THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

MECHANISM OF HOST DEFENSE AGAINST CANDIDA IN PATIENTS WITH AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE I.

by

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“Time not important.Only life important.”

(The fifth Element, Luc Besson, 1997)

CONTENT

1. INTRODUCTION

The term „chronic mucocutaneous candidiasis (CMC)" was introduced in the late 1960s, and defines a heterogenous collective group of inherited conditions characterized by chronic, recurrent or persistent superficial mucocutaneous infections, caused by members of the *Candida* genus, mostly the commensal *Candida albicans*, an ubiquitous, opportunistic yeast; affecting the skin, nail sand mucosal surfaces (Maródi 1997, Puel et al. 2012, Soltész et al. 2013). *Candida* change from commensal to pathogen, only if the homeostasis between the virulence of the fungus and the resistance of the host immune systemis damaged (Romani 2011). CMC frequently occurs in early childhood and newborn age, and is also common in patients with broad and profound, acquired or inherited T-cell deficiencies (Puel et al. 2012). Principally, the most common manifestation is recurrent candidiasis of oral and eosophageal epithelial surfaces, and in case of CMC, patients rarely develop disseminated or systemic invasive *Candida* infections (Maródi et al. 2012). Severe oropharyngeal candidiasis remains common in HIV-infected patients and was the most frequent opportunistic fungal infection in these patients before the introduction of effective antiretroviral treatments. It is also seen in patients with immunosuppressive, antibiotic or steroid treatments (Puel et al. 2012). The first sporadic CMC cases were described in the 1960s but its genetic background remained unknown until recently (Soltészet al. 2013). The first familial cases, typically with inheritence as an autosomal dominant trait or, rarely, as an autosomal recessive trait in some consanguineous families, were reported in the 1970s. Over the next 40 years, other sporadic and familial cases were reported, suggesting that CMC results from gene lesions in at least some patients (Puel et al. 2012, Soltész et al. 2013). Invasive candidiasis, dermatophytosis, bacterial infections of the respiratory tract, staphylococcal diseases of the skin and autoimmune components have been reported as additional symptomes in CMC patients (Puel et al. 2012). The further genetic and immunological dissection of thepathogenesis of CMC should delineate the function of various genes, controlling and mediating the IL-17 signalisation and Th17 cell development (Puel et al. 2010, Puel et al. 2012). Predisposition to mucosal and skin candidiasis is a hallmark of several primary immunodeficiency disorders (PIDs), especially associated with impaired CD4+IL-17+ T cell immunity (Maródi et al. 2012, Puel et al. 2012, Soltész et al. 2013). Nevertheless, CMC may be the only or the principal disease manifestation in patients with some immundeficiencies, such as AD IL-17F and AR IL-17RA deficiencies (Puel et al.2012, Soltész et al.2013), and gain-of-function

(GOF) mutations of STAT1 (Liu et al. 2011, Soltész et al. 2013, Tóth et al. 2012). This rare condition of essentially isolated CMC (about 1/100 000 individuals) nowadays is often referred to as „CMC-disease" (CMCD) (Puel et al. 2012). The complex group of CMC syndromes can be subclassified according to spreading and distribution and by the underlying pathomechanism. Above all, CMC is diagnosed clinically, and by *in vitro* isolation and cultivation of *Candida* from smear, and the molecular diagnosis can be revealed by functional and mutational analysis, based on the known underlying signal transduction pathways and genetic defects (Eyerich et al. 2008).

Autoimmune polyendocrine syndromes (APS) are rare disorders, characterized by the coexistence of at least two endocrine gland insufficiencies mediated by autoimmune mechanisms. Additionally, nonendocrine immune components may be present (Cutolo 2014). According to the Neufeld and Blizzard Classification of 1980, there are four main types of APS (Neufeldet al. 1980). The first description of the assotiation between hypoparathyroidism and candidiasis was reported by Thorpe and Handley in 1929, the condition was commonly referred to as Schmidt's syndrome, after the German pathologist who studied autoimmunity to more than one endocrine organ (Thorpe et al.1929, Gupta et al. 2012). APS associated with CMC is a rare autosomal recessive disease and is also referred to as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy - (APECED) syndrome, or APS I (Neufeld et al. 1981, Husebye et al. 2009). Research into APS I has shed new light on normal self-tolerance induction in the thymus and how its breakdown leads to autoimmunity (Anderson et al. 2002). APS I is caused by a loss-of-function mutation of the autoimmune regulator gene (*AIRE*), resulting the production of a mutant AIRE protein, which allows autoreactive Tcells to escape negative selection in the thymus, leading to autoimmunity to various endocrine organs and the production of autoantibodies against self-antigens, enzymes, which have important role in synthesis of hormons and neurotransmitters, and various cytokines (Peterson et al., 2004, Husebye et al. 2009, Kisand et al. 2010, Kisand et al. 2011). APS I is classically defined by the association of at least two of three major disease components: CMC, primary hypoparathyroidism and autoimmune adrenal insufficiency, which was not related to the syndrome until 1946 (Ahonen et al. 1990, Perniola et al. 2000, Husebye et al. 2009, McManus et al. 2011).

1.1.Host defense mechanisms on body surfaces

1.1.1.*Candida albicans*

The approximately 200 species of 'yeast-like' fungi in *Candida* genus represents a highly heterogenic group of microbas. Taxonomically, the *Candida* genus is in the class Deuteromycetes, and the characteristic of *Candida* species is their ability to grow polymorphically, either in the form of budding yeasts (blastoconidia) or filament (hyphae or pseudohyphae) (Romani et al. 2003, Williams et al. 2013). *Candida* species can differ greatly in terms of their biochemistry, morphology composition and, importantly, their ability to instigate human infection (Romani et al. 2003). Approximately 20 *Candida* species have been associated with causing candidiasis in humans. The species most frequently isolated from humans and the causative agent of the majority of infections is *Candida albicans*. *C. albicans* is an opportunistic pathogen and generally exists as a harmless commensal of humans, primarily on moist mucosal surfaces, particularly of the gut, vagina, and oral cavity.The complex dimorphic pathogen colonizes the mucosal surfaces of healthy individuals. Use of a broad-spectrum antibiotic, hormonal imbalances, and poor nutrition may also be contributory factors (Romani et al. 2003, Williams et al. 2013). The fungal cell wall is a dynamic structure that is continuously changing, throughout the cell cycle and morphological transition (Romani et al. 2003, Romani et al. 2011).*Candida* produce several factors that are potent regulators of the host inflammatory response. β-(1,3)-glucans are exposed in the bud scar of *C.albicans* yeast, but are masked on hyphae, thus favouring fungal escape from recognition of β-glucans. By masking or subverting the host detection systems, may avoid inflammation, and this contributes to fungal adaptation and opportunism (Williams et al. 2013).

1.1.2.The role of innate immune mechanisms

Candida is a complex pathogen, and the host immune system uses various cells, surface receptors and signalling pathways to trigger an efficient defence against *Candida* (Netea et al 2010). Innate immune mechanisms are used by the host to respond to a range of fungal pathogens in a rapid and conserved manner. Molecules of fungal cell wall are the main source of pathogen-associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs) of host immune cells (Maródi 1997, Romani et al. 2011). The

major cell wall components, found in all medically important fungi are β-glucans, especially β-(1,3)-glucans with varying numbers of β-(1,6)- branches, N-acetyl-glucoseamine (chitin) and mannans (Díaz-Jiménez et al. 2010, Romani et al. 2011). During the course of a fungal infection, host PRRs are stimulated by fungal PAMPs in different combinantions, depending on the fungal species and on host cell types. This receptors are located on professional phagocytes; granulocytes, monocytes-macrophags and on dendritic cells, as well as non-immune cells. The recognizing process is followed by activation of intracellular signalling pathways and stimulation of pro-inflammatory and anti-inflammatory mediator cytokines. Chemokines and cytokines are released, and inflammatory cells consistently accumulate at the site of *Candida* invasion. Nevertheless, effector immune mechanisms are triggered, as well as initiation of adaptive immunity through the anti-*Candida*T helper (Th) cell responses (Netea et al. 2010, Romani et al. 2011, Maródi et al. 2012, Drummond et al. 2011, Mencacci et al. 1999, Stambach et al. 2003, Szolnoky et al. 2001, von Bernuth et al. 2006).

1.1.3. Cell mediated immunity

1.1.3.1. CD4+ interleukin-17+ T helper cells

CD4+IL-17+ T helper cells (Th17 cells) have the most important function in the host response against *Candida*, but they are also associated with the pathogenesis of many autoimmune and allergic disorders (Ahlgren et al. 2011, Cypowyj et al. 2012, Puel et al 2010, Puel et al. 2012). In fungal infection, Th17 cell activation occurs throug the SYK-CARD9-MYD88 and mannose receptor signalling pathways in DCs and macrophages, and it is inhibited by the RAF and TRIF-type I IFN pathways (Romani et al. 2011). Recenst studies showed that IL-23 promotes the production of IL-17 by activated T cell subset development (Aggarwal et al. 2003, Langrish et al. 2005). On the basis of the characteristic cytokine profile, these cells were subsequently called ThIL-17 or Th17 cells. Gene-expression analysis showed that Th17cells has a distinct gene-expression profile**,** compared to Th1 cells. This IL-23-driven cells expressed high amounts of IL-17A, IL-17F and TNFα. The outstanding differences predicted, that Th1 and Th17 cells probably have distinct immune functions. Subsequent experiments confirmed that Rorc, which encodes RORγt, a retinoid orphan nuclear receptor, isspecifically expressed in this new cell line. Some autohors further demonstrated that RORγt synergizes with RORα to promote differentiation and function of Th17 cells, and the RORγ

upregulation depend on the function of STAT3 (McGeachy et al. 2008). Naiv T cells, activated in the presence of TGF-β, provided by regulatory T cells (Treg) and IL-6 begin differentiation towards the Th17 cell subset; IL-6 upregulates IL-21 and IL-23R to further their Th17 development. In the absence of IL-6, TGF-β induces regulatory T cells (Noack et al. 2014).

1.1.3.2. CD4+ interleukin-22+ T helper cells

CD4+IL-22+ T helper cells (Th22 cells) belong to a new class of leukocytes with selective effects on epithelial surfaces. This characteristic functional profile of Th22 cells is mediated by distinct cytokines (Liu et al. 2009). Th22 cells lack production of IFN-γ, IL-4 and IL-17, but they secrete TNF-α and their lead cytokine IL-22. IL-22 is a glycoprotein belonging to the IL-10 cytokine family, which binds to a heterodimer of the IL-10 receptor β, (IL-10Rβ) and the IL-22 receptor (IL-22R). While IL-10Rβ is widely expressed, IL-22R expression is limited to epithelial cells, thus ensuring tissue-specific effects of IL-22 (Zelante et al. 2011). In line with that observation, Th22 cells are enriched in the skin of inflammatory disorders such as atopic eczema and psoriasis (Koga et al. 2008). Some authors demonstrated that IL-22-producing CD4+ T cells are persistent in human PBMC and expressed mainly by memory CD4+ T cells (Liu et al. 2009). Consistent with this findings, a previous study reported that IL-22 mRNA expression was induced by activated memory CD4+ T cells. It has been demonstrated that IL-17A was required for anti-*C. albicans* host defense, and the Th17 cells specific for *C. albicans* were persistent in human peripheral blood. PBMC from healthy individuals could produce IL-22 in response to *C. albicans* yeast or hyphae. IL-22-producing cells specific to *C. albicans* displayed a memory phenotype with the expression of CD45RO memory IL-22-producing cells may be pathogenspecific (Liu et al. 2009). Whereas *C. albicans*, either yeast or hyphae, induced both IL-22 and IFN-γ at the similar level in PBMC from healthy individuals. It has been reported that yeast and hyphae have different capacities to induce the differentiation of Th1 or Th17. Moreover, recently was found that IL-22-producing CD4+ T cells specific for *C. albicans* were different from Th1, Th2 and Th17 cell subsets (Liu et al. 2009).

1.1.4. Cytokines involved in host defense against *Candida* on body surfaces

1.1.4.1. Interleukin-17 cytokine family

The IL-17 cytokine family consists of 6 members (IL-17A through IL-17F). The receptors for IL-17 include a family of 5 receptors (IL-17RA through IL-17RE). Heterodimers of IL-17RA and IL-17RC constitute the receptors for IL-17A and IL-17F. IL-17A and IL-17F are preferentially produced by Th17 cells (along with IL-21 and IL-22). The main Th17 cytokines, IL-17A and IL-17F coordinate tissue inflammation through the induction of proinflammatory cytokines and chemokines, (IL-1, IL-6, TNF-a, granulocyte colony-stimulating factor, and GM-CSF), chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7), antimicrobial peptides (defensins and S100), and matrix metalloproteinases (MMP1, MMP3, and MMP13) from the endothelium, epithelia, and fibroblasts (Kawaguchi et al. 2004). As a result, Th17 cells can promote granulopoiesis and recruitment of neutrophils to sites of infection and provide host defense against extracellular bacteria and fungi. Activated CD4+ IL-17+ T cells produce the proinflammatory effector cytokines IL-17A, IL-17F, IL-22, and IL-26. IL-17A binds as a homodimer or alternatively as an IL-17A–IL-17F heterodimer to its receptor IL-17RA, which can be found in various tissues (such as lung, spleen, kidney, and liver). IL-17A is also expressed by $CD8^+$ T cells, γδ T cells, NK cells, B cells, neutrophils, fibroblasts, epithelial cells, vascular endothelial cells, myelomonocytic cells, and bone marrow stromal cells. Finally, IL-17A has been shown to be produced by intestinal Paneth cells, whereas colonic epithelial cells have been shown to express IL17F mRNA, suggesting that a variety of cell types can play roles in host defense against fungi and extracellular bacteria.The highest overall amino acid sequence identity (50%) is found between IL-17A and IL-17F. Like IL-17A, IL-17F acts on a large variety of cells and induces a similar panel of proinflammatory cytokines and chemokines (Kawaguchi et al. 2004, Akdis et al. 2009).

1.1.4.2. Interleukin-22

IL-22, a member of the IL-10 family is considered to be expressed predominantly by Th17 cells and, to some extent, by Th1 cells. In addition, high levels of IL-22 production have been detected in memory CD4+ T cells (Eyerich et al. 2011, De Luca et al. 2010, Zelante et al. 2011). IL-22 signals through a receptor complex, IL-22R1/IL-10R2, for which IL-10R2 is highly expressed in immune cells. In contrast, IL-22R1 is not expressed on either resting or

stimulated immune cells, but specifically on epithelial and some fibroblast cells in peripheral tissues such as gastrointestinal, respiratory system and skin. IL-22 up-regulates the production of antimicrobial proteins such as human β-defensin (hβD). Furthermore, IL-22 induces antimicrobial peptides such as β defensin 2 and S100 proteins. Th22 cells induce genes belonging to the innate immune response in primary human keratinocytes, and this induction is dependent on the synergistic action of TNF-α and IL-22 (Eyerich et al. 2011). Recently have been demonstrated that IL-22 and TNF-α act on primary human keratinocytes via synergistic induction of MAP kinases and transcription factors of the AP-1 family, and that this induction results in an effective protection of the epidermal barrier after infection with *C. albicans* (Eyerich et al. 2011). New findings on the IL-22-dependent pathway, focusing on IL-22's role in the regulatin of host-fungus interactions at mucosal surfaces. This findings suggest, that IL-22 regulates intestinal and skin homeostasis and mucosal wound healing via activation of tha JAK/STAT pathway, which results in tyrosine phosphorylation of epithelial STAT3 and directly targets gut epithelial cells to release of S100A8 and S100A9 peptides, which have important antifungal and anti-inflammatory effects. Mentionend findings showed that IL-22 production can occur in the relative absence of Th17 cells, and clearly pointing to mechanisms of IL-22 production outside of the Th17 pathway (Zelante et al. 2011).

