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LETTER TO EDITOR

Persistently Increased Anti-cytokine Antibodies Without Clinical Disease in a Boy with APS1 Genotype

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In patients with autoimmune polyendocrine syndrome type 1 (APS1), chronic mucocutaneous candidiasis (CMC) is usually the earliest and most common clinical feature, especially in those homozygous for the c.769C > T "Finnish" allele (R257X) [1]. Notably, ~ 100% of APS1 patients have high titer IgG autoantibodies (autoAbs) that neutralize type I interferons, especially IFN-ω and IFN-α subtypes [1, 2]. Though rare in other conditions, these autoAbs were further implicated recently by their ~ 10% prevalence in 987 other cases with severe coronavirus disease (Bastard et al., Science, 2020). These autoAbs against type I interferons have proved to be early and highly reliable diagnostic markers for APS1, regardless of the exact clinical picture or autoimmune regulator gene (*AIRE*) mutation [3]. However, there are conflicting reports about the relative contributions of altered TH17/TH22 function and IL17A/F and IL-22 autoAbs to the CMC. The absence of CMC in one rare unaffected sibling "S1"—despite high levels of IL-17F and IL-22 autoAbs—is intriguing [2, 4]. On the other hand, IL-22-neutralizing antibodies appeared to be pathogenic in a mouse model of oropharyngeal candidiasis, when their transfer led to delayed clearance of yeasts from the oral cavity [5].

The purpose of the current study was to revisit this male sibling S1, who is still unaffected at age 12 (APS-23 in ref. [2]; S1 in ref. [4] and hereafter), especially to re-evaluate the relative roles of autoAbs to IL-17 and IL-22, and of cells producing these cytokines, in APS1. S1 was originally tested for autoAbs and *AIRE* mutations at age 7 months [2] because one of his siblings had presented with severe APS1 manifestations, starting with CMC from late infancy. Both were c.769C > T homozygotes. This sister had died 16 years ago, at age 10, of adrenal failure and severe lung damage due to CMC. She received Nystatin orally but no systemic anti-fungal agents were applied. She also received oral hydrocortisone, supplementation of sodium and potassium, and fluticasone inhalation. Their healthy brother and sister are aged 30 and 32 and both are c.769C > T heterozygotes. The parents are healthy and confirmed repeatedly that they are not aware of any consanguinity in the family. S1 was born at term weighing 3560 g. He received all vaccines of the Hungarian mandatory vaccination program, including measles, mumps, rubella, and Bacillus Calmette-Guérin vaccines, without complications. He has never received an influenza vaccine. Recently, he has received two doses of mRNA-based coronavirus vaccine (Pfizer) without complication. At age 1 year, he had a febrile seizure associated with a viral infection of the upper respiratory tract, but with no subsequent complications. At age 6, he developed a mild and transient white plaque on the left buccal mucosa which was considered as possible mucosal candidiasis, but it was not checked microbiologically and disappeared after a short course of therapy with Nystatin. He has needed no other treatments. Similar mucosal lesions have never recurred since. Totally unlike his sister, S1 has been developing and growing normally and has never shown any APS1-related disease manifestation or endocrine biochemical changes except for mild hyperphosphatemia between 12 and 36 months of age (1.66–1.89 mmol/L; normal: 0.8–1.45 mmol/L). In stark contrast, his older sister's features resembled those in Finnish patients (77% of whom are c.769C > T homozygous), where symptom onset is from age 0.2 months to 18 years (median, 3.3 years) [6]. In accordance, 94% of US APS1

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patients (with different mutations though) had at least one symptom by age 10 but by age 15 all of them was symptomatic [7]. Altogether, according to large cohort studies, the clinical penetrance of the c.769C > T allele at the homozygous state is very high in childhood.

Subject S1 tested weakly positive for autoAbs against adrenal autoantigen cytochrome 21-hydroxylase (CYP21A2) at 11 and 28 months of age [4], but not thereafter. He has recently tested negative for autoAbs against CYP21A2, CYP1A2, CYP2A6, glutamic acid decarboxylase 65 (GAD-65), protein disulfide isomerase-like protein of the testis (PDILT), aromatic L-amino acid decarboxylase (AADC), trans-glutaminase 4 (TGM4), and tear lipocalin-1 (LCN-1), and also against IL-1A, IL-5, and IL-6. In addition, recent serum concentrations of parathormone were 5.24 pmol/L (normal, 1.6–6.9); calcium, 2.35 mmol/L (normal, 2.10–2.6); phosphorous, 1.59 mmol/L (normal, 0.95–1.85); calcitonin, 7.2 ng/L (normal, < 10 ng/L); alkaline phosphatase, 696 U/L (normal, 180–800); TSH, 5.37 mU/L (normal, 0.3–4.2); cortisol, 437 nmol/L (normal, 260–720); and ACTH, 27.6 ng/L (normal, < 75). Subject S1 is clinically euthyroid.

