypoparathyroidism, and adrenal insufficiency in particular, with some of these symptoms being caused by pathogenic autoantibodies (auto-Abs). A genome-wide mapping approach led to the identification of APS-I–causing mutations in the *AIRE* gene in 1997 (Finnish-German APECED Consortium, 1997; Nagamine et al., 1997). Autoimmunity in patients with APS-I may be accounted for by the key role of *AIRE* in tolerance. *AIRE* is expressed in the thymus, where it contributes to the expression of peripheral antigens (Anderson et al., 2002; Mathis and Benoist, 2009). This gene has also been shown to be expressed in secondary lymphoid organs, where it also contributes to tolerance (Gardner et al., 2008). Surprisingly, most patients with APS-I suffer from chronic mucocutaneous candidiasis (CMC) without displaying any marked susceptibility to any other pathogen. The product of the *AIRE* gene is not involved in any known cellular pathway governing host defense. The pathogenesis of CMC in patients with APS-I has thus remained both intriguing and elusive.

High titers of auto-Abs against some type I IFNs, including IFN-α and -ω in particular, are found in all patients (Meager et al., 2006; Meloni et al., 2008). These auto-Abs are a hallmark of APS-I and are therefore useful for diagnostic purposes (Husebye et al., 2009). However, they confer no particular overt predisposition to viral diseases, perhaps because of the large number of redundant type I IFN species, resulting in incomplete neutralization of the overall antiviral activity of IFNs by the auto-Abs. It has been suggested that these auto-Abs contribute to CMC in APS-I patients (Meager et al., 2006). However, this is now thought unlikely because of the absence of CMC in patients with various forms of STAT1 and TYK2 deficiency and impaired responses to type I IFNs, and in patients with various forms of NEMO, UNC-93B, and TLR3 deficiencies and impaired production of type I IFNs (Minegishi et al., 2006; Zhang et al., 2008; Chapgier et al., 2009). Nevertheless, based on this observation, we hypothesized that CMC in patients with APS-I might result from autoimmunity to cytokines other than type I IFNs potentially involved in protective immunity to *Candida albicans* in the skin and mucosae.

Recent studies in the mouse have suggested that IL-17 cytokines, such as IL-17A, IL-17F, and IL-22 (Korn et al., 2009), may be important in host defense against *Candida* (Conti et al., 2009; van de Veerdonk et al., 2009). However, it is difficult to assess mucocutaneous immunity to *Candida* in mice (Netea et al., 2008), in which IL-17 cytokines seem to be important for the control of various other pathogens, particularly in the lungs and gastrointestinal tract (Dubin and Kolls, 2008; Khader et al., 2009). Stimulation with *Candida* in vitro leads to the preferential generation of IL-17A– and IL-22–producing human T cells (Acosta-Rodriguez et al., 2007; Liu et al., 2009). Furthermore, patients with STAT3 deficiency display a predisposition to CMC (and staphylococcal disease) and lack IL-17–producing T cells (de Beaucoudrey et al., 2008; Ma et al., 2008; Milner et al., 2008; Minegishi et al., 2009). Similarly, some patients with IL-12p40 or IL-12Rβ1 deficiency present CMC (together with mycobacteriosis and salmonellosis) and have lower than normal proportions of IL-17–producing T cells (de Beaucoudrey et al., 2008). Finally, patients with rare defects of CARD9, which normally controls the production of IL-17 in response to dectin-1 and -2 stimulation by *Candida*, suffer from candidiasis (LeibundGut-Landmann et al., 2007; Glocker et al., 2009; Robinson et al., 2009). We therefore hypothesized that autoimmunity to IL-17 cytokines might account for CMC in patients with APS-I.