1.1.5. The aryl hydrocarbon receptor theory

Recent study has shown that IL-22 is required for the control of *C. albicans* growth at mucosal sites in the absence of Th1 and Th17 cells (De Luca et al. 2010, Zelante 2011). This study suggested that IL-22 produced by NKp46+ innate lymphoyd cells expressing the AhR was found to directly target intestinal epithelial cells. The AhR is a ligand-activated transcription factor that mediates IL-22 production, through a variety of indole derivates which act as endogenous ligands for AhR, which ligands are generated through conversion from diatery thryptophan by commensal intestinal microbes. Thus, the tryptophan metabolism pathway is likely exploited by commensals and the mammalian host to increase immunological fitness in response to fungi. Vaginal epithelial cells also produce S100 proteins following interaction with *C. albicans*, suggesting the possible involvement of IL-22 in mucosal candidiasis on other surfaces of body. The supposition that IL-22 production in the gut is driven by commensals, may provide novel mechanistic insights on how intestinal dysbiosis and mucosal candidiasis following antibiotic therapy. So, IL-22 production in the

mucosa may be a primitive mechanism of resistance against fungi under conditions of limited inflammations. This results support a model, in which the AhR-IL-22 axis, in conjunction with the IL-17-Th17 cell pathway, controls initial fungal growth and epithelial cell homeostasis, through primitive antifungal effector mechanisms, such as the release of defensins and antimicrobial peptides (Romani et al. 2011, Zelante et al. 2011).

1.2. Pathogenesis of chronic mucocutaneous candidiasis

Infectious susceptibility that is primarily limited to patients with CMC represents a unique subset of immune deficiency (Maródi et al. 2012, Puel et al. 2010, Puel et al. 2012). CMC may accompany various other infectious diseases in patients with almost any broad and profound T-cell primary immunodeficiency, moreover, recently several complex human diseases have been identified, which confirmed that impair Th17 cell differentiation and inbornerrors of Th17-IL-17 immunity can result in susceptibility to CMCD, as one manifestation of a more complex syndrome (Puel et al. 2012, Liu et al. 2011). Recent dissection has shed light on the molecular mechanisms of mucocutaneous immunity to *C. albicans*. CMC is a part of infectious symptomes in neutrophil defects, which are associated with systemic candidiasis and susceptibility to a wide variety of bacteria. CMC disease and invasive candidiasis have also been described in case of homozygous nonsense mutation in CARD9, a signaling protein that along with SYK, is downstream of the antifungal receptors Dectin-1, Dectin-2, and Mincle (Maródi et al. 2012). Rare syndromes with characteristic features and CMCD resulted by mutations in Signal Transducer and Activator of Transcription 3 (STAT3) (autosomal dominant (AD) hyper-IgE syndrome (HIES)) and mutations in the dedicator of cytokinesis 8 gene (DOCK8) (autosomal recessive (AR) HIES) more confirmed the role of Th17 cell deficiency in predisposition to CMC (Maródi et al. 2012). Furthermore, APS I, which is also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), and some cases of thymoma also result in susceptibility to CMC through autoantibody production and impairment of Th17 cytokines functions (Kisand et al. 2010, Kisand et al. 2011, Puel et al. 2010., Ng et al. 2010) IL-12 receptor β1 (IL-12Rβ1) deficiency, the most common form of Mendelian susceptibility to mycobacterial disease. IL-12Rβ1 deficiency is characterized by childhood-onset mycobacteriosis and salmonellosis; recurrent thrush caused by *Candida* species has been found high percent of earlier reported cases. CMC has also been observed in almost 10% of

patients with IL-12p40 deficiency (Maródi et al. 2012). T cells from patients with IL-12Rβ1 mutations cannot respondto either IL-12 or IL-23 cytokine signals because these cytokines bind IL-12Rβ1. Patients with IL-12p40 deficiency, a subunit shared by IL-12 and IL-23, might be prone to CMC because of impaired IL-23–dependent immunity, which is important for the differentiation of IL-17–producing T helper lymphocytes. A Japanese patient with autosomal recessive HIES has been reported and carries a homozygous nonsense mutation in TYK2, which encodes TYK2. The patient presented with atopic dermatitis, markedly increased serum IgE titers, and recurrent infections with *S. aureus*, herpes simplex virus (HSV), BCG, and *Candida* species, and his PBMCs did not respond normally to stimulation with IL-6 and IL-10. Patients with TYK deficiency, presented disseminated BCG infection and zoster but did not display staphylococcal or candidal disease has been reported (Maródi et al. 2012).CMCD but without any other severe infection or sever noninfectious disease manifestation have been described in patients with gain-of-function STAT 1 mutations (Liu et al. 2011, Soltész et al. 2013, Tóth et al. 2012). Investigations in 2 families led to the discovery of new genetic defects of IL-17–mediated immunity (Puel et al. 2010, Puel et al. 2012). In a Moroccan patient born to consanguineous parents, an AR premature stop codon mutation of the *IL-17RA* gene, leading to complete loss of protein expression, was found to cause CMCD with neonatal onset. The healthy parents and siblings of this patient were heterozygous for a Q284X mutation of the IL-17RA protein. The AD mutation *S65L* in the IL-17F protein was identified by the same group in several patients of an Argentinean family;this mutation was found to impair the function of both homodimers (IL-17F/F) and heterodimers (IL-17A/F) containing the mutant isoform. Recent report about human Dectin-1 deficiency and mucocutaneous fungal infections suggested that familial CMC can be caused by a homozygous or heterozygousTyr238X defect of the β-glucan receptor Dectin-1. However, both homozygous and heterozygous early stop codon mutations in Dectin-1 have subsequently been found in healthy subjects (Maródi et al. 2012).

1.3. Autoimmune polyendocrine syndromes

The term autoimmune polyendocrine syndromes (APS), also called polyglandular autoimmune syndromes (PAGS) are a heterogenous group of rare diseases comprise a wide spectrum of different conditions in which patients present multiple endocrine hypofunctions and nonendocrine autoimmune disease components (Cutolo 2014, Kahaly et al. 2012). The

nature of APS has been based on the presence of lymphocyte infiltration inthe affected gland, organ-specific antibodies inthe serum, cellular immune defects and an association with the human leucocyte antigen (HLA) DR/DQ genes or immune response genes. Genetic susceptibility is necessary but not sufficient to produce disorder (Kahaly et al. 2012). In 1980, Neufeld and Blizzard developed the first classification of polyglandular failure (Neufeld et al. 1980). Neufeld and Blizzard's classification distinguishes two broad categories, APS type I and APS type II (APS I and APS II). An additional group, APS type III (APS III), was subsequently described. APS III, in contrast to APS I and II, does not involve the adrenal cortex. In APS III, autoimmune thyroiditis occurs with another organ-specific autoimmune disease, but the syndrome cannot be classified as APS I or II (Kahaly et al. 2012, Neufeld et al. 1981).

Table 1. Neufeld-Classification of Autoimmune Polyendocrine Syndromes (1980)

APS I	CMC, primer hypoparathyroidism, adrenal insufficiency
APS II	Autoimmune adrenal insufficiency, autoimmune thyroid disease, diabetes
APS III	Autoimmune thyroid disease and other endocrine insufficiency, except adrenal hypofunction and hypoparathyreosis
APS IV	Two or more organ specific endocrine autoimmune disease (which don’t fall into type others)

Neufeld and Blizzard's classification distinguishes two broad categories, APS type I and APS type II (APS I and APS II). An additional group, APS type III (APS III), was subsequently described.

1.3.1. Autoimmune polyendocrine syndrome type I

1.3.1.1. Epidemiology

APS I is rare syndrome, approximately 500 patients have been reported worldwide (Weiler et al. 2012). The highest prevalence was found in small closed communities, among the Iranian Jewish community (1:9000), in Sardinia (1:14000) and in Finland (1:25000) (Weiler et al. 2012, Peterson et al. 2004, Perheentupa et al. 2006, Myhre et al. 2001). In most cases, signs and symptoms of the disease appear in infancy or childhood and affected patients typically develop three to seven different disease manifestations. In addition to the major criteria, minor disease components are also common. These signs and the major components of disease often develop in a particular chronological order (Myhre et al 2001, Sarkadi et al. 2014). CMC commonly begins before the age of 5 years, followed (usually years later) by autoimmune hypoparathyroidism before the age of 10 years and adrenal insufficiency before the age of 15 years (Myhre et al. 2001, Weiler et al. 2012). The complet triad usually presentapproximately only in 50 % of patients at the age of 20, 55% at age 30, and 40% at age of 40. Studies revealed that the earlier the first manifestation appears, the greater the number of disease components. The female-to-male ration various in different reports, from 0,8:1, to 2,4:1 (Weiler et al. 2012, Neufeld et al 1981, Ahonen et al. 1990).

1.3.1.2. Clinical manifestations

- ***CMC***

Chronic superficial infection with *C. albicans* is the most common manifestation in APS I, which is particularly common in patients carrying the Finnish homozygous allele R257X, and rare in Iranian Jews in whom the infection is seldom reported (Kisand et al. 2011, Collins et al. 2006, Peterson et al. 2004). In most cases, CMC is the first of the major components of APS I to appear, often occuring befor age 5, frequently develops during the first year of life and its severity is variable. It preferably affects the oral mucosa, oral candidiasis, followed by the involvement of the skin and nails (Myhre et al. 2001, Wolff et al. 2007). Chronic oral candidiasis may lead to hyperplastic CMC with thick white or grey plaques of yeast and hyperkeratosis, and atrophic form with thin mucosa and leukoplakic areas (Rosa et al. 2008,

Rautemaa et al. 2007). *Candida* infection may cause superficial mucous membrane lesions in the esophagus, serious inflammation with strictures, painful swallowing, retrosternal pain and a sensation of food becoming stuck in the chest. In several cases, squamous cell carcinoma of the mouth or eosophagus has been reported in association with mucocutaneous candidiasis of the oropharyngeal cavity and the esophagus, suggesting that persistent mucosal candidiasis may has carcinogenic potential in the absence of other risk factors (Rosa et al. 2008, Rautemaa et al. 2007, Uittamo et al. 2009). Symptomatic intestinal candidiasis includes abdominal pain, flatulence and diarrhea. Female patients may suffer from vulvovaginitis. In the majority of cases, the infection is limited to no more than 5 % of the skin surface. Candidiasis of the nails results in thickening and discoloration, paronychia and nail loss. Generalized candidiasis has been reported in patients on immunsuppressive medication (Rautemaa et al. 2007).

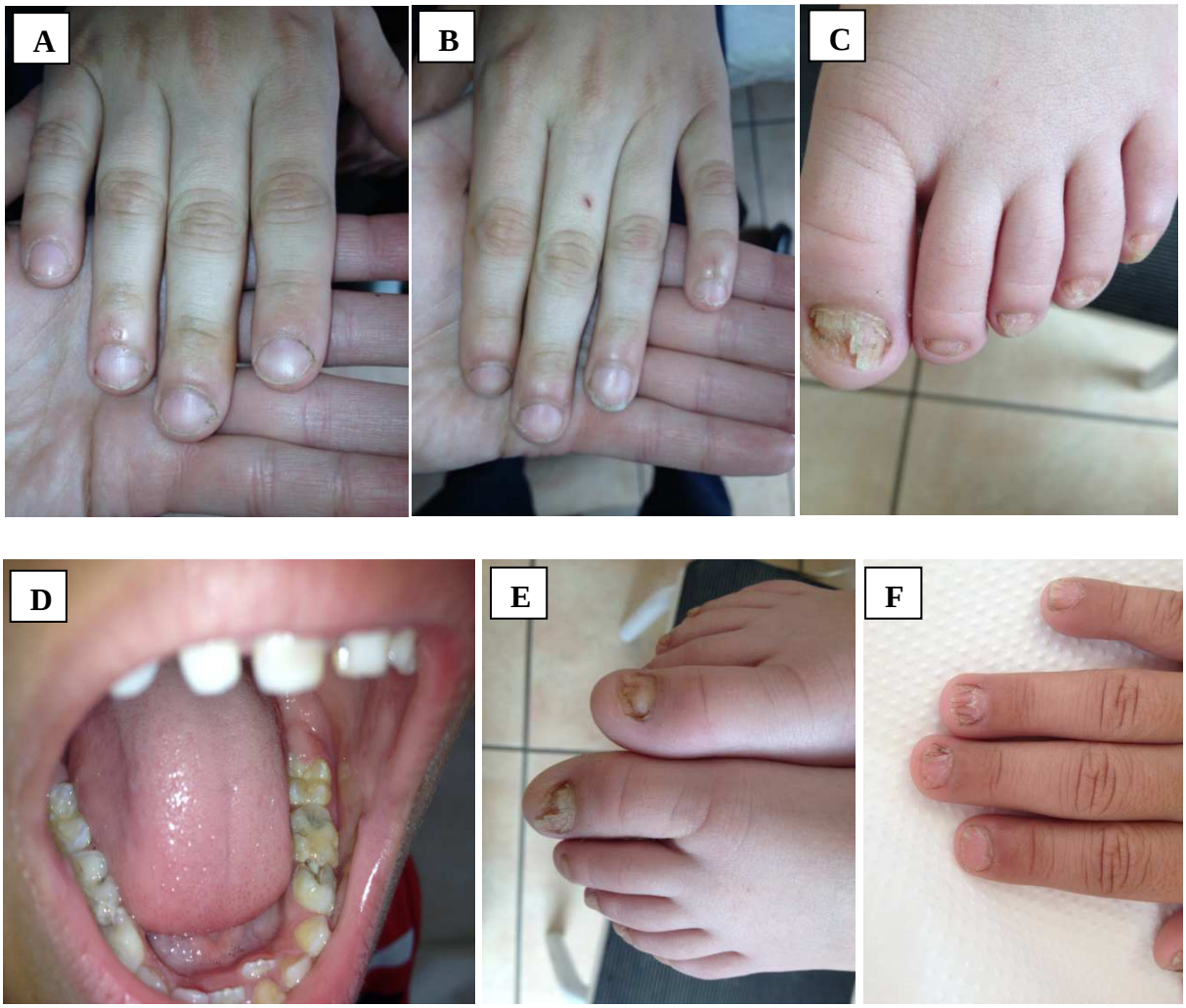


Figure 1. Clinical manifestations of APS I patients. Recently diagnosed two young patients predominantly presents the components of ectodermal dystrophy: onychomycosis and nail

dystrophy in a 5-year old boy (Fig. 1A-C, E). Enamel hypoplasia and nail dystrophy in a 10-year old boy (Fig. 1D, F).

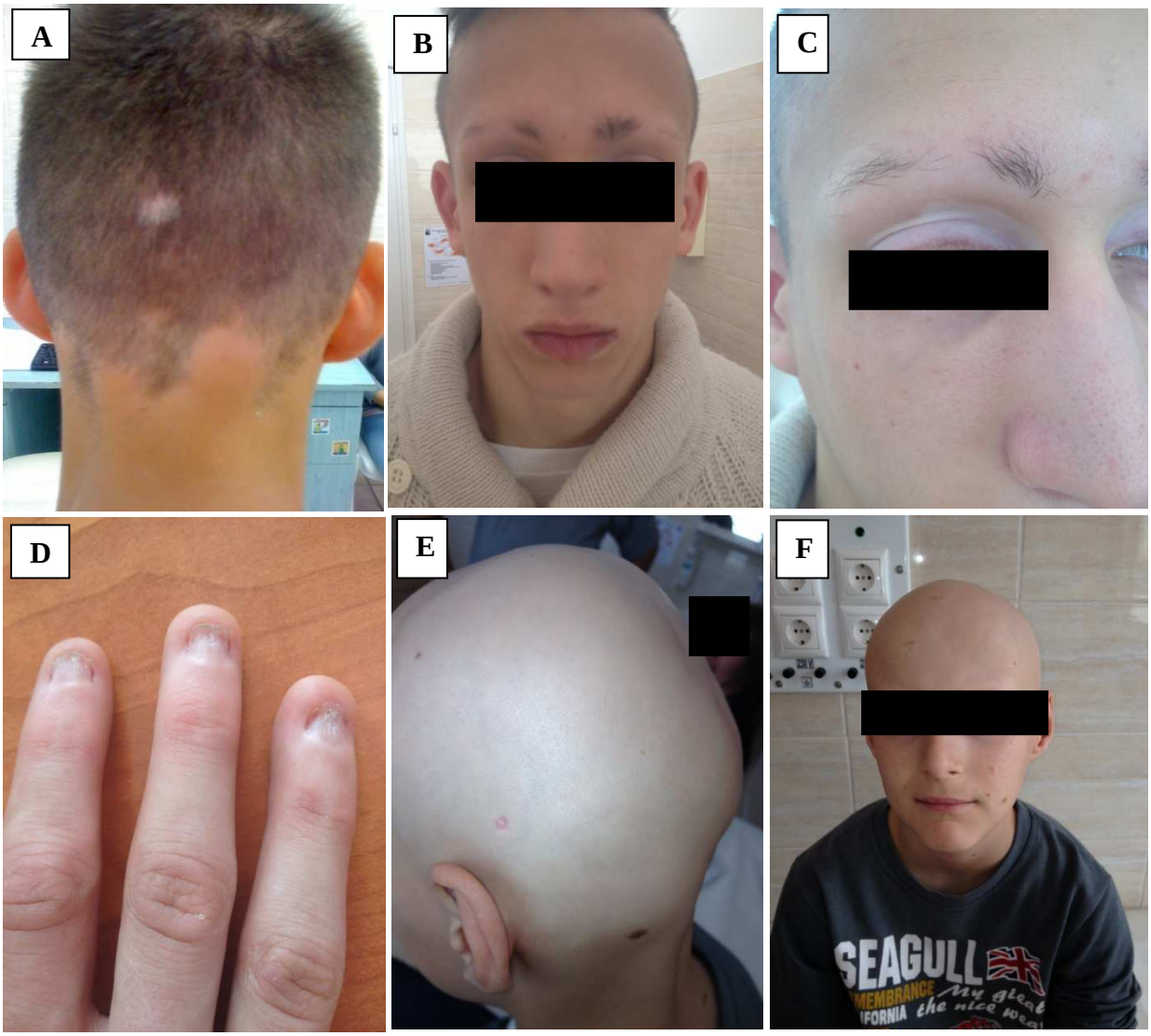


Figure 2. Clinical manifestations of APS I patients. Alopecia areata in a 21-year old boy, involved the scalp, eyebrows and eyelashes (Fig. 2A, B, C). Severe nail dystrophy on hands (Fig. 2D), progrediating vitiligo on the periorbital area and mild angular cheilitis in same patient (Fig. 2B, C). Alopecia universalis in a 10-year old boy (Fig. 2E, F).