In striking contrast, by age 7 months, S1 already had persistently high titer autoAbs against IFN-ω and IFN-α2 and lower (but increasing) titers against IL-17F, though not against IL-17A (Fig. 1A, binding tested by ELISA). Cell-based assays revealed neutralizing autoantibodies against IL-22 already in the earliest sample and the maturation of type I IFN neutralization capacity to high titers by age 17 months (Fig. 1B). AutoAbs against type-I IFNs, IL-22, and IL-17F were still high at the age of 12 (Fig. 1C; red symbols), when we confirmed that his serum neutralized IL-17F and IL-17A/F in spite of the lack of anti-IL-17A (Fig. 1D). Unusually, S1 also had autoAbs against IL-12 from age 7 months, reaching high titers from 17–30 months (Fig. 1A) but disappearing by age 12 (Fig. 1C). Anti-IL-12 has been found in 4 of 68 mainly Finnish and clinically typical APS1 patients tested, each detected only in their early childhood samples. The early appearance of IL-12 autoAbs and their waning with time is intriguing but probably unrelated to CMC.

Since S1 still showed no signs of APS1 by 12 years of age, we tested his peripheral blood lymphocytes for IL-17A- and IL-22-producing cells, after stimulating with phorbol-myristate-acetate + ionomycin followed by intracellular cytokine staining (Fig. 1E). Both Th22 and Th17 cell percentages were within the healthy control range (Fig. 1E, F, red symbols). However, they did not show the complete absence noted in APS1 patients with CMC (Fig. 1F), to which we hypothesized their deficiency might contribute, especially in combination with autoAbs neutralizing IL-22 and/or IL-17 s. It is tempting to speculate that S1 is being protected from CMC by these few remaining Th22 or Th17 cells, which might possibly be more frequent in his mucosal tissues. On the other hand, they were detected after very potent stimulation, and their responses to *Candida* antigens might have been much weaker or even undetectable.

The boy reported here is unusual in the medical literature as he has had APS1-typical IFN-ω, IFN-α2, IL-22, and IL-17F autoAbs at least since age 7 months [4], but still shows no clinical or biochemical signs of APS1 even 12 years later. It could be argued that S1 has APS1 but it is non-penetrant in him. However, that has never been reported before in R257X homozygous siblings of APS1 patients, to our knowledge. Indeed, he clearly has been penetrant serologically (for IFN-α, IL-22, and IL-17F autoAbs) for at least 10 years, but neither clinically nor biochemically penetrant for APS1—which is unique.

Despite candidiasis in most patients being the earliest clinical manifestation, the order of occurrence of major disease components varies between patients. Furthermore, CMC is not present in 100% of the patients and the match between CMC status and the presence of anti-IL-17F and/or IL-22 autoAbs is not absolute but correlative. These findings further underlie that incomplete penetrance of CMC in the biallelic APS1 even in the case of longstanding presence of Th17-related autoantibodies is possible. This has been also illustrated recently for autoAbs against IFNs in patients with APS1 and severe COVID-19 [8].

Taken together, we believe that autoAbs neutralizing IL-22 and IL-17F may not be sufficient by themselves to precipitate CMC in subjects with APS1 genotypes. Our data suggest that the lack of anti-IL-17A autoAbs and the presence of IL-22-positive cells together may also have a role in protecting against CMC. The role of aberrant Th1 responses in precipitating CMC in APS1 patients is warranting further studies.