RESULTS AND DISCUSSION

We searched for auto-Abs against IL-17 cytokines (IL-17A, IL-17F, IL-22, and IL-26, which is absent from mice) or against IL-17–inducing cytokines (IL-1β, IL-6, IL-21, IL-23, and TGF-β) in 33 patients diagnosed with APS-I on the basis of autoimmune polyendocrinopathy, circulating auto-Abs against IFN-α and –ω, and the presence of two mutant *AIRE* alleles (Table I). CMC was observed in 29 out of the 33 patients (Table I). Multiplex particle-based flow cytometry revealed a high fluorescence intensity (FI; >1,000, arbitrary definition) of IgG auto-Abs against IL-17A in the plasma of 22 patients, with a high FI of auto-Abs against IL-17F in 31 patients and of auto-Abs against IL-22 in 30 patients (Fig. 1 and Table I). All 33 patients had significant ($P = 6.4 \times 10^{-9}$, 4.6×10^{-12}, and 2.16×10^{-11}, respectively) levels of auto-Abs against at least one of these three cytokines. 5 patients had a significant reaction against a single cytokine, 6 had a reaction against two cytokines, and 22 had a reaction against all three cytokines. No auto-Abs against IL-17A, IL-17F, and IL-22 were found in plasma samples from the 37 healthy individuals tested. Similarly, no auto-Abs against any of these three cytokines were found in plasma samples from another 103 patients with various autoimmune conditions (Fig. S4, A and B). No auto-Abs against IL-1β, IL-6, IL-23, or IL-26 (whether as a monomer or a dimer) were found in the APS-I patients (Fig. S3, A–D). There were no auto-Abs against IL-12, IFN-γ, or GM-CSF (Fig. S3, E–G), consistent with the lack of mycobacterial disease and alveolar proteinosis in patients with APS-I. No auto-Abs were found against any of the other cytokines tested, including IL-10, IL-18, IFN-β, and TNF (Fig. S3, H–K). As expected, all APS-I patients had auto-Abs against IFN-α (Fig. 1 and Table I) and IFN-ω (not depicted). In addition, no IgA antibodies, a key element of mucosal immunity, directed against IL-17A, IL-17F, or IL-22 were detected in the plasma of 21 APS-I patients by classical ELISA (not depicted).

We also ran classical ELISA, with plasma samples diluted by a factor of five more than in the multiplex assays. 4 out of

the 30 patients tested had high titers of IgG (OD > 1) auto-Abs against IL-17A ($P = 0.056$), 10 patients had high titers of auto-Abs against IL-17F ($P = 1.4 \times 10^{-7}$), and 12 patients had high titers of auto-Abs against IL-22 ($P = 1.23 \times 10^{-7}$; Fig. S1 and Table I). 17 patients had auto-Abs against at least one of these three cytokines, including 10 patients with auto-Abs against a single cytokine, 5 patients with auto-Abs against two cytokines, and 2 patients with auto-Abs against all three cytokines. No such auto-Abs were detected in the 37 healthy controls tested. This assay was not used to test patients with various autoimmune diseases. There were no detectable auto-Abs against TGF-β1, IL-6, IL-21, IL-23, or IL-26, or against IL-12 or IFN-γ in any of the APS-I patients (Fig. S2, A–G). Thus, in both ELISA and multiplex particle-based flow cytometry, the only auto-Abs detected in the 33 patients with APS-I tested were directed against IFN-α, IFN-ω, IL-17A, IL-17F, and IL-22 (Fig. 1, Fig. S1, and Table I). Moreover, all 33 patients with APS-I had auto-Abs against at least one out of three IL-17 cytokines (IL-17A, IL-17F, and IL-22). High titers of auto-Abs against all three of these cytokines were found in 22 patients.

We performed Western blotting to assess the specificity of these auto-Abs. Plasma samples from five patients with APS-I and auto-Abs against IL-17A, IL-17F, and/or IL-22, as detected by ELISA and multiplex particle-based flow cytometry, and from a healthy individual were tested for their ability to recognize rIL-17A, rIL-17F, and rIL-22 on Western blots. The three cytokines were clearly recognized by plasma samples from patients but not by the control plasma (Fig. 2). The plasma samples from patients did not recognize IL-23 in a control assay. Finally, we investigated whether the auto-Abs against IL-17A neutralized this cytokine in a simple