- **Hypoparathyroidism**

Hypoparathyroidism is usually the first during the cours of APS I and it has been reported in 70-93% of the cases (Weiler et al.2012). Hypoparathyroidism is the second most common major component of APS I and usually the first endocrine component, with a prevalence of

one-third at age 5, two-thirds at 10 years and 85% at 30 years of patients in the Finnish series. Hypoparathyroidism generally occurs within 10 years of the onset of candidiasis, but it may manifest at any time in adulthood. It varies according to gender, affecting 98% of female patients, but only 71% of male patients. Interestingly, when adrenal insufficiency is the first endocrinopathy, susceptibility to HP appears reduced. Many symptoms of hypocalcaemia are nonspecific, such as paresthesias and muscle cramps. Seizures may occure in more severe cases. It is more common in females than males. Symptoms of hypocalcaemia may be vague for a long time consisting of muscle cramps during infections, periods of mild paraesthesia and clumsiness. Moreover, APS I patients without hypoparathyroidism should have plasma calcium (P-Ca) and phosphate determined at least annually, even in the absence of symptoms. Biochemically, the combination of hypocalcaemia, hyperphosphataemia with normal or low parathyroid hormone (PTH) and with normal plasma creatinine gives the diagnosis (Perhentupaa et al. 2006, Gylling et al. 2003).

- **Primary adrenal insufficiency**

Adrenocortical failure generally occurs after the onset of candidiasis and/or hypoparathyroidism, with a peak incidence between the ages of four and 12 years, but hormone levels may fluctuate for years. Adrenal insufficiency (AI) is preceded by the production of anti-21-hydroxylase antibodies. The prevalence of AI is 60-100%, with a peak incidence around 12 years of age. It is a lifethreatening condition that should be rapidly recognized and treated. Symptomes are fatigue, weight loss, salt craving, hypotension, abdominal pain and increased pigmentation of the skin. It is important to be aware that hypocorticolism and hypoaldosteronism may appear years apart (Myhre et al. 2001, Neufeld et al. 1981, Betterle et al. 1998, Scherbaum et al. 1982).

- **Hypogonadism**

Hypogonadism appears in 12-60% of the APS I patients, with a prevalence three times higher among females, possibly due to the blood-testis barrier that protects Leydig cells from an autoimmune attack. Half of the affected female patients have primary amenorrhea with absence or early interruption of spontaneous pubertal development, and others develop premature menopause (Ahonen et al. 1990, Perheentupa 2004).

- **Autoimmune thyroiditis**

Hypothyroidism is relatively uncommon, affecting no more than 30% of the APS I patients. It develops more often following puberty and by middle age, usually before the age of 30. A higher frequency of autoimmune thyroiditis is observed in patients with APS I caused by a specific mutation, the G228W missense mutation (Cetani et al. 2001, Neufeld et al. 198).

- **Type-1 diabetes mellitus**

Diabetes mellitus has been described in up to 15% of the APS I patients. The highest prevalence was seen in Finnish patients. This syndrome is part of the heterogenous spectrum of diabetes mellitus (Ahonen et al. 1990, Neufeld et al. 1981, Betterle et al 1998, Weiler et al. 2012, Zhang et al. 2011).

- **Pituitary failure, lymphocytic hypophysitis**

Single or multiple pituitary defects have occasionally been described in APS I. Growth hormone deficiency is the most commonly reported defect, but central diabetes insipidus and gonadotropin or ACTH deficiencies have also been seen. Autoimmune hypophysitis rarely affects patients (Ahonen et al. 1990, Al-Herbish et al. 2000).

- **Chronic atrophic gastritis and pernicious anaemia**

Autoimmune gastritis is characterized by gastric mucosa atrophy, selective loss of parietal cells from the gastric mucosa, and submucosal lymphocyte infiltration. Based on intrinsic factor deficiency vitamin B12 deficiency may be impaired, leading to pernicious anaemia (Weiler et al. 2012, Ekwall et al. 1998).

- **Intestinal dysfunction and malabsorption.**

The prevalence of intestinal dysfunction varies from 15 up to 22%. The symptoms consist of chronic or periodic constipation, chronic or recurrent diarrhae and malabsorption, which cause weight loss, growth retardation and erratic absorption of medications. Although it has been considered a nonendocrine manifestation, new evidence indicate that an autoimmune attack against cells of the gastrointestinal endocrine system actually occurs in APS I. Besides,

malabsorption may be attributed to a variety of causes, such as celiac disease, failure of exocrine pancreas or intestinal infections. In some cases, diarrhea is caused or worsened by the presence of hypoparatyroidism, since hypocalcaemia may cause a functional deficit in cholecystokinin (Weiler et al. 2012, Perheentupa 2006).

- **Autoimmune hepatitis, nephritis**

Hepatitis has been decribed in 8-26% of the cases. Severity is variable, ranging from an asymptomatic course with spontaneous regression to fulminant necrotizing disease with extremely high mortality. Tubulointerstital nephritis has been sporadically described, sometimes with progression to terminal renal failure and need for kidney transplantation (Weiler et al. 2012, Myhre et al. 2001, Perheentupa 2006).

- **Ectodermal dystrophy**

The components of ectodermal dystrophy are keratoconjunctivitis, dental enamel hypoplasia and punctate nail defects. Keratoconjunctivitis appears in 10-35% of patients, usually before the age of 5. It is extremely rare among Iranian Jewish patients. Affected individuals suffer from intense photophobia, blepharospasm, lacrimation and blurred vision. Progression to blindness may occur. Corneas show irregular confluent opacities with bulbar injection of the conjunctiva, and subsequent superficial corneal neovascularisation. Enamel hypoplasia of permanent teeth is observed in approximately 80% of the patients. This defect predisposes them to further damage and cavities. Punctate nail dystrophy was also described in APS I patients (Husebye et al. 2009, Ahonen et al. 1990).

- **Vitiligo and alopecia**

Vitiligo may develop since the first month after birth and is present in 8-25% of the patients. Extent is highly variable, from spots to almost all corporal surfaces. Another component of the syndrome is alopecia, which may appear since first years, with increasing prevalence to 40% by middle age. It involves the scalp, eyelashes, eyebrows, axilla and pubis. Alopecia may be present as transient hairless scalp patches, but it may become extensive (Husebye et al. 2009, Weiler et al. 2012, Collins et al. 2006).

- **Vasculitis**

Recurrent maculopapular, morbilliform or urticarial erythema and rash with fever has also seen in APS I. Skin biopsies in some cases revealed lymphoplasmacytic vasculitis (Weiler et al. 2012). .

1.3.1.3. Diagnostic criteria

The diagnosis of APS I is based on the presence of at least two of three major clinical manifestations (Ahonen et al. 1990, Husebye et al. 2009, McManus et al. 2011, Perniola et al. 2000). When relatives of patient are analyzed, only one condition is required for APS I diagnosis. Recently some authors have proposed that genetic studies and anti-interferon (IFN) antibodies should be included as diagnostic criteria. In most cases, signs and symptoms of the disease appear in infancy or childhood and affected patients typically develop three to seven different disease manifestations. In addition to the major criteria, minor disease components are also common (Weiler et al. 2012, Perheentupa et al. 2006). These signs and the major components of disease often develop in a particular chronological order. CMC commonly begins before the age of 5 years, followed (usually years later) by autoimmune hypoparathyroidism before the age of 10 years and adrenal insufficiency before the age of 15 years (Ahonen et al. 1990, Husebye et al. 2009, Myhre et al. 2001). That may confuse their diagnosis (Al-Herbish et al 2000); once APS I is suspected, the consequent delays can be reduced by testing for the recently identified autoantibodies neutralizing IFN-αs and IFN-ω. These are valuable markers, as they are found in almost 100 % of APS I patients'sera, regardless of their exact *AIRE* mutations, clinical features or disease durations, but neither in unaffected heterozygous siblings nor in a wide range of other autoimmune disorders (Meager et al. 2006, Meloni et al. 2008, Kisand et al. 2010, Puel et al. 2010). By contrast, autoantibodies against IL-17F (~75 %) and IL-22 (>90 %) correlate with both the patients' CMC and their loss of Th17/Th22 cells (Kisand et al 2010). These antibodies are highly selective for these particular cytokines; although many others have been screened (IFN-γ, IL-1α/β, IL-2, IL-4, IL-6, IL-9, IL-10, IL-12, IL-18, IL17B/C/D, IL-21, IL-23, IL-26, TNF or TGF-β1), the occasional binding autoantibodies against IFN-γ, IL12, IL23 and TNF-α never neutralized their corresponding cytokines (Meager et al. 2006, Meloni et al. 2008).

1.3.1.4. Molecular background of autoimmune polendocrine syndrome type I

Functions ofthe autoimmune regulatorgene

The autoimmune regulator (*AIRE*) has been the focus of intense research since mutations in the gene were identified and reported in 1997 independently by two groups, as the cause of APS I. Key insights into the mechanism by which mutations in *AIRE* affect tolerance have come through the development of *AIRE*-deficient mouse models, namely each knockout strain develops lymphocytic infiltrate and autoantibodies (Gardner et al. 2008, Nishikawa et al. 2010, Eldershaw et al. 2011). The role of *AIRE* in central tolerance was first suggested through experiments demonstrating that expression of *AIRE* is largely restricted to a rare and special cell type in the thymic medulla. The thymic medulla represents a determining site for the induction of T cell tolerance, in particular, *AIRE*-expressing medullary thymic epithelial cells (mTECs) provide a spectrum of tissue-restricted anitgens (TRAs) that, through both direct presentation and cross-presentation by dendriticcells, purge the developing T cell repertoire of autoimmune specificities (Gardner et al. 2008, Danso-Abeam et al. 2011). While TRA expression in the thymic medulla had been previously documented, the finding specific molecular mediator to drive expression suggested endogenous tolerogenic function. Additional experiments have demonstrated that thymic TRA expression is likewise able to drive regulatory T cell conversion, with the alternative fates likely depending on antigen quantity and TCR affinity (Ilmarinen et al. 2008, Nishikawa et al. 2010). Several alternative mechanisms of central tolerance were proposed for *AIRE*, however the TRA-transcription function appears to be the most robust, and has been subsequently extended to multiple endogenous self-antigens from various tissues, including for example insulin 2 salivary protein, desmoglein 3, seminal vesicle secretory protein and interphotoreceptor retinoid-binding protein. Nevertheless, several groups have found extrathymic *AIRE* expression in multiple peripheral locations, and have suggested that *AIRE* is expressed in the periphery and does have a functional role. *AIRE* expressing stromal cells from the lymph nodes have been demonstrated to express TRAsin a tolerogenic form, and this process is *AIRE*-dependent, for at least a subset of TRAs. The lymph node stroma mediates TRA immune tolerance, including both *AIRE*-dependent and *AIRE*-nondependent mechanisms (Eldershaw et al. 2011, Poliani et al. 2004).

The gene encodesan 57,7 kDa protein. Three isoforms of the AIRE protein are described.: AIRE-1 as the full-lenght functional protein, and AIRE-2 and AIRE-3 are shorter forms

generated by alternative splicing. Based on its domain structure, the AIRE protein is belived to be a transcriptional factor. It has two zinc fingers of plant homeodomein (PHD)-type motifs, is a modular domain existing as a single or multiple unit in a number of nuclear proteins, nclude a leukocyte-specific component of the nuclear body Sp140, and proteins involved in chromatin mediated transcriptional modulation such as Mi-2 autoantigen, ALL-1, ATRX, TIF1 or KRIP-1.Homology of AIRE with known proteins was generally restricted to this PHD domain, extended structural similarity could be observed with the groud of human Sp100 proteins. Besides a 100 residue N-terminal region, AIRE-1, NucP41/75 and DEAF-1/supressin shared a putative DNA-binding segment referred as the SAND domain, located upstream of the PHD fingers and spanning 80 residues (Eldershaw et al. 2011, Rinderle et al. 1999, Ilmarinen et al. 2008).

Selection mechanisms of thymocytes

After the recruitment to the thymus, T cell precursors proliferate and differentiate to produce a large pool of immature CD4+CD8+ thymocytes that resides within the thymic cortex (Lee et al. 2007). As a result of the random recombination of gene segments at T cell receptor (TCR) –α and TCR-β loci, CD4+CD8+ thymocytes express a diverse repertoire of αβTCRs, and so are required to undergo selection events based on αβTCR specificity (Nishikawa et al. 2010). Positive selection rescues thymocytes capable of self-MHC recognition from cell deth, with the lineage commitment mechanism ensuring that changes in receptor expression result in the generation of CD4+ and CD8+ cells recognizing MHC class II and MHC class I, respectively. Newly selected thymocytes are then screened further their ability to recignizes self-peptide/MHC complexes, with negative selection puring the αβTCR repertoire of potentially autoreactive specificities. Such combined selection events ensure that T cell production inthe thymus leads to the generation of naive T cell pool that recognizes self-MHC molecules but is able to discriminate between self and nonself antigens (Danso-Abeam et al. 2011). Positive and negative selection events occure within specialized intrathymic environments defined by distinct stromal components: Positive selection signal are provided by cortical thymic epithelial cells (cTECs), a function that is least in part due to their expression of a unique MHC-bound self-peptide repertoire generated by cTEC-specific expression of thymus-specific serine protease gene (Prss16) and the β5 thymo-proteosomal subunit. In contrast, the medulla provides a microenvironment where self-tolerance is imposed, through both negative selection and FoxP3+ regulatory T cell (Treg) production (Danso-Abeam et al. 2011,

Eldeshaw et al. 2011, Gardner et al. 2008). AIRE have been shown to play a role in both processes, highlighting their importance inthe avoidance of autoimmunity.