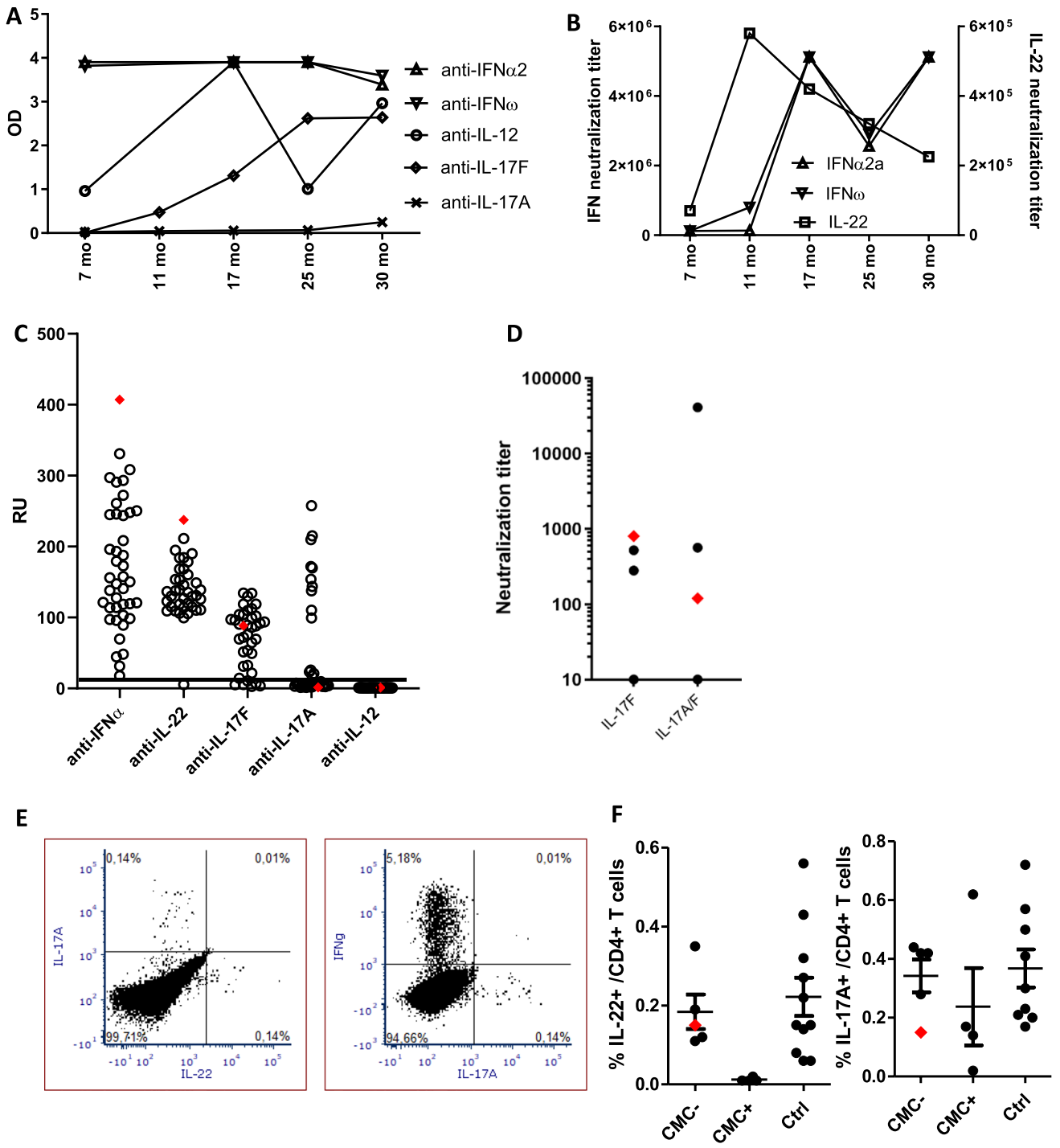

Fig. 1 Dynamics of cytokine-binding (**A**, tested with ELISA as in ref. [10] or neutralizing autoAbs (**B**, tested with cell-based bioassays in S1 from 7 to 30 months of age; his autoAb levels at 12 years of age (red diamonds) are compared with those of Finnish APS1 patients (**C**, tested with luciferase-based immunoprecipitation assay. Horizontal line represents the cutoff level. **D** Neutralizing titers against IL-17F (10 ng/mL) and IL-17A/IL-17F heterodimer (5 ng/mL) were tested using cytokine-stimulated NCTC 2544 keratinocytes; the titer is the serum dilution that halved the concentration of GRO-α in the supernatant (tested with ELISA as described in ref. [4]. S1 serum (12 years of age, red diamond) was tested along with samples from 3 APS1 patients: one negative against both IL-17A and anti-IL-17F (no neutralizing capacity); one with anti-IL-17F but negative against IL-17A (moderate neutralization of IL-17F and IL-17A/F); one positive against IL-17A and IL-17F (moderate neutralization of IL-17F and high of IL-17A/F). **E** Intracellular cytokine staining (IL-17A, IL-22, and IFN-γ as a stimulation control) after PMA and ionomycin stimulation (method in ref. [4]) of PBMC of subject S1 at 12 years of age. CD4 + T cells were gated. **F** Frequencies of IL-22 + and IL-17A + cells among CD4 + T cells from S1 (red diamond) and two healthy controls (Ctrl) tested in parallel; their data are displayed together with those of the APS1 patients tested for ref. [4]

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Author Contribution Melinda Erdős and Jaanika Kärner conducted the experiments and collected clinical data. Melinda Erdős and László Maródi performed the clinical investigation and followed subject S1. László Maródi and Kai Kisand designed the experiments and wrote the manuscript with contributions from the whole study group.

Data Availability Data and materials used in this study are available on request.

Declarations

Ethical Approval This study was approved by the institutional ethics committee of the University School of Medicine, Debrecen, Hungary.

Consent Statement The parent signed an informed consent to conduct the study and for data publication.

Competing Interests The authors declare no competing interests.

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