Table I. APS-I patients tested

Patients	Gender	Age	Origin	*AIRE* genotype	Auto-Abs against							Mucocutaneous candidiasis phenotype	
					Luminex				ELISA			Age of onset	Clinical features
					IFN-α	IL-17A	IL-17F	IL-22	IL-17A	IL-17F	IL-22		
APS-I 1	M	12 yr	Saudi Arabia	845insc/845insc	8,584	7,406	1,874	11,810	0.11	0.21	2.41	18 mo	Mouth, nails, esophagus
APS-I 2	M	9 yr	Africa	c958del/c958del	1,070	1,264	23,963	13,661	0.03	2.16	2.41	6 yr	Nails, scalp, eyes
APS-I 3	M	13 yr	Africa	c958del/c958del	6,467	4,824	28,271	13,265	0.03	2.61	2.51	–	Nails
APS-I 4	F	26 yr	North Africa	ex6_8del/ex6_8del	10,836	828	1,765	761	0.02	0.11	0.26	16 yr	Nails, scalp
APS-I 5	M	25 yr	North Africa	ex6_8del/ex6_8del	12,785	1,448	16,980	2,758	0.04	1.33	0.26	7 yr	Nails, scalp, digestive tract
APS-I 6	F	10 yr	North Africa	ex6_8del/ex6_8del	3,705	1,998	16,742	5,691	–	1	1.09	1 mo	Mouth, eyes, vulva
APS-I 7	M	10 yr	France	R257X/P539L	20,109	27,523	25,409	18,446	2.29	1.95	2.83	2 mo	Mouth, esophagus, respiratory tract
APS-I 8	F	21 yr	Ireland	964del13/964del13	14,169	192	1,645	5,914	0.02	0.13	0.21	3 yr	Mouth, scalp, skin
APS-I 9	M	20 yr	Ireland	964del13/964del13	12,325	455	6,135	11,345	0.03	0.31	2.06	3 yr	Mouth, nails, perineum
APS-I 10	F	29 yr	UK	964del13/964del13	9,371	734	15,140	615	0	0.11	0.03	4 yr	Mouth, esophagus, nails
APS-I 11	F	36 yr	UK	964del13/964del13	12,078	148	4,439	102	0.01	0.15	0.16	Childhood	Mouth, esophagus, vagina
APS-I 12	F	41 yr	UK	964del13/964del13	6,098	17,201	24,837	9,749	0.16	0.47	0.64	4 yr	Mouth, nails, vagina, anus
APS-I 13	F	18 yr	Ireland	964del13/964del13	13,379	367	548	2,708	0.02	0.1	0.18	Childhood	Skin, scalp
APS-I 14	M	12 yr	Ireland	964del13/964del13	13,555	24,983	17,131	22,225	0.36	0.35	1.06	Few weeks	Mouth, nails, scalp
APS-I 15	M	18 yr	UK	964del13/964del13	16,906	28,484	25,907	20,585	2.34	0.5	1.07	3 yr	Mouth, nails
APS-I 16	M	18 yr	UK	964del13/964del13	15,473	28,262	23,926	12,034	1.89	0.52	0.3	3 yr	Mouth, nails
APS-I 17	M	13 yr	Ireland	964del13/964del13	8,086	805	23,917	14,558	0.02	0.64	0.91	5 yr	Mouth, nails
APS-I 18	F	10 yr	UK	964del13/964del13	13,053	5,282	27,409	15,236	0.03	1.52	0.61	Few weeks	Mouth
APS-I 19	M	6 yr	UK	964del13/964del13	13,797	13,588	28,025	13,575	0.25	1.84	1.83	Birth	Mouth, perineum
APS-I 20	M	17 yr	UK	964del13/964del13	11,946	11,272	25,904	2,129	0.08	0.39	0.12	7 yr	Penis, perineum
APS-I 21	M	9 yr	UK	964del13/964del13	13,127	752	12,048	18,039	0.08	0.44	1.11	2 yr	Esophagus, nails
APS-I 22	F	52 yr	UK	964del13/c.769C>T	8,012	955	22,886	4,502	ND	ND	ND	Childhood	Mouth
APS-I 23	M	16 mo	Hungary	R257X/R257X	23,216	20,035	4,531	21,012	0.55	0.08	2.31	–	None
APS-I 24	M	18 yr	Hungary	R257X/C449fsX479	23,486	1,708	28,012	18,937	0.05	1.97	0.45	–	None
APS-I 25	M	14 yr	Hungary	R257X/L323fsX373	23,923	2,025	9,169	5,259	0.06	0.15	0.18	–	None
APS-I 26	F	21 yr	Hungary	R257X/R257X	23,120	12,359	28,834	11,583	0.43	2.86	0.16	–	None
APS-I 27	M	28 yr	Canada	964del13/964del13	21,225	737	7,576	5,047	0.02	0.09	0.04	3 yr	Mucosa, skin, nails, eyes, scalp
APS-I 28	F	31 yr	Canada	964del13/964del13	22,324	26,214	25,064	1,178	0.91	0.78	0.03	Birth	Mucosa, skin, nails, eyes, scalp
APS-I 29	F	16 yr	Canada	delT253/R257X	24,099	2,268	13,563	15,899	0.08	0.79	0.14	2 yr	Nails
APS-I 30	M	19 yr	Canada	delT253/R257X	21,442	153	448	1,264	0.03	0.04	0.24	3 yr	Mucosa, skin, nails
APS-I 31	F	5 yr	Canada	964del13/964del13	22,798	26,120	28,667	19,954	1.99	>3	1.83	6 mo	Mucosa
APS-I 32	F	31 yr	UK	964del13/964del13	14,718	2,155	27,222	14,358	ND	ND	ND	15 yr	Mouth, nails
APS-I 33	F	7 yr	UK	964del13/964del13	15,332	25,648	26,358	22,808	ND	ND	ND	4 yr	Mouth, nails, scalp