Mutations in the autoimmune regulator gene

To date more than 60 different mutations have been identified. These mutations include nonsense and missense mutations, small insertions and deletions leading into frame shifts, and splice site mutations. Although mutations are spread throughout the coding region of the gene some hotspots emerge, including the more common and recurrent mutations R257X and 967-979del13bp (13bp del) (Myhre et al. 2001, Peterson et al. 2004, Puel et al. 2010, Tóth et al. 2010, Wolff et al. 2007). Some of the identified mutations have been shown to affect subcellular localization or transactivation properties of the protein, thus providing insights into the functional properties of the predicted protein motifs (Rinderle et al. 1999). Recent studies demonstarted that the wild-type AIRE gene product has the ability to be targeted into the nucleus, where it is found associated with distinct speckled sub-domains in the nucleoplasm (Eldershaw et al. 2011). Nuclear factors have been shown to be localized in discrete macromolecular domains within the cell nucleus, the distribution in punctate domains has been described previously for many transcription factors or transcription-associated factors, where this pattern is likely to represent protein complexes on target sites of the chromatin (Rinderle et al. 1999). The speckled pattern is thought to reflect the transcriptional activity of the cell, and the number of speckles observed for AIRE is possibly indicative of multiple interaction sites on the chromatin. The sub-nuclear localization observed for the AIRE protein would be in agreement with its putative function as a transcriptional regulator. Recent evidences showed that wild-type AIRE interacts with structural components of the cytosplasmic compartment, observations confirmed that stained AIRE fibers are found partly co-localizing with the vimentin filamentous system but that coincidence was even more striking with microtubules (Rinderle et al. 1999). Vimentin is the major protein constituent of cytoplasmic intermediate filaments maintaining cell shape and cytoskeletal integrity. Microtubules are highly dynamic structures that play a key role in cell mophogenesis in the formation of mitotic spindles. N-terminal AIRE fragments deleted for the PHD domain show altered subcellular, nuclear localization, suggesting that the mutations of AIRE may elicit their primary effect in the nucleus (Rinderle et al. 1999, Eldershaw et al. 2011, Danso-Abeam et al. 2011).

2. AIMS OF THE STUDY

1. To analyse the clinical data of autoimmune poliendocrine syndrome type I patients and their family members by focusing on disease manifestations, laboratory findings and organ specific autoantibody profiles. We studied how early their various manifestations and autoantibodies appeared,and analyzed the correlation between organ specific autoantibody production andorgan functions.

2. To investigate the genetic heterogenity and genotype-fenotype correlations of 19 autoimmune poliendocrine syndrome type I patients from 7 different countries.

3. To measure anti-cytokine autoantibody levels to interleukin-17A, interleukin-17F, interleukin-22 and type I interferons in sera of autoimmne poliendocrine syndrome type I patients, and to analyze the correlation between anti-cytokine autoantibody levels and the clinical manifestations, especially chronic mucocutaneous candidiasis. We planned to show chronological relationship between the production of autoantibodies and the development of clinical signs of autoimme polyendocrine syndrome type I.

4. To study anti-*Candida* cytokine responses of autoimmune polyendocrine syndrome type I patients, by measuring interleukin-17 and interleukin-22 secretion by *Candida*-exposed peripheral blood mononuclear cells.

5. To examine in vitro differentiation of CD4+interleukin-17+ /interleukin-22+ T helper cells.

6. To follow up a patient (P3) from 7 week of age who carried R257X/R257X mutation ofautoimmune regulator genein order to detect the very first clinical and laboratory manifestations of autoimmune polyendocrine syndrome type I.

3. MATERIALS AND METHODS

3.1. Patients

We tested 19 patients from seven countries: six patients from Hungary (P1-P6), three patients from the USA (P7-8, P13), three patients from Finland (P9-10, 14), two patients from Sardinia (P11, P19) two patients from Norway (P15, P18) and two patients from Russia (P16-17) and one patient from Azerbaijan (P12). With informed consent and ethics committee approval, sera were collected at diagnosis of APS I or soon afterwards, or beforehand in three, at first sampling-time, as yet unaffected sieblings of known patients.

Patient 1

This 10-year-old boy had nail dystrophy, dental enamel hypoplasia and nail pitting from the age of 3.5 years. At the age of 4 years, he presented chickenpox and developed alopecia totalis, involving the scalp, eyelashes and eyebrows, which has persisted ever since. Routine laboratory tests showed persistent hyperphosphatemia and high thyroid-stimulating hormone (TSH) levels, but serum calcium concentration and liver enzyme activities were normal. The patient has not had mucosal or skin candidiasis. An adrenal cortex antibody assay showed persistent weak positivity without signs of autoimmune adrenal insufficiency.

Patient 2

This 7-year-old girl (the sister of Patient 1) developed muscle cramps, recurrent upper respiratorytract infections and oral and nail candidiasis caused by *C. albicans*at 18 months of age. Calcium and vitamin D supplementation was initiated. At 4 years of age, blood tests showed low PTH concentration, hyperphosphatemia, hypergammaglobulinemia and high levels of liver enzymes. At the age of 5 years, P2 had a low serum cortisol concentration and developed iron deficiency with hypochromic microcytic anemia. Laboratory and genetic tests excluded celiac disease and DiGeorge syndrome. This patient had organ-specific autoantibodies against liver-kidney microsomal enzymes and the adrenal cortex. She was treated with anti-fungal agents (borax-glycerin, nystatin, fluconazole), parenteral and oral calcium and vitamin D derivatives from early childhood.

Table 2. Patients with clinically and genetically diagnosed APS I.

P	Gend.(M/F)	Age (year)	Origin	Mutation	Manifestations
P1	M	10	Hun	R257X h.	ED, EH, Alo
P2	F	7	Hun	R257X h.	CMC, HP, Alo, Urt, Mal
P3	M	5	Hun	R257X h.	no symptomes
P4	M	27	Hun	R257X/13 bp del	HP, AI, Ker, CMC
P5	M	22	Hun	R257X/13 bp del	HP, AI, CMC
P6	F	9	Hun	R257X h.	HP
P7	M	12	USA	13 bp del h.	HP, AI, ED
P8	F	12	USA	C302Y/nd.	HP
P9	F	16	Fin	R257X/13 bp del	HP, AI, CMC, Mal
P10	F	43	Fin	R257X h.	Urt, HP, CMC, Mal, Vit, AI, OF, DM
P11	F	5	Sard	R139X h.	CMC, HP, AH
P12	M	10	Azeri	520X h.	AI, Alo, CMC
P13	F	6	USA	13 bp del/R92W	Ret, HP, CMC, Aspl, AH, AI
P14	F	36	Fin	R257X h.	CMC, HP, Thyr
P15	M	17	Nor	R257X/13 bp del	CMC, HP, Ker, Alo, AI, Thyr
P16	M	6	Rus	R257X h.	CMC, Mal, Alo, AI
P17	M	11	Rus	R257X h.	CMC, AI, HP, Epil, ED, EH
P18	M	20	Nor	R257X/13 bp del	CMC, HP, Bleph, Mal
P19	M	4	Sard	R139X h.	CMC, AH, AI

P, patient; Gend., Gender; M, male; F, female; Age, Age of sampling; Hun, Hungary; Fin, Finland; Sard, Sardinia; Azeri, Azerbaijan; Nor, Norway; Rus, Russia; h., homozygous; nd,

nodetectated; ED, ectodermal dystrophy; EH, enamel hypoplasia; Alo, alopecia; CMC, chronic mucocutaneous candidiasis; HP, hypoparathyroidism; Urt, urticaria; Mal, malabsorption; AI, adrenal insufficiency; Ker, keratopathy; Vit, vitiligo; OF, ovarian failure; Ret, retinal dystrophy; Aspl, asplenia; AH, autoimmune hepatitis; Thyr, thyroid disease; Epil-epilepsy; Bleph, blepharitis.

At 5 years of age, given the persistence of hypertransaminasemia and treatment-resistant candidiasis, we initiated intravenous immunoglobulin therapy (IVIG), at a dose of 560 mg/kg monthly. One week after the first session of IVIG treatment, oral candidiasis had improvedand the patient remained symptom-free for 2 weeks. In the following month, before IVIG treatment, laboratory tests showed a decrease in liver enzyme levels and a normal serum calcium concentration. One year after the first IVIG infusion, the patient presented with fever once or twice per week (T_{max}:39.3 °C), followed by an urticarial rash on her arms and legs. Symptoms ceased spontaneously after 12 to 24 h. Laboratory tests showed high levels of liver enzymes (GOT 78 U/l, GPT 54 U/l) and C-reactive protein (16.4 mg/l; normal: <5 mg/l), a high white blood cell count (12.49 G/l; normal range: 4.5–11.5 G/l) and granulocytosis (9.14 G/l; normal range: 1.9–7.7 G/l). IgG and IgM levels were 38.4 g/l and 2.35 g/l, respectively, and serum protein electrophoresis excluded monoclonal gammopathy. Serological tests for Epstein-Barr virus, cytomegalovirus, hepatitis A-, B-, C-, and E virus infections were negative. Recurrent maculopapular, morbilliform and urticarial erythema with fever have also been reported in other cases of APS I with skin biopsy in some cases revealing lymphoplasmacytic vasculitis. Liver protection therapy (silimarin, vitamin B complex) and local antifungal treatment with borax-glycerin and nystatin were initiated. Four weeks after the cessation of IVIG, serum Ig and liver enzyme levels gradually decreased, but the oral candidiasis remained resistant to local and oral treatment. Patients 1 and 2 were from the same family and had the same *AIRE* mutation, but had completely different clinical phenotypes. Patient 1 developed alopecia totalis after contracting chickenpox. He also has nail dystrophy without *Candida* infection. Patient 2 has been suffering from CMC since the age of 18 months. She also developed impaired liver function at the age of 4 years.

Patient 3

This 5-year-old boy was first seen at our department at 3weeks of age. His sister was diagnosed with APS I at 2 years of age, she had had severe CMC since early infancy, recurrent respiratory infections, adrenal and parathyroid insufficiency. She died from adrenal failure during an episode of acute bacterial infection and dehydration. Based on the family history and in response to parental requests, we performed molecular genetic analysis and regular follow-up for this boy. From the ages of 12 to 36 months he had mild persistent hyperphosphatemia. Determinations of serum immunoglobulin isotypes showed low IgA and high IgG levels. Weakly positive results were obtained only once for anti-adrenal and anti-islet cell organ-specific autoantibodies. This 5-year-old patient has no clinical signs of disease, but has produced high levels of anti-cytokine autoantibodies since infancy. Endocrine organ involvement differed considerably between these three APS I patients. Patient 1 had no endocrine organ deficiency or electrolyte imbalance, or other signs of autoimmune disease, despite the persistent detection of anti-adrenal autoantibodies. Patient 2 has hypoparathyreosis with low PTH concentration, hypocalcemia and hyperphosphatemia. She has high liver enzyme and serum IgG and IgM levels. Patient 3 showed subclinical signs of hypoparathyreosis, with slightly high phosphate levels and high levels of IgG. He had a transiently low cortisol concentration, with no anti-adrenal autoantibodies. The GAD antibody was detected during a transitional period.

Patient 4

This 27-year-old man had recurrent sinusitis and allergic atopic dermatitis from childhood. He developed hypoparathyroidism and hypocalcemia at the age of 9.5 years and adrenal hypofunction at 11 years of age. His parents are healthy and his only sibling (Patient 5) also has APS I. At the age of 14 years, he was diagnosed with corneal degeneration and cataracts and subsequently underwent several ophthalmological operations and corneal abrasion. He has suffered from recurrent non-purulent conjunctivitis and atopic allergic asthma. This patient has not had chronic mucosal or skin candidiasis; he presented with intermittent angular cheilitis since the age of 21 years, with no microbiological confirmation of a candidial etiology. He occasionally has palpitations and weakness, and cardiological examination revealed mitral prolapse with mild regurgitation. He has been on regular calcium andactive

vitamin D supplementation, with hydrocortisone replacement since the development of endocrinopathy during childhood.

Patient 5

This 22-year-old man (the brother of patient 4) presented with weakness of the extremities and muscle cramps associated with a severe episode of viral gastrointestinal infection at theage of 11 years. Chvostek's sign was positive; laboratory tests showed hypocalcemia and very low parathormone levels and the patient was diagnosed with hypoparathyreosis. At the age of 17 years, he developed isolated nail candidiasis of the right thumb. Candidial infection recurred several times at this anatomic location, but the patient has no mucosal, cutaneous or nail candidiasis at any other site. At the age of 21 years, this patient presented with persistent and progressive weight loss, and laboratory tests revealed hypoadrenia, with high levels of ACTH and lower levels of cortisol. His endocrine status has stabilized on substitution therapy.

Patient 6

This 9-year-old girl presented with recurrent upper respiratory tract infections and purulent otitis media and was frequently treated with antibiotics between the ages of 18 months and5 years. Bilateral paracentesis was performed several times and, at the age of 5 years, a tube (grommet) was implanted, resulting in significant clinical improvement. The patient's speech and communication development and social adaptation were delayed and audiology analysis revealed mixed-type hearing impairment necessitating the use of a hearing aid. At the age of 8 years, this patient presented with muscle cramps and tetany in association with an acute episode of viral gastrointestinal infection. Laboratory tests revealed hypocalcemia and hypoparathyreosis. Until the age of 3 years, P6 presented short episodes of mild soor oris and vulvovaginitis about once per year, which have not occurred since.

3.2. Laboratory methods

3.2.1. Serum samples

Blood was taken under aseptic conditions and serum was isolated by centrifuging blood at 2,500 rpm (550×g) for 10 min at room temperature. Aliquots of the samples from the patients and healthy controls were frozen at −20 °C until use. Routine Laboratory Assays Anti-endocrine organ antibody levels, clinical chemistry and immunology parameters were determined by routine laboratory assays with freshly isolated serum.

3.2.2. Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from the patients and controls. After density gradient centrifugation and removal of the mononuclear cell layer, the cells were washed several times in Krebs-Ringer Phosphate Puffer. The cells were resuspended in Dulbecco's modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO). PBMCs (5×10^5 cells/well) were incubated either alone or in the presence of heat-killed *C. albicans*, at 37 °C, in 96-well round-bottomed culture plates 5 days. After the incubation, the plates were centrifugedand the supernatants were removed and stored at −20 °C for the determination of cytokine concentrations.

3.2.3. Preparing *Candida* suspension

C. albicans (ATCC 10231) was maintained on Sabouraud dextrose agar at 4°C and stationary-phase cultures were prepared by inoculating 2 ml of DMEM. Heat-killed *Candida* was prepared by inoculating 5 ml SalSol (TEVA, Debrecen, Hungary) with *Candida* and incubating the culture at 56°C for 60 min. We checked that the heat inactivation was effective by transferring various dilutions of *Candida* suspension onto Sabouraud dextrose agar and incubating for 48 h.The *Candida* suspension was then centrifuged at4,000×g for 10 min and the cell pellet was resuspended in Krebs-Ringer phosphate buffer with dextrose (KRPD)/ in DMEM. The density of heat-inactivated *Candida* was adjusted to 10^6/mlwith a McFarland densitometer.

3.2.4. Routine laboratory assays

Anti-endocrine organ antibody levels, clinical chemistry and immunology parameters were determined by routine laboratory assays with freshly isolated serum.

3.2.5. Analysis of autoimmune regulator gene mutations

Genomic DNA was sequenced by amplifying the exons and flanking intron regions of *AIRE* by PCR. Amplicons were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed with an ABI PRISM 3130 capillary sequencer (Applied Biosystems). Sequence variations were described with respectto a reference sequence (GenBank accession No. ENST00000291582) for *AIRE* cDNA, in which the c.1 position corresponds to the A of the ATG translation initiation codon. Mutations are designated as recommended by den Dunnen and Antonarakis (den Dunnen et al. 2001).

3.2.6. Organ specific antibody assays

Organ-specific autoantibodies against 21-hydroxylase (21OH), side-chain cleavage enzyme (SCC), aromatic L-amino acid decarboxylase (AADC), NACHT leucine-rich-repeat protein 5 (NALP-5), tryptophan hydroxylase (TPH) and glutamic acid decarboxylase (GAD-65) were assayed by radio-immunoassays, as for cytokine autoantibodies (Wolff et al. 2010). In RIA, levels of autoantibodies are estimated based on a positive control and a negative control. A threshold value is created based on the mean of a pool of healthy blood donors (N=50–150). This assay has the drawback of giving fairly high background signals, but is a very effective semi-quantitative autoantibody assay toolwhich demands little time and volume of sample. Some patient sera were assayed for ovary, adrenal and islet cell antibodies by routine indirect immunofluorescence tests.

3.2.7. Anti-cytokine antibody assays

Levels of serum autoantibodies binding to IL-17A, IL-17F, IL-22, IFN-α and IFN-ω were determined by ELISA. Briefly, cytokine samples (IL-17A, IL-17F and IL-22 from Peprotech, EC, London; INF-α and IFN-ω from Rocky Hill, NJ, USA) were diluted in PBS (0.1µg/ml). We then coated Nunc MaxiSorp immunosorbent 96-well flat-bottomed plates with 100µl of

cytokine suspension/well and incubated the plates overnight at 4°C. The supernatant was removed and the nonspecific binding sites were blocked by incubation for 2 h at room temperature with 100 µl of blocking buffer (0.1 % Tween-20, 3 % bovine serum albumin in PBS) per well. The plates were then washed four times with washing buffer (0.1 %Tween-20 in PBS). Serum samples were diluted 1:1000 in blocking buffer, and 100 µl of the dilutions was added to wells and incubated for 1 h at room temperature. The plates were washed four more times. Alkaline phosphatase-conjugated goat anti-human IgG was diluted 1:10000 in antibody buffer, and 100 µl was added to each well. The plates were then incubated for 1 h at room temperature. The plates were washed five times with washing buffer and antibody binding was visualized by adding a solution of the substrate, p-nitrophenol, according to the manufacturer's protocol and determining absorbance at 405 nm, with a Multiskan EX photometer (Thermo Scientific,Shanghai, China), after incubation in the dark for 4.5 min.

3.2.8. Immunglobulin G antibody subclass assay

Autoantibody subclasses were determined as described previously (Kärner et al. 2013, Hendrix et al. 2011). In brief, streptavidin agarose resin (Invitrogen,1µl per well) was incubated for 1 h with biotin-conjugated anti-human subclass-specific antibodies (from BD Pharmingen against IgG1, IgG2 and IgG4, and from Invitrogen against IgG3) in 96-well Multi Screen filter HTS plates (Millipore, Bedford, MA, USA), and washed. At the same time, APS I or control sera (1:25) were incubated for 1 h with 10^5 luminescence units of luciferase-linked IL-22 or IFN-α 2a; any immune complexes were then captured onto the coated beads. After washing, their luminescence intensities were measured in a 1450 MicroBeta TriLux Liquid Scintillation Counter & Luminometer (Perkin Elmer Life Sciences, Waltham, MA, USA).

3.2.9. Measurement of cytokine concentrations

The concentrations of IL-17A, IL-17F, IL-22 and TNF-α in the supernatants of *Candida* -exposed PBMCs were determined by sandwich enzyme-linked immunosorbent assays (Quantikine, R&D Systems, MN, USA). All experiments were performed in duplicate or triplicate.

3.2.10. Differentiation and flow cytometry analysis of interleukin-17 and interleukin-22 producing T cells

PBMCs from patient and healthy control were purified by gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare). PBMCs were washed 3 times with 1X Phosphate buffer saline (PBS; Sigma-Aldrich) and suspended in RPMI-1640 supplemented with 10% foetal bovine serum (FBS; Sigma-Aldrich). Adherent cells were removed by incubation at 37°C for 4 hours in RPMI. Non-adherent leukocytes were stimulated with 2 µg/ml anti-CD3 (Miltenyi Biotec, Bergisch Gladbach, Germany) and treated with a cocktail of different cytokines: recombinant human interleukin (IL)-23 (20ng/ml), IL-1β (10 ng/ml), IL-6 (50 ng/ml), and human transforming growth factor (TGF)-β (5 ng/ml); all from PeproTech. After two days, RPMI medium containing with cytokines listed above and plus IL-2 (22 ng/ml) were added into the cells and another two days we added again RPMI medium with cytokines. The following day, the cells were treated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) and 10^{-5} M ionomycin (IMC; Sigma-Aldrich) in the presence of 1 µg/ml GolgiPlug (Sigma-Aldrich) for 6 hours at 37°C. The cells were then washed with washing buffer (2% FBS-bovine serum albumin (BSA)-PBS). For the surface labelling the cells were stimulated with allophycocianin (APC)-conjugated mouse anti-hCD4 IgG_1 mAb (BD, San Jose, CA, USA) or Peridinin chlorophyll (PerCP)-conjugated mouse anti-hCD3 IgG_1 mAb (BD) for 30 min at 4°C. They were incubated with Fixation Medium A (Invitrogen, Camarillo, CA, USA) for 15 min at 4°C. The cells were then washed with washing buffer and stained in Perm Medium B (Invitrogen) with phycoerythrin (PE)-conjugated mouse anti-human IL-17A IgG_1 mAb (R&D Sytems, Minneapolis, MN, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-human IL-22 IgG_1 mAb (R&D Sytems) for 30 min at 4°C and after washing steps resuspended in 1% paraformaldehyde to analyse with AccuriC6 cytometer (BD).

4. RESULTS

4.1. Clinical presentation

Sample were tested from 19 APSI patients (P1-P19).Among these 19 subjects, we have noted mild or severe CMC in 14, HP in 13 and 11 had AI. The mean onset-ages of the major or minor manifestations were 45,7 months (range 12 months–11years). The mean onset-ages for CMC were 61,6 months (range 12 months-21years), for HP were 63,7months (range 18 months-11 years), and for AI were 110,8 months (range 48 months-25,4 years). 7 of the 19 patients presented the classical diagnostic triad of APS I, before 7 years of age, 15 of the 19 subjects fulfilled the traditional diagnostic criteria for APS I, having at least two of the CMC, HP and AI triad; in addition, P19 had CMC plus an affected sibling at onset. In 18 subjects, mutations were detected in both *AIRE* alleles, but only in one allele in P8, who only had HP and type I interferon autoantibodies. Based on the presence of these type I interferon anti-cytokine autoantibodies, which are present in almost 100% of APS I patients and which are not present in case of heterozygous sieblings, we suppose that P8 carries a compound heterozygous mutation, but with PCR and sequencing we found a pseudo-negative result on the other allele of *AIRE* gene. Unfortunately, we don’t have additional sample of this patient to continue the additional analysis. Subjects P1, despite homozygosity for the R257X *AIRE* mutation, still only has dental enamel dysplasia, alopecia universalis and neail dystrophyby age 10, whereas his sister (P2) had developed both CMC and HP till the age 18 months.

Table 3A. Laboratory parameters for APS I patients

							Age at measurement (years)					
		Patient 1				Normal range	Patient 2					Patient 6
Variable	Units	7.5	8	8.5	9		4	5	5.5	6	6.5	9
Sodium	mmol/l	142	142	136	136	(133-150)	148	144	143	137	132	142
Potassium	mmol/l	4.1	4.6	4.4	4.4	(3.5-5.3)	4	4.4	3.8	4.6	4	3.9
Chloride	mmol/l	101	101	106	101	(99-111)	104	101	102	104	94↓	104
Calcium	mmol/l	2.32	2.46	2.29	2.51	(2.1-2.6)	1.7↓	2.1	2.45	1.8↓	1.57↓	2.01↓
Phosphate	mmol/l	1.53↑	1.5↑	1.48	1.5↑	(0.8-1.45)	2.48↑	2.1↑	-	1.75↑	2.38↑	2.19↑
Iron	umol/l	-	-	7	-	(6.6-26)	7.4	-	-	4↓	3.4↓	12.9
GOT	U/l	35	35	32	28	(<40)	83↑	233↑	95↑	73↑	78↑	37
GPT	U/l	18	23	20	19	(<40)	76↑	245↑	84↑	51↑	54↑	26
Alkaline phosph.	U/l	456	558	357	172	(<720)	462	-	-	134	483	212
IgG	g/l	11.49	12.1	11.6	12.3	(5.4-15.1)	17.46↑	19.9↑	20.6↑	19.9↑	38.4↑	12.2
IgA	g/l	1.1	1.37	1.41	1.13	(0.52-3.25)	1.52	-	2.04	2.58	2.49	1.15

IgM	g/l	0.71	0.72	0.78	0.6	(0.52-1.5)	1.47	1.78↑	1.64↑	1.59↑	2.35↑	2.64↑
ACTH	ng/l	-	14.1	<20	<20	(<75)	<12.8	<12.8	12.9	<20	-	22.2
Cortisol	nmol/l	319	188.4	207.4	432.1	(138-690)	248.8	118.9↓	129.8↓	285	-	216.5
TSH	mU/l	4.3	2.22	2.27		(0.3-4.2)	2.88	-	2.39	1.29	-	2.13
Adrenal antibody		pos.	pos.	pos.	pos.		neg.	-	pos.	neg.	-	neg.
GAD antibody		-	neg.	neg.	neg.		-	-	neg.	neg.	-	neg.
TPO antibody		neg.	neg.	-	-		neg.	neg.	-	-	-	neg.
TG antibody		neg.	-	-	-		-	neg.	-	-	-	-

Table 3B. Laboratory parameters for APS I patients (cont.)

		Age at measurement (years)							
		Patient 3							Normal range
Variable	Units	1	1.5	2	2.5	3	3.5	4	
Sodium	mmol/l	145	140	142	140	138	141	135	(137-150)
Potassium	mmol/l	4.8	4.4	4.8	4.6	4.1	4.0	4.2	(3.5-5.3)
Chloride	mmol/l	108	103	106	103	103	104	102	(99-111)
Calcium	mmol/l	2.39	2.47	2.39	2.4	2.54	2.41	2.48	(2.1-2.6)

Phosphate	mmol/l	1.7↑	1.89↑	1.66↑	1.58	1.76↑	1.62↑	1.44	(0.8-1.6)
Iron	umol/l	3.5↓	12.4	-	14.3	16.3	13.9	17.2	(10.6-28.3)
GOT	U/l	37	36	34	28	31	29	24	(<40)
GPT	U/l	14	11	11	12	14	17	16	(<40)
Alkaline phosph.	U/l	441	-	439	418	492	228	174	(<720)
IgG	g/l	18.18↑	19.82↑	12.8	-	11.1	-	9.01	(5.4-15.1)
IgA	g/l	0.21↓	0.11↓	0.26↓	-	0.23↓	-	0.32↓	(0.52-3.25)
IgM	g/l	0.89	1.29	1.06	-	1.04	-	1.02	(0.52-1.5)
ACTH	ng/l	25.8	30.9	61.3	27.5	32.4	34	25.9	(<75)
Cortisol	nmol/l	437.1	342.6	190.2↓	207.1↓	260.2	213↓	277.1	(260-720)
PTH	pmol/l	2.94	4.62	3.07	2.7	3.69	-	2.38	(1.6-6.9)
TSH	mU/l	3.03	1.88	3.08	4.76	3.47	2.88	5.88	(0.3-4.2)
Anti-adrenal antibody		-	-	neg.	neg.	neg.	neg.	-	
GAD antibody		-	-	pos.	neg.	pos.	neg.	neg.	
TPO antibody		-	-	-	-	neg.	-	neg.	
TG antibody		-	-	-	-	-	neg.	-	

Table 3C. Laboratory parameters for APS I patients (cont.)

Variable	Units	Age at measurement (year) Patient 4			(Normal range)	Patient 5		
		22	24	26		18	20	22
Sodium	mmol/l	147	-	142	(136-146)	146	139	135↓
Potassium	mmol/l	3.6	-	3.8	(3.5-5.3)	4.6	4.7	4.3
Chloride	mmol/l	102	-	99	(99-111)	104	99	96↓
Calcium	mmol/l	1.88↓	1.07↓	2.02↓	(2.1-2.6)	2.19	2.09↓	2.19
Phosphate	mmol/l	-	1.28	1.45	(0.8-1.45)	-	1.42	1.55↑
Iron	umol/l	-	-	20.7	(10.6-28.3)	-	-	24
GOT	U/l	-	-	20	(<40)	-	50↑	25
GPT	U/l	-	-	16	(<40)	-	72↑	36
AP	U/l	-	-	77	(40-115)	-	276↑	224↑
IgG	g/l	-	-	12.7	(7.0-16.0)	-	-	13.2
IgA	g/l	-	-	2.94	(0.7-4.0)	-	-	3.36
IgM	g/l	-	-	0.83	(0.4-2.3)	-	-	0.52

ACTH	ng/l	165.8↑	79↑	150↑	(<75)	21.9	44.4	262.1↑
Cortisol	nmol/l	883↑	1032.2↑	649.2	(138-690)	265.7	251.2	165
TSH	mU/l	2.65	1.33	2.49	(0.3-4.2)	0.989	1.53	1.81
PTH	pmol/l	-	-	0.57↓	(1.6-6.9)	0.59↓	0.59↓	0.51↓
Adrenal antibody		-	-	pos.		-	-	pos.
GAD antibody		-	-	neg.		-	-	neg.
TPO antibody		-	-	-		-	-	-
TG antibody		-	-	-		-	-	-

GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ACTH, adrenocorticotropic hormone; PTH, parathyroid hormone; TSH; thyroid-stimulating hormone; GAD, glutamic acid decarboxylase; TPO, thyreoperoxidase; TG, thyroglobulin; AP, alkaline phosphatase.

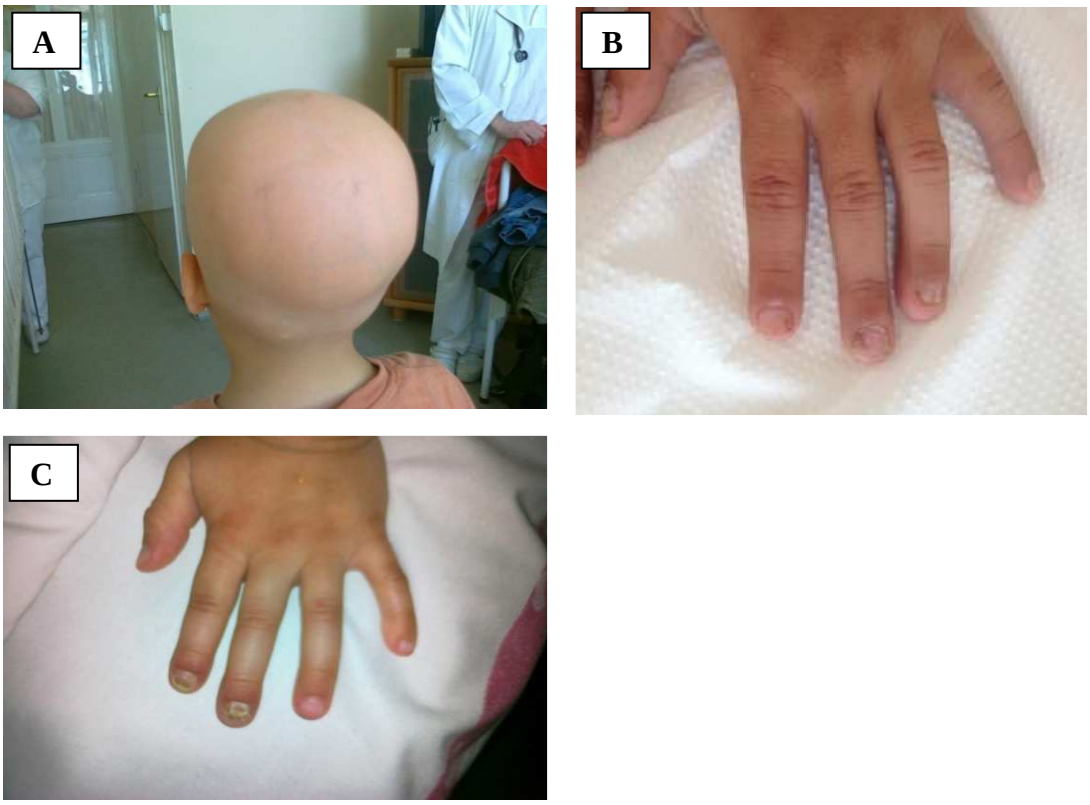


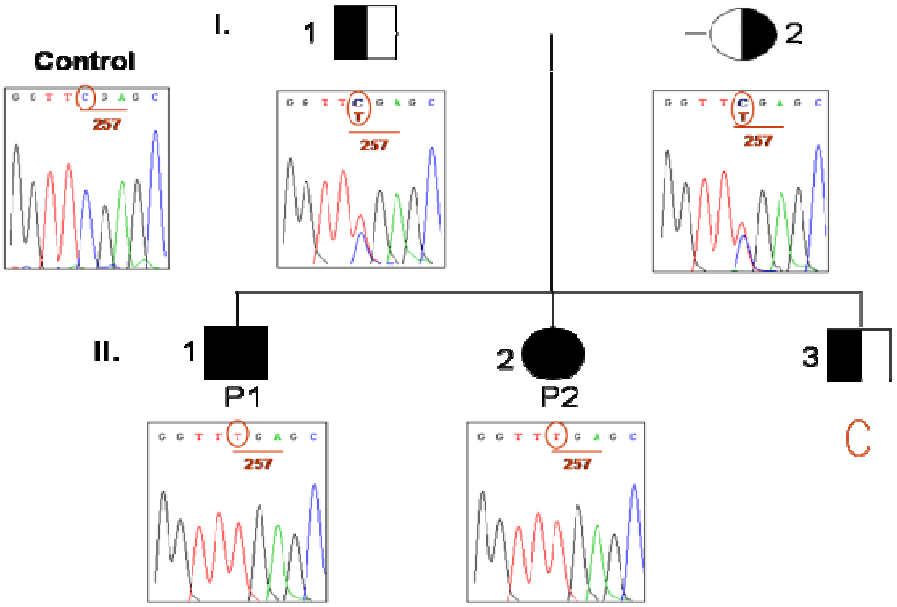
Figure 3. Clinical manifestations of APS I patients. Alopecia universalis and nail dystrophy of Patient 1 (Fig. 3A, B). His sister (Patient 2), carring the same mutation; suffered from recurrent oral, and chronic nail candidiasis (Fig. 3C) from early childhood.