Sex; age; country of origin; *AIRE* genotype; levels of auto-Abs against IFN-α, IL-17A, IL-17F, and IL-22 measured by Luminex (FI values) or classical ELISA (OD values); and candidiasis phenotype are shown.

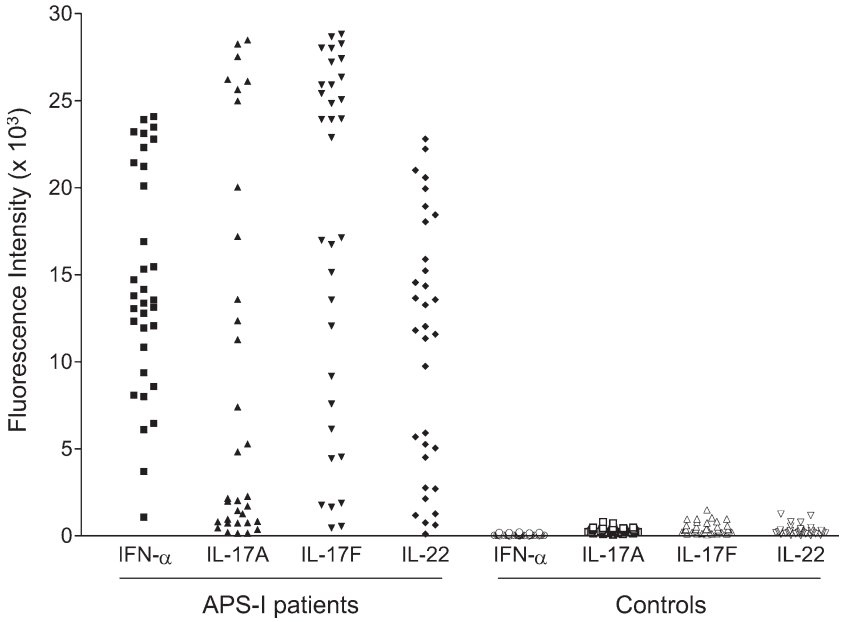

Figure 1. High titers of auto-Abs against IFN-α, IL-17A, IL-17F, and IL-22 in the plasma from patients with APS-I. Anti–IFN-α, –IL-17A, –IL-17F, and –IL-22 circulating IgG titers were measured by multiplex particle-based flow cytometry in 33 samples from patients with APS-I and in 37 samples from healthy controls. FI is plotted on the y axis. Representative data for two experiments are shown.