4.2. Genetic data

Eight patients (P1, P2, P3, P6, P10, P14, P16, P17 on Table 2.) were found to be homozygous for the c.769C>T nonsense mutation of the *AIRE* gene, which replaces the arginine codon in position 257 with a stop codon (R257X/R257X). The available, testedparents of Patients P1, P2 (Fig. 4A), and P3 (Fig. 4B) and the mother of Patient 6 (Fig. 4D) were heterozygous for the mutant allele, consistent with autosomal recessive inheritance for this trait. Heterozygosity was also detected in a male sibling of Patients 1 and 2 (Fig. 4A), in a brother and a sister of Patient 3 (Fig. 4B), and in one of the two brothers of Patient 6 (Fig. 4D). Genomic DNA sequencing ofsamples from the mother and two siblings of Patient 3 revealed a silent mutation in one allele of *AIRE* in addition to the disease-causing mutation located in the other allele (Fig. 4C). Five patients (P4, P5, P9, P15, P18 on Table 2.) carry the c.769C>T/c.1344delC or

c.1344delC/c.769C>T compound heterozygous mutations in the *AIRE* gene. P7 were found to be homozygous for the c.1344delC. P8 is compound heterozygous, for the C302Y mutation, mutation on the other allel was not detected. P11 and P19 for the R139X and P12 for the 520X mutations. P13 carry the c.1344delC/R92W compound heterozygous mutation in the *AIRE* gene (Table 2.).

Figure 4A



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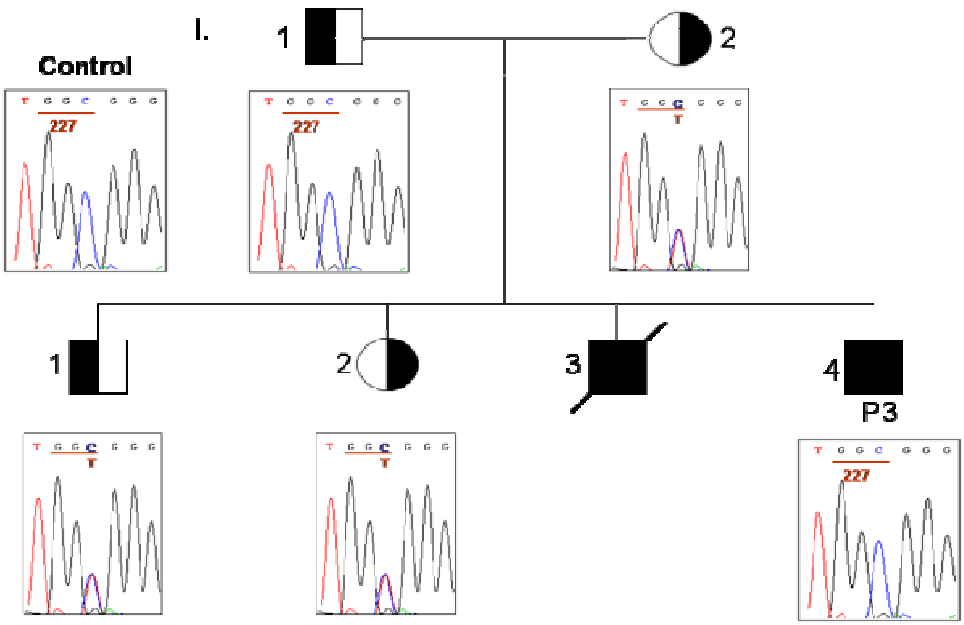


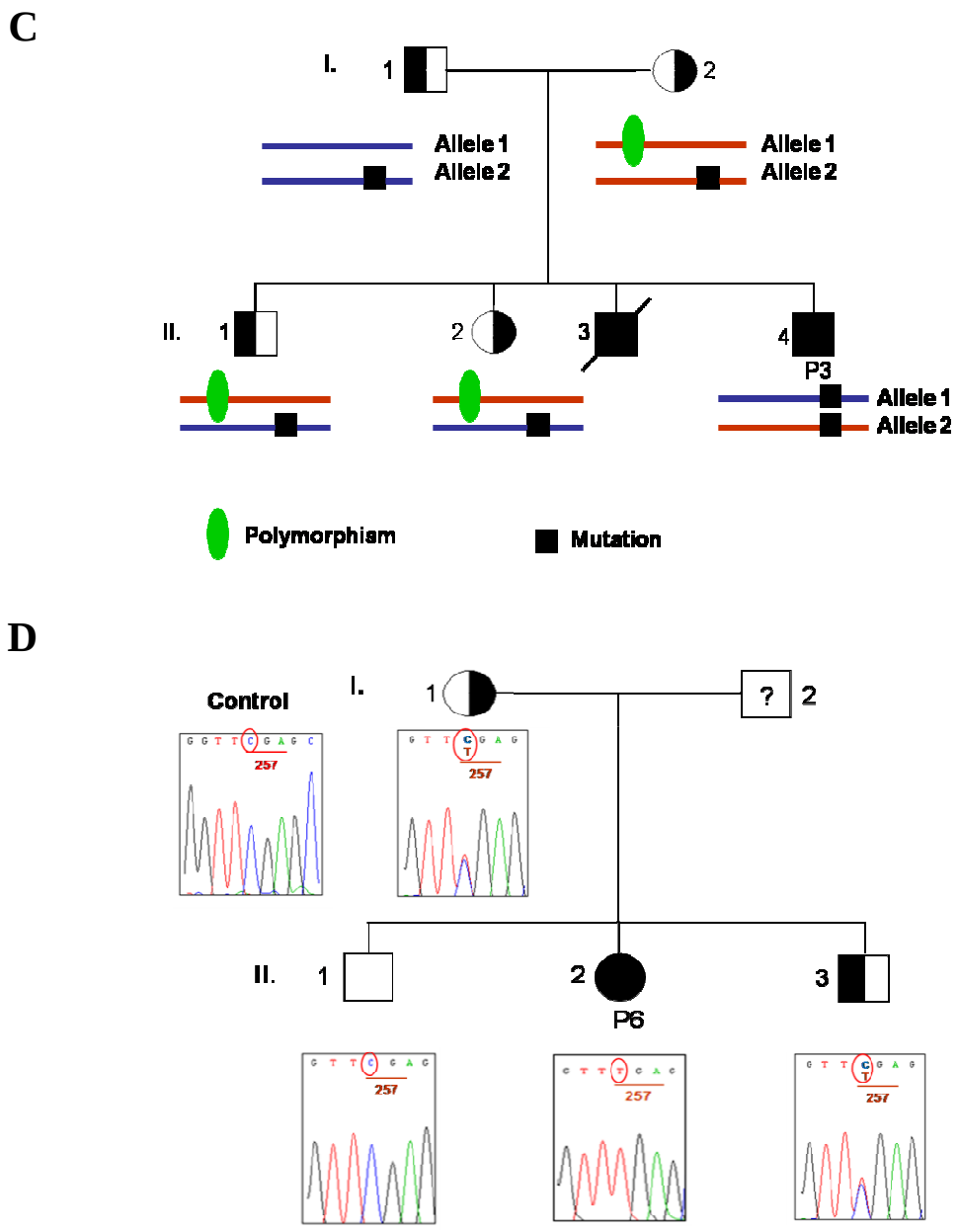


Figure 4. Pedigree and AIRE sequence variants of Patients 1, 2 (Fig 4A), 3 (Fig 4B, C) and 6 (Fig. 4D). Patients with APA I (II/1 and II/2 on Fig 4A) are homozygous for the c.769C> T nonsense mutation predicting a stop codon in the *AIRE* gene, whereas the asymptomatic parents (I/1 and I/2 on Fig 4A), and brother (II/3 on Fig 4A) are heterozygous. (Fig 4C) Schematic representation of the c.769C>T (R257X) mutation (black squares) and a silent sequence variant (c.681C>T; p.G227G; green ovals) in the family of Patient 3 (see also Fig. 3B). (Fig 4B) The mother (I/2) and two children (II/1 and II/2) are heterozygous for both the pathologic and silent mutations, whereas the father is heterozygous (I/1) and Patient 3 (II/4) is homozygous for the disease-causing mutation. (Fig 4D) Pedigree and mutation of Patient 6.

4.3. Measuring of cytokine- and organ specific autoantibody concentrations

4.3.1. Autoantibodies against cytokines

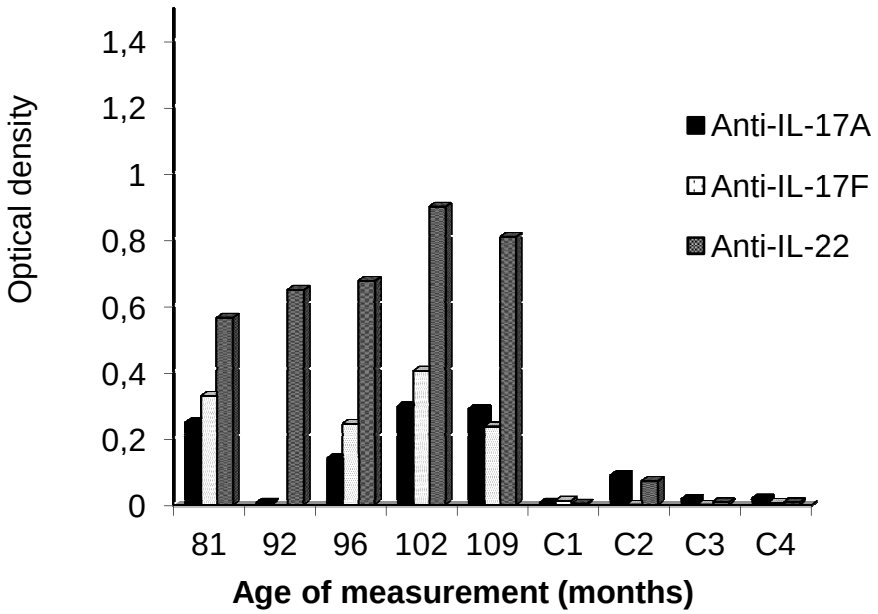
Patient 3 tested negative for all the antibodies in our panel at the age 6 weeks. However, by 7 months of age, his neutralization titers were already medium-high against IFN-α, IFN-ωand also IL-22. Only by 11 months did autoantibody levels begin to rise against IL-17F, and even later against IL-17A. By age 6 months, Patient 19 already tested modestly positive against IFN-α and IFN-ω, and marginally against IL-22. By 30 months, he was also strongly positive against IL-17A, IL- 17F and especially IL-22. He developed CMC only at 14 months, followed 16 months later by autoimmune hepatitis, a feature noted early in 6 of 22 Sardinian APS-I patients. P1 had high level autoantibodies against IFN-α, IFN-ω, IL17-F and IL-22 at age 6.5 years, although he had only dental manifestations and alopecia even at age 10. Of the APS I. patients, 13 had moderate-high autoantibody levels against IFN-ω in their first samples; 9 of these were also strongly positive against IFN-α. The one exception was P13, whose antibodies against IFN-ω were found 3 years later, and at lower titers, than those against IL-17F and IL-22, and she remained negative against IFN-α. Since all subjects had IFN-ω and/ or IFN-α autoantibodies. Antibody levels were already high against both IL-22 and IL-17F in 13 of the 16 patients when first tested, but only against IL-22 in an Azeri boy (P12) homozygous for a c.1497delT-mutation, probably leading to a frameshift with a premature termination in codon 520. In the USA patient with only a single identified C302Y mutation (P8), we found antibodies only against IFN-ω, and only against IFN-α and IFN-ω in a Russian boy (P16) homozygous for the common Finnish mutation, R257X. Unusually, he and his R257X homozygous compatriot (P17) had recurrent respiratory and enteric infections since age 24 months and birth, respectively. We found autoantibodies against IL-17F and/or IL-22 in 14 of the 16 subjects with CMC; in at least 6 of those, they were strongly positive before its onset. Their titers were also high in P1, P3 and P7, who still did not have CMC at ages 5, 10 and 12 years, respectively. The Russian P16 is the one exception who has CMC without detectable binding antibodies to Th17 cytokines.

Moreover, we tested and follow-up six Hungarian subjects for autoantibodies to IFN-α, IFN-ω, IL-17F and IL-22, and to IL-17A.

4.3.1.1. Autoantibodies against interleukin-17A, interleukin-17F and interleukin-22

OD values for autoantibodies against IL-22 were higher for all patients than for healthy controls. In Patient 1, anti-IL-22 values increased gradually between the ages of 81 and 102 months. OD values for anti-IL-22 antibodies were slightly higher in the adult patients than in children. By contrast, the levels of anti-IL-17A autoantibodies differed considerably between patients. These levels were higher in Patient 2 than in the other patients. Patient 2, who had presented CMC since the age of 18 months, had persistently high anti-IL-17A levels. Patient 2 was referred to our center at the age of 4 years, and unfortunately no serum samples were available for antibody testing before this age. Her brother, who had no signs of CMC (Patient 1), had low or undetectable levels of anti-IL-17A antibodies over the period of observation. Patient 3, with no signs of mucosal or skin candidiasis, also had low or undetectable anti-IL-17A antibody levels over a period of 4 years. Similarly, OD values for anti-IL-17A antibodies in Patients P4, P5, and P6, who had candidiasis of a single fingernail beginning at the age of 17 years (P5), angular cheilitis of unknown etiology since the age of 21 years (P6) or three short episodes of mucosal candidial disease during the first 3 years of life (P6), were negligible, as shown by comparison with control values and with those for Patient 2, who had prolonged, severe CMC. OD values for IL-17F were intermediate, between those obtained for anti-IL-17A and anti-IL-22 antibodies, and were not correlated with the severity of CMC. Experiments were performed to measure OD values of anti-IL-17A and anti-IL-22 in sera of Patients 1, 2 and 3 by using varying serum concentrations. Data of these experiments confirmed the differences in OD values found with a single dilution of sera.