biological assay. We incubated fibroblasts from a healthy control with up to 50 ng/ml rIL-17A in the presence of plasma from healthy individuals, or from a patient with APS-I and auto-Abs against IL-17, as detected by ELISA and Western blotting. We then measured the induction of IL-6 by ELISA on the supernatant. Strikingly, even strong dilutions of plasma samples from patients (up to 0.1%; not depicted) completely abolished fibroblastic cell responses to such high concentrations of rIL-17 (Fig. 3), whereas control plasma had no effect. Lower concentrations of IL-17A failed to induce IL-6, even in the presence of control plasma (unpublished data). The lack of robust bioassays precluded assessments of the possible neutralizing effects of auto-Abs against IL-17F and IL-22. Thus, all patients with APS-I tested had high titers of auto-Abs against IL-17A, IL-17F, and IL-22 in their plasma, and these auto-Abs were specific and neutralizing, at least in the patients tested. These data suggest that most patients with APS-I carry neutralizing auto-Abs against IL-17A, IL-17F, and IL-22.

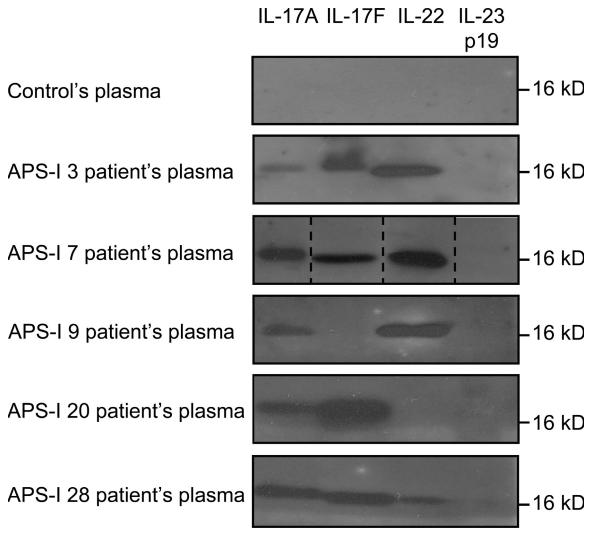

Figure 2. Specific auto-Abs against IL-17A, IL-17F, and/or IL-22 in the plasma from patients with APS-I. Western blot against rIL-17A, rIL-17F, rIL-22, and rIL-23 were performed using plasma from a healthy individual or from five patients with APS-I, diluted 1:500. Representative data for three experiments are shown. Dashed lines indicate that intervening lanes have been spliced out.

APS-I patients may display auto-Abs against IL-17 cytokines as a result of impaired AIRE-dependent tolerance induction if the expression of the corresponding cytokine genes is normally controlled by AIRE in the thymus or in secondary lymphoid organs. Whatever the mechanism, these data suggest that impaired IL-17 immunity may account for CMC in patients with APS-I. In particular, these data are consistent with the impaired IL-17 immunity reported in other patients with known inborn errors of immunity predisposing to CMC (and other infectious diseases). Indeed, patients with low proportions (*IL12B*, *IL12RB1*) or a lack of (*STAT3*) IL-17–producing circulating T cells suffer from mild (*IL12B*, *IL12RB1*) or severe (*STAT3*) CMC, respectively. Patients with APS-I and auto-Abs against IL-17 cytokines do not suffer from severe staphylococcal disease, unlike STAT3-deficient patients (Freeman and Holland, 2009). Impaired IL-17 immunity probably contributes to staphylococcal disease in STAT3-deficient patients, as epithelial cells in the skin and

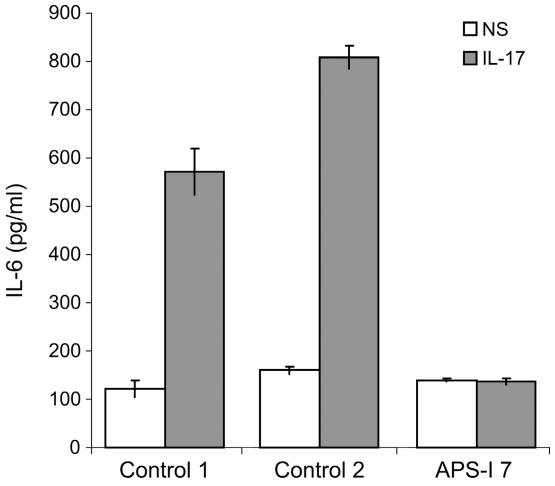

Figure 3. Neutralizing auto-Abs against IL-17A in the plasma from a patient with APS-I. IL-6 production, after 48 h of stimulation with 50 ng/ml IL-17A, by control SV-40–transformed fibroblasts incubated with 10% plasma from two controls or from one patient (APS-I patient 7; means and errors bars from duplicates are shown). The data shown are representative of two experiments.