Figure 5A



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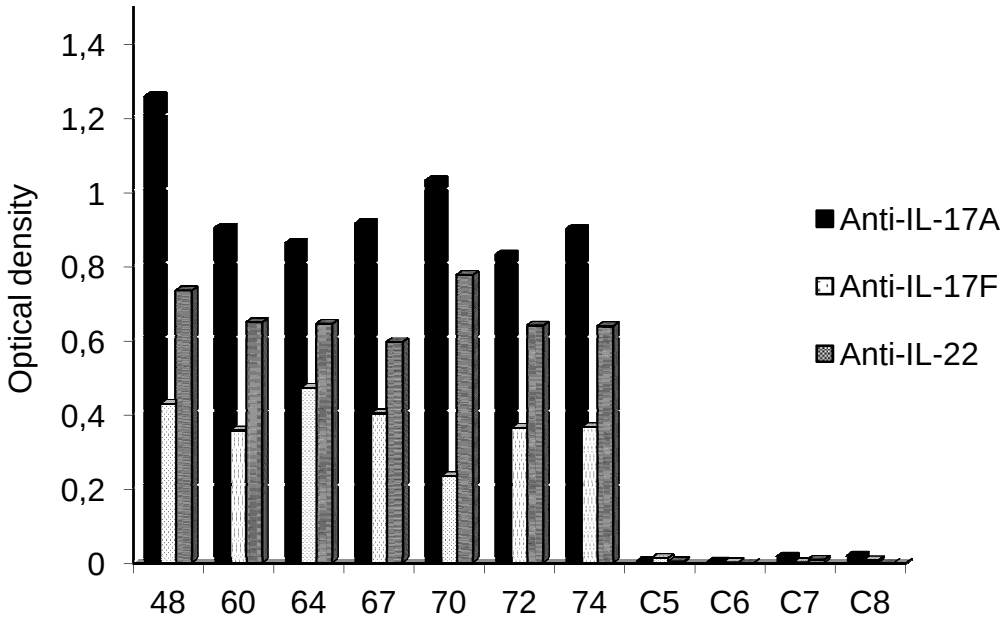

Age of mesurement (months)

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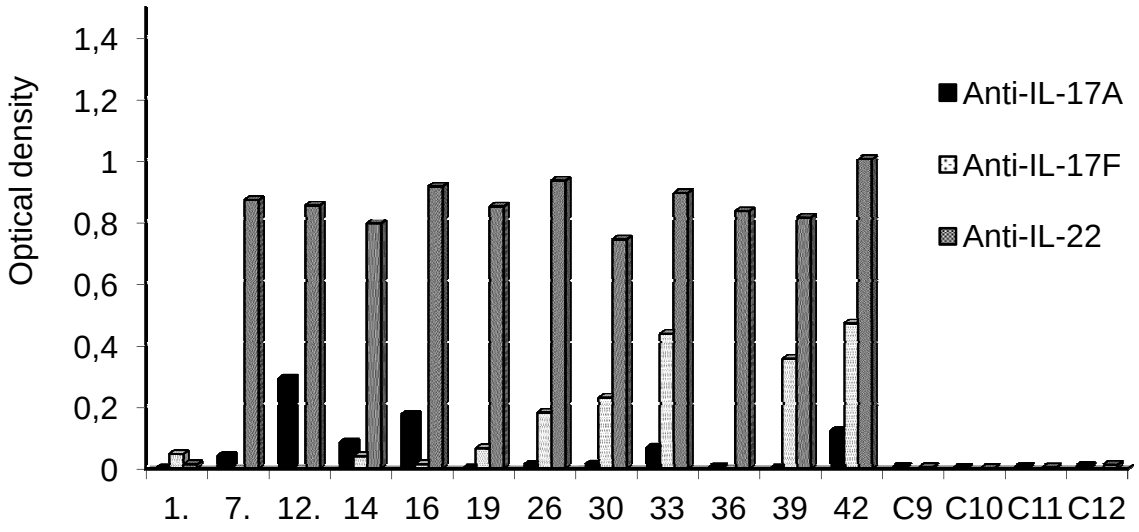

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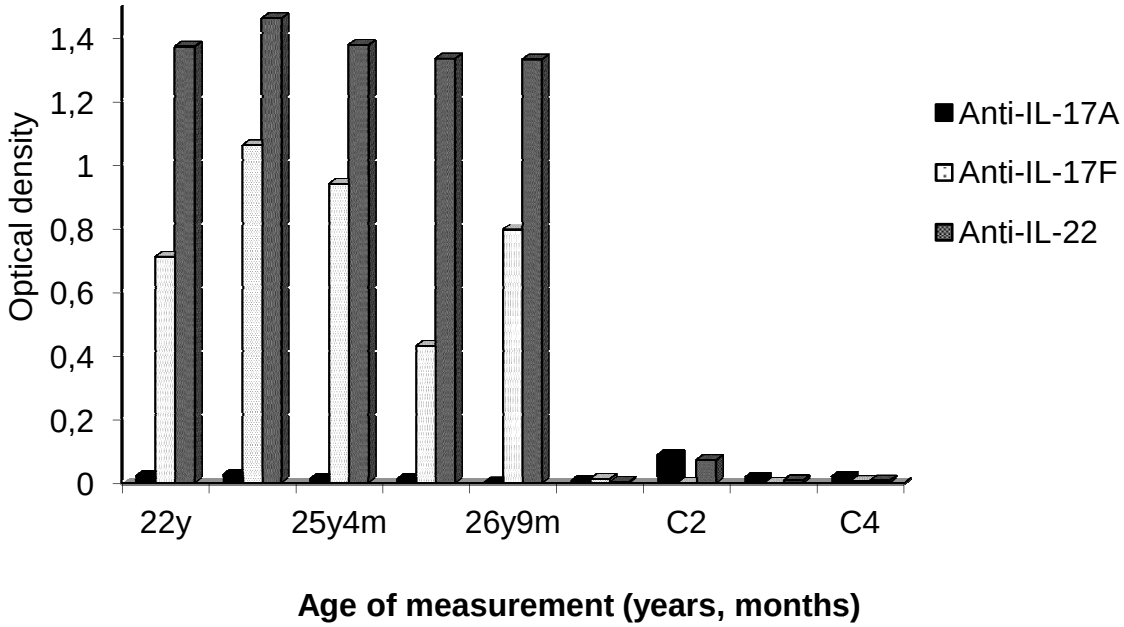
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Anti-IL-17F
Anti-IL-22
Age of measurement (years, months)

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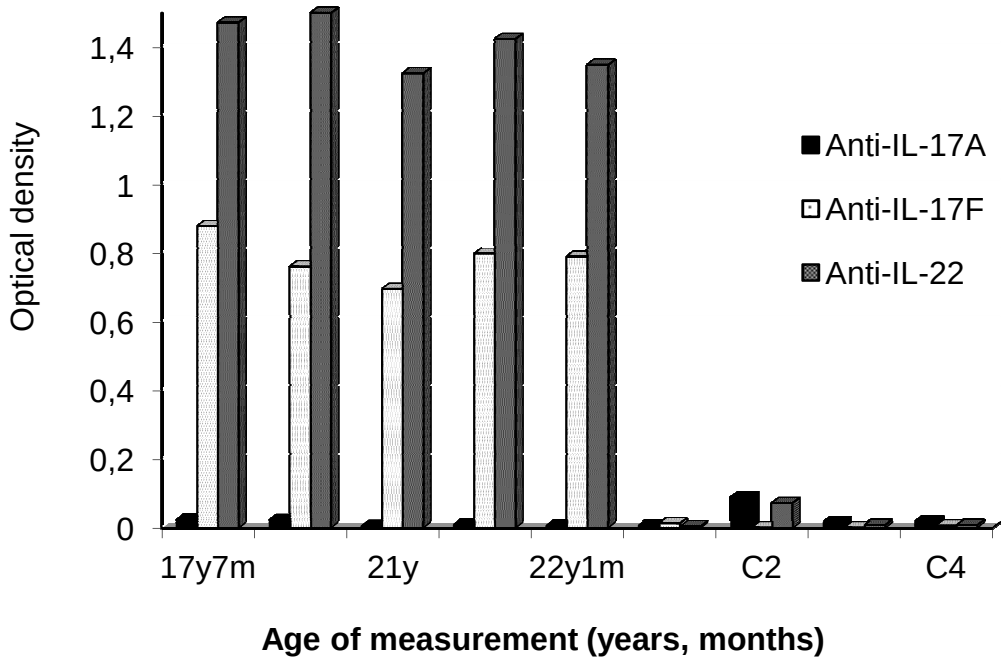
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Anti-IL-17F
Anti-IL-22
Age of measurement (years, months)

F

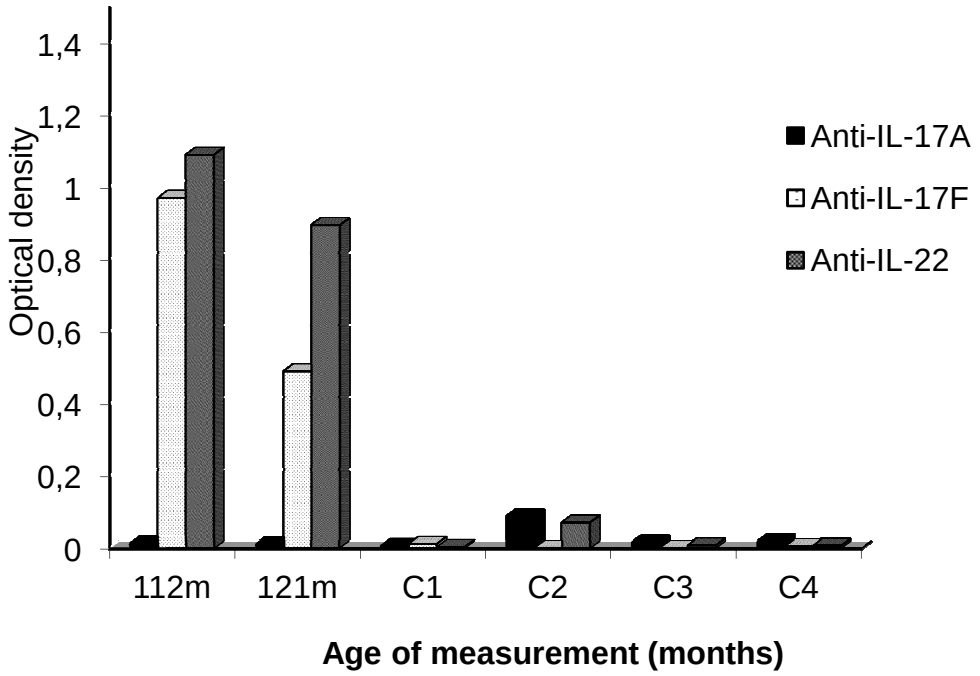
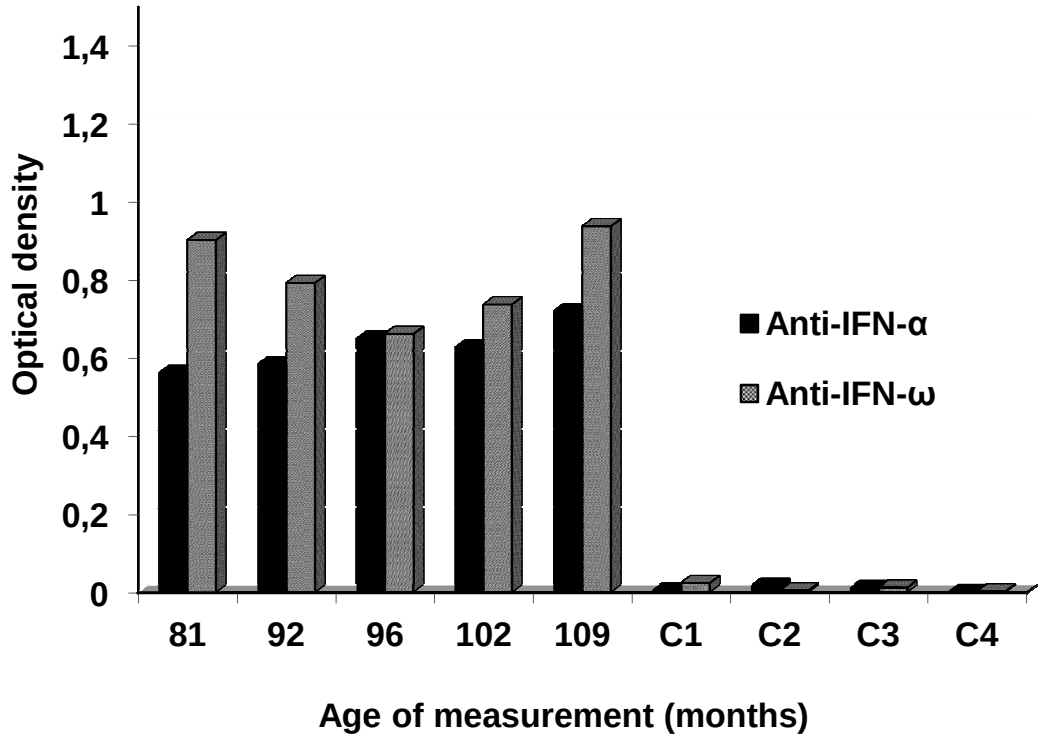


Figure 5. Autoantibodies to IL-17A, IL-17F and IL-22 in sera of APS I patients (next page). In Patients 1, 3, 4, 5, and 6 (Fig. 5A and 5D-4F) with no candidiasis or with mild and transient CMC, levels of anti-IL-17A autoantibodies, as measured by OD values, were low or undetectable, contrasting with the persistently high levels of anti-IL-17A antibodies in Patient 2 (Fig. 5B), who presented with severe and persistent CMC from the age of 18 months. Anti-IL-22 antibody levels were high and similar in all patients (Fig. 5A-5F). We obtained similarly high OD values for anti-IL-17F antibody levels in the sera of APS-1 patients with severe oral and therapy-resistant nail candidiasis and intermittent angular cheilitis (Fig. 5B), patients with mild intermittent angular cheilitis or mild, isolated nail candidiasis (Fig. 5D, E) and patients without candidiasis (Fig. 5 A,C,F).OD values for anti-IL-17F autoantibodies were higher than anti-IL-17A values for the sera of all patients without severe CMC (Fig. 5A, 5C, 5D, 5E, 5F), but were lower in the serum of Patient 2 with severe candidiasis (Fig. 5B).

4.3.1.2. Autoantibodies against type I interferons

OD values for antibodies against IFN-α and IFN-ω were measured in three patients, and were high and similar in Patients 1 and 3. In Patient 2, with CMC, the low anti IFN-ω antibody level contrasted with the high level of anti-IFN-α antibodies. In Patient 3, who was followed up from early infancy, we observed a gradual increase in the levels of IFN-α and IFN-ω antibodies until the age of 14 months. Autoantibody production began at the age of 7 months and sustained increases in the levels of these antibodies resulted in high OD values later on.

Figure 6A



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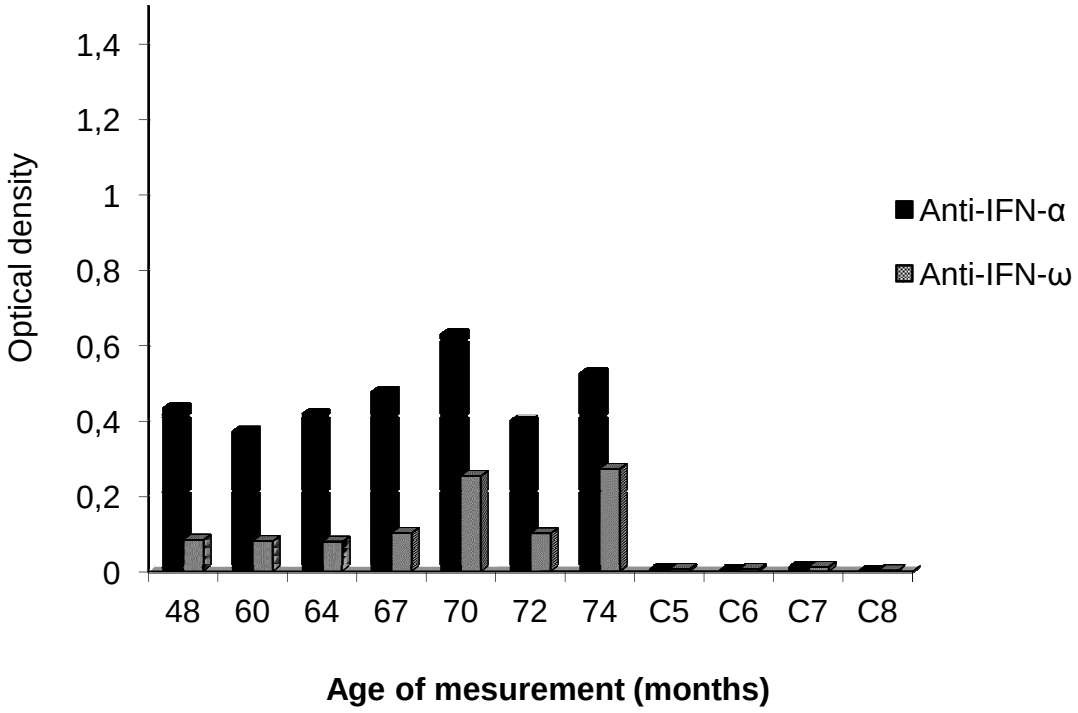
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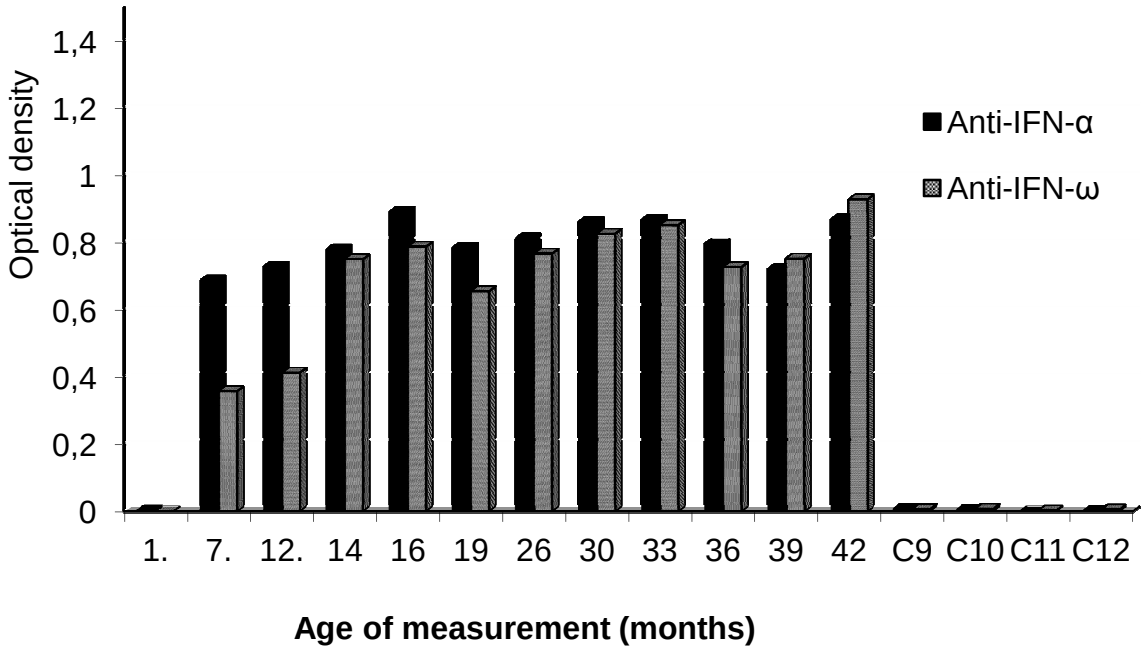
Age of mesurement (months)

C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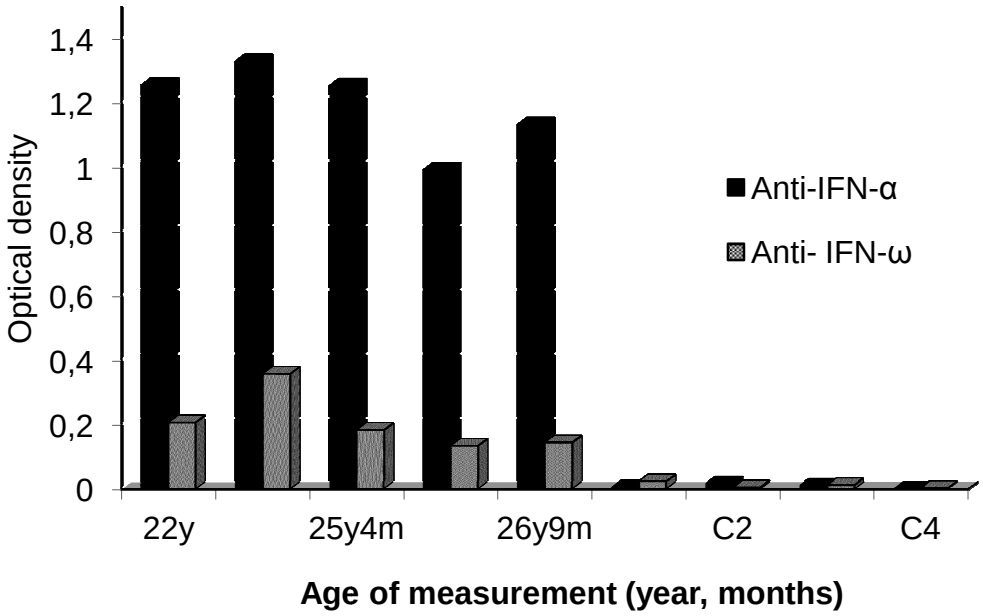
Age of measurement (months)

D



Age of measurement (year, months)

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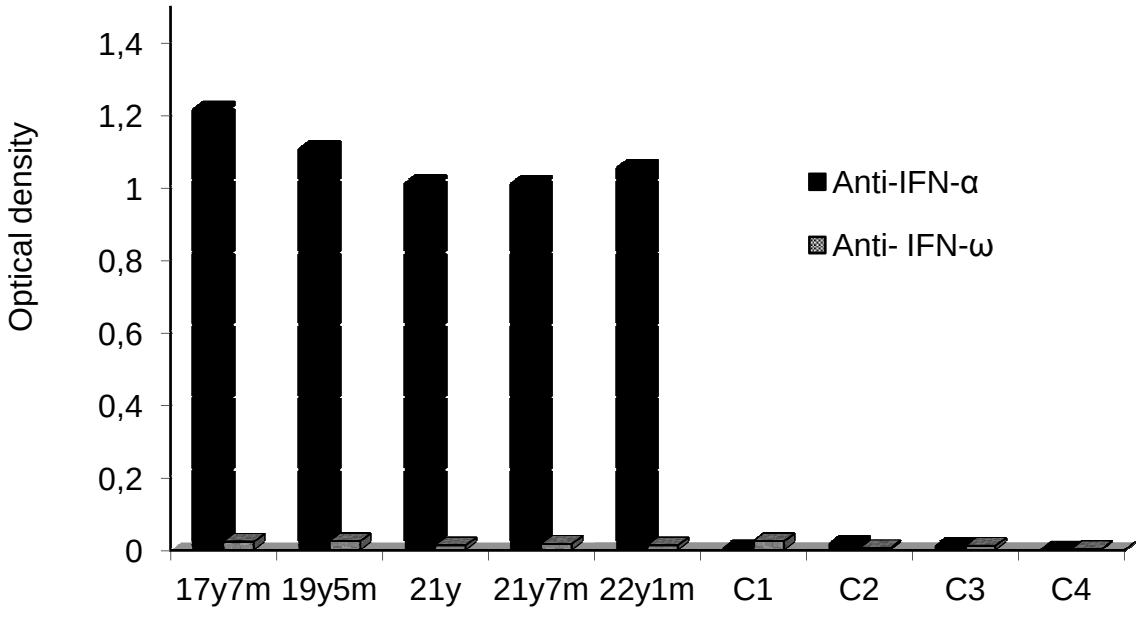


Age of measurement (year, months)

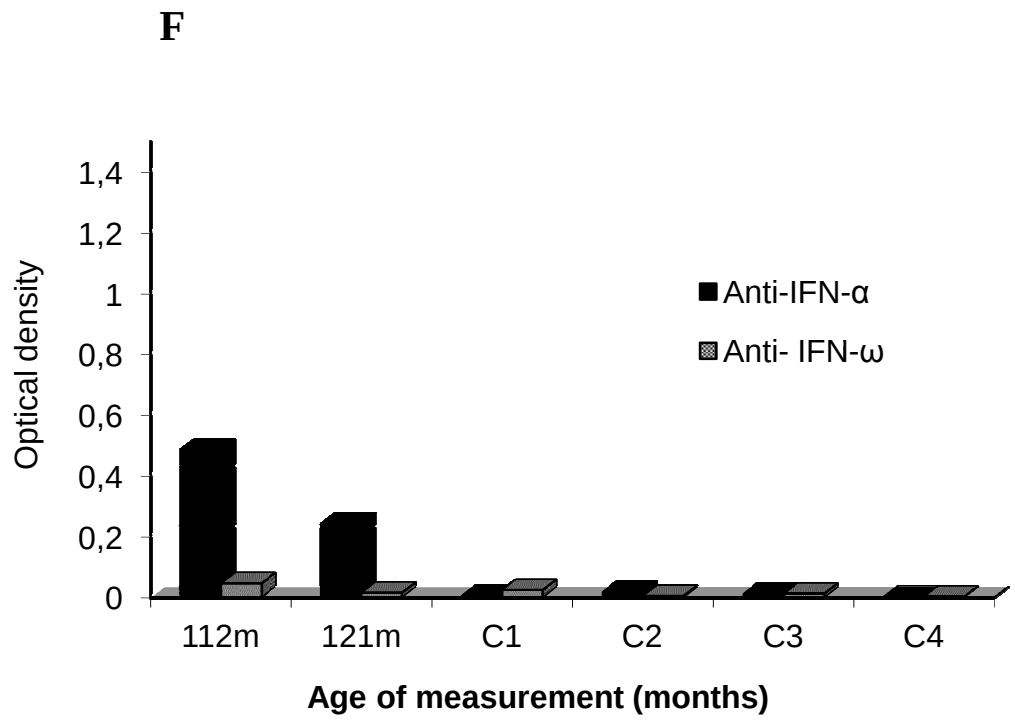


Figure 5. Anti-cytokin autoantibodies to type-1 interferons. In Patients 1 and 3 (Fig. 6A and C), the OD values for both anti-IFN-α and anti-IFN-ωantibodies were persistently high. Serum OD values for anti-IFN-α and anti-IFN-ωantibodies were relatively low in sera of Patient 2 and 6 (Fig. 6B, 6F), contrasting with the high anti-IL-17A and anti-IL-22 antibody levels of Patients 2. In patient 4 and 5 (Fig. 6E, F), we measured persistently high anti-IFN-α ODs, but interestingly low anti-cytokine antibodies to IFN-ω.

4.3.2. Immuglobulin Gsubclass analysis of interferon-α2 and interleukin-22 antibodies

Kärner et al have recently reported high proportions of IgG4, in addition to IgG1, among the autoantibodies against IFN-α2 and IL-22 in APS-I patients. IgG1 was again prevalent in all the autoantibodies detected in 6 of the present subjects (Kärner et al. 2013). Surprisingly, against IL-22, there were already substantial proportions of IgG4 at 7 months in P3 (with traces of IgG2 and IgG3); also as early as 42 months in P13, where they exceeded those of IgG1. Against IFN-α2, IgG4 again constituted a substantial proportion in P3 (at 30 months) and P10 (30 months), but not in the two other seropositive patients (Table 4).

Table 4. Immunglobulin G subclasses of autoantibodies against IL-22 and IFN-α2

	IgG1	IgG2	IgG3	IgG4	Ratio IgG4:IgG1
IL-22 signals in binding assay × 10^{-3}					
P3 7 m	94.21	6.01	1.44	17.61	0.19
P325 m	48.43	2.11	1.33	83.03	1.71
P330 m	4.76	0.26	0.15	7.24	1.52
P196 m	0.55	0.23	0.13	0.71	1.28
P19 60 m	0.14	0.32	0.12	0.15	1.12
P10 36 m	7.86	0.27	0.16	0.75	0.1
P1342 m	2.09	0.40	0.13	10.24	4.89
P1372 m	2.24	0.36	0.14	11.52	5.15
P1548 m	5.78	0.19	0.19	0.28	0.05
P1560 m	8.04	0.20	0.17	0.39	0.05
IFNα signals in binding assay× 10^{-3}					
P3 30 m	2.25	0.18	0.12	1.47	0.65
P19 6 m	1.60	0.16	0.13	0.14	0.09
P8 60 m	0.11	0.15	0.11	0.10	0.94
P10 36 m	2.97	0.19	0.17	1.28	0.43
P13 42 m	0.11	0.11	0.11	0.12	1.05
P1372 m	0.18	0.13	0.11	0.12	0.66
P15 48 m	0.53	0.14	0.16	0.14	0.26
P1560 m	2.24	0.12	0.12	0.11	0.05

We found high proportion of IgG1 and IgG4 among the IL-22 and IFN-α anti-cytokine autoantibodies, in conjunction with the published literature.

4.3.3. Organ-specific autoantibodies

We detected high levels of autoantibodies against adrenal cortex or steroid hydroxylases in 13 subjects—including all of the 11 with AD; they preceded its onset by 2–3 years in P15 and P13 and by over 18 years in P11; they were also stronglypositive in 3 of the other subjectswho do not yet have AD. Antibodies to NALP-5 were found in 3 of the 6 subjects who had HP (among a total of 10 tested); in P8, they preceded its onset by 6 months. Antibodies to ovaries, or specifically to SCC, were detected in 3 of the 12 subjects tested (weakly in one), including P11 who has clinical ovarian failure (OF). Autoantibodies against pancreatic islets or GAD-65, and thyroid tissue or antigens, were also tested in all subjects. They were detected against islets in P11, over 31 years before onset of diabetes; also against GAD-65 in 4 others who do not yet have diabetes. TPO and Tg antibodies were found in one of two patients with thyroid disease (P14) 5 years before its onset. TPH antibodies were detected in 2 patients, AADC in 5 and TH in 3, but they are too few to correlate with manifestations. Overall, endocrine autoantibodies were found surprisingly early in P19, P13 and P2 (at 6, 24 and 48 months respectively), but only later in P10, P14 and P15.

4.4. Release of cytokines by *Candida*-exposed blood cells

We measured the release of IL-17A, IL-17F, IL-22 and TNF-αby PBMCs after 5 days of stimulation with heat-killed *Candida*. PBMCs from patients released negligible or small amounts of anti-cytokine antibodies on exposure to *Candida*, compared to results of healthy controls (C on Figure 7, means values of controls), who presented high levels of IL-17A, IL-17F and IL-22, and higher levels of TNF-α.These data suggest an impaired Th17-typeresponse to Candidain patients with APS. To prove the possibility of a broader defect of cytokine release by *Candida*-exposed PBMCs, we measured concentrations of TNF-α in supernatants as positive control. The data of these control experiments showed that release of TNF-α by cells from patients and controls were comparable (Figure 7). These results suggest an isolated impairment of Th17 cell functions and cytokine secretion.

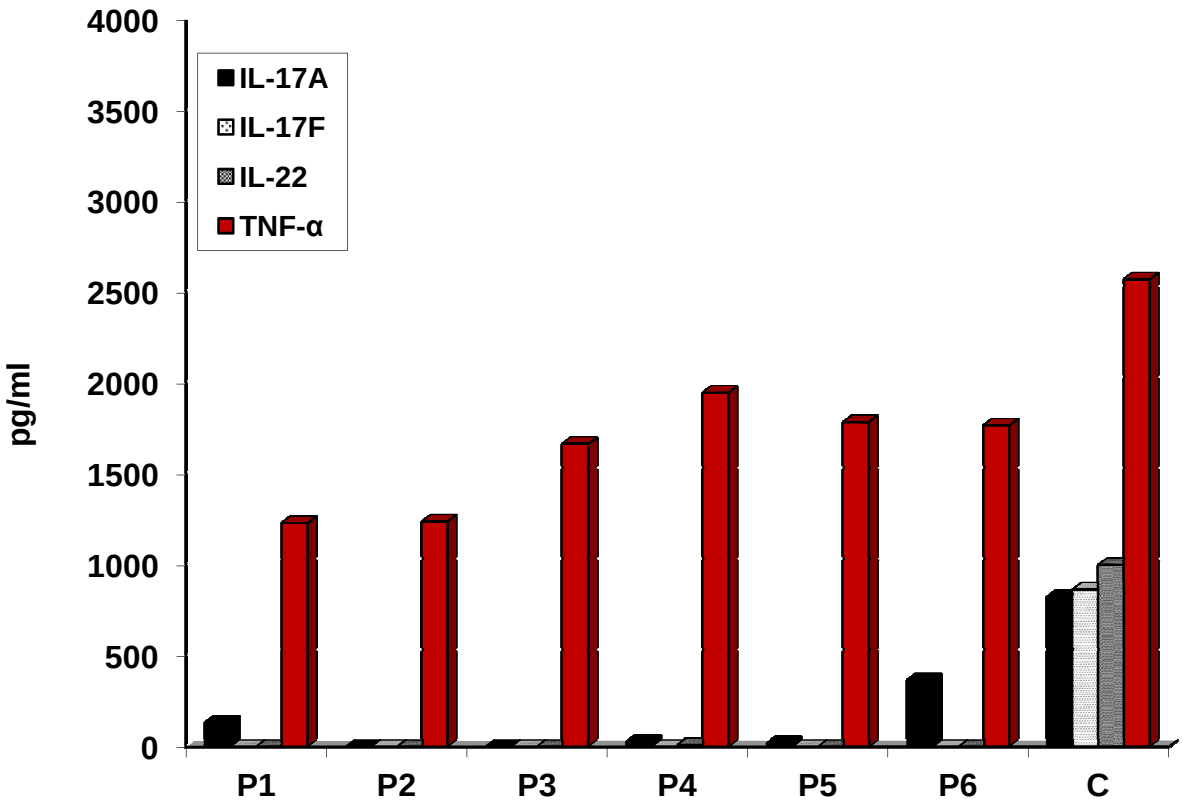