lungs, the organs most frequently affected by staphylococcal infection in these patients, specifically rely on IL-17 stimulation for the induction of known antistaphylococcal target genes (Minegishi et al., 2009). The lack of overt staphylococcal disease in most APS-I patients may result from residual IL-17 immunity, as observed to an even greater extent in patients with IL-12p40 and IL-12Rβ1 deficiencies who also control staphylococcal infections normally (Filipe-Santos et al., 2006). In any case, proof that human IL-17 cytokines play an essential role in immunity to *Candida* will require the identification of mutations specifically impairing IL-17 immunity in other patients with inherited, isolated CMC without APS-I (Ryan et al., 2008; Hong et al., 2009). The recent observation that some patients with isolated CMC have smaller than normal proportions of IL-17–producing T cells and produce low levels of IL-17 suggests that this may be plausible (unpublished data; Eyerich et al., 2008).

Various human diseases seem to be caused by the production of auto-Abs against cytokines. Patients with pulmonary alveolar proteinosis were shown to display auto-Abs against GM-CSF in 1999 (Kitamura et al., 1999). Mutations in the GM-CSF receptor were subsequently found in other patients, establishing a causal relationship between impaired GM-CSF immunity and alveolar proteinosis (Martinez-Moczygemba et al., 2008; Suzuki et al., 2008). Patients with mycobacterial diseases and inherited IFN-γR1 deficiency were first reported in 1996 (Filipe-Santos et al., 2006). Autoimmune phenocopies, with auto-Abs against IFN-γ, were subsequently found in other patients (Döffinger et al., 2004; Kampmann et al., 2005; Patel et al., 2005). We recently reported a patient with auto-Abs against IL-6 and staphylococcal disease (Puel et al., 2008). There may be a causal relationship between auto-Abs against IL-6 and staphylococcal disease, as STAT3- and Tyk2-deficient patients suffer from staphylococcal disease and have a poor cellular response to IL-6 (Minegishi et al., 2006, 2007, 2009). However, no germline mutation in the gene encoding IL-6 or its receptor has yet been reported in similar patients. This description of patients with auto-Abs against IL-17 cytokines is the fourth reported example of pathogenic auto-Abs against human cytokines. Other auto-Abs against cytokines not tested in this study, including some that would impair the development of IL-17–producing T cells, may also contribute to the development of CMC in APS-I patients.

MATERIALS AND METHODS

Patients. 33 patients with APS-I (18 male and 15 female patients; Table I and Table S1), 103 patients with endocrine/autoimmune disorders, and 37 healthy controls (15 male and 22 female subjects) were enrolled in this study, with informed consent and approval obtained by the Necker Hospital and Medical School Institutional Review Board (IRB) and the Rockefeller University IRB.

Plasma and serum samples. Plasma and serum samples from the patients and controls were frozen at −20°C immediately after collection.

ELISA. ELISA was performed as previously described (Puel et al., 2008). In brief, 96-well ELISA plates (MaxiSorp; Thermo Fisher Scientific) were coated by incubation overnight at 4°C with 2 μg/ml rIL-6, rIL-12p70, rIL-17A, rIL-17F, rIL-21, rIL-22, rIL-23, rIL-26 monomer, rIL-26 dimer, rTGF-β, and rIFN-γ (R&D Systems). Plates were then washed (PBS/Tween 0.005%), blocked by incubation with the same buffer supplemented with 5% nonfat milk powder, washed, and incubated with 1:500 dilutions of plasma samples from the patients or controls for 2 h at room temperature (or with specific mAbs as positive controls). Plates were thoroughly washed. Horseradish peroxidase (HRP)–conjugated Fc-specific IgG fractions from polyclonal goat antiserum against human IgG or IgA (Nordic Immunological Laboratories) were added to a final concentration of 2 μg/ml. Plates were incubated for 1 h at room temperature and washed. Substrate was added and OD was measured.

Multiplex particle-based flow cytometry. Recombinant human cytokines (rIL-1β, rIL-6, rIL-10, rIL-12p40, rIL-12p70, rIL-17A, rIL-17F, rIL-18, rIL-22, rIL-23, rIL-26 monomer, rIL-26 dimer, rIFN-α, rIFN-β, rIFN-γ, rTNF, and rGM-CSF; R&D Systems) were covalently coupled to carboxylated beads (Bio-Plex; Bio-Rad Laboratories). Beads were first activated with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (Thermo Fisher Scientific) in the presence of *N*-hydroxysuccinimide (Thermo Fisher Scientific), according to the manufacturer's instructions, to form amine-reactive intermediates. The activated beads were incubated with the corresponding cytokines at a concentration of 20 μg/ml in the reaction mixture for 3 h at room temperature on a rotator. Beads were washed and stored in blocking buffer (10 mM PBS, 1% BSA, 0.05% NaN_3). Cytokine-coupled beads were incubated with plasma or serum from patients for 1 h in 96-well filter plates (MultiScreenHTS; Millipore) at room temperature in the dark on a horizontal shaker. Fluids were aspirated with a vacuum manifold and beads were washed three times with 10 mM PBS/0.05% Tween 20. Beads were incubated for 30 min with a PE-labeled anti–human IgG-Fc antibody (Leinco/Biotrend), washed as described, and resuspended in 100 μl PBS/Tween. They were then analyzed on an analyzer (Bio-Plex) using Bio-Plex Manager 3.0 software (both from Bio-Rad Laboratories). Successful coupling of the cytokines to their respective bead sets was verified with specific mAbs.

Western blotting. We subjected 500 ng rIL-17A, rIL-17, rIL-22, rIL-23 (R&D Systems), or BSA to SDS-PAGE (10% acrylamide) under reducing conditions. The protein bands were electroblotted onto nitrocellulose membranes (iBlot Gel Transfer Stacks; Invitrogen). Membranes were blocked by

incubation for 1 h at room temperature with PBS supplemented with 5% BSA and 0.05% Tween 20, and were washed and incubated overnight at 4°C with plasma samples from a patient or a control diluted 1:500 in PBS, 5% BSA, 0.01% Tween 20. The membranes were washed three times and incubated for 1 h at room temperature with HRP-coupled anti–human IgG and used at a final concentration of 0.67 μg/ml. The membranes were washed three times and developed for ECL (GE Healthcare).

Neutralization assay. SV-40–transformed control fibroblasts were plated in 24-well plates (60,000 fibroblasts/well) in 0.5 ml DMEM/10% FCS (Invitrogen). On the following day, cells were washed in 1× PBS (Invitrogen) and incubated with 10% plasma (from a control or a patient) in DMEM. They were left unstimulated or were stimulated with 50 ng/ml rIL-17A (R&D Systems). The supernatants were collected 24 and 48 h after stimulation, and IL-6 production was assessed by ELISA (Sanquin Mast Diagnostic) according to the manufacturer's instructions.

Online supplemental material. Fig. S1 shows anti–IL-17A, –IL-17F, and –IL-22 IgG auto-Abs measured by ELISA in plasma from APS-I patients and controls. Fig. S2 shows anti–TGF-β1, –IL-6, –IL-21, –IL-23, –IL-12, –IFN-γ, and –IL-26 IgG auto-Abs measured by ELISA in plasma from controls and APS-I patients. Fig. S3 shows anti–IL-1β, –IL-6, –IL-23, –IL-26, –IL-12, –IFN-γ, –GM-CSF, –IFN-β, –IL-10, –IL-18, and -TNF IgG auto-Abs measured by multiplex particle-based flow cytometry in plasma from healthy controls and APS-I patients. Fig. S4 shows anti–IFN-α, –IL-17A, –IL-17F, and –IL-22 IgG auto-Abs in 100 patients suffering from autoimmune and/or endocrine disorders. Table S1 shows the autoimmune/endocrine phenotypes and treatments of each APS-I patient tested. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20091983/DC1.

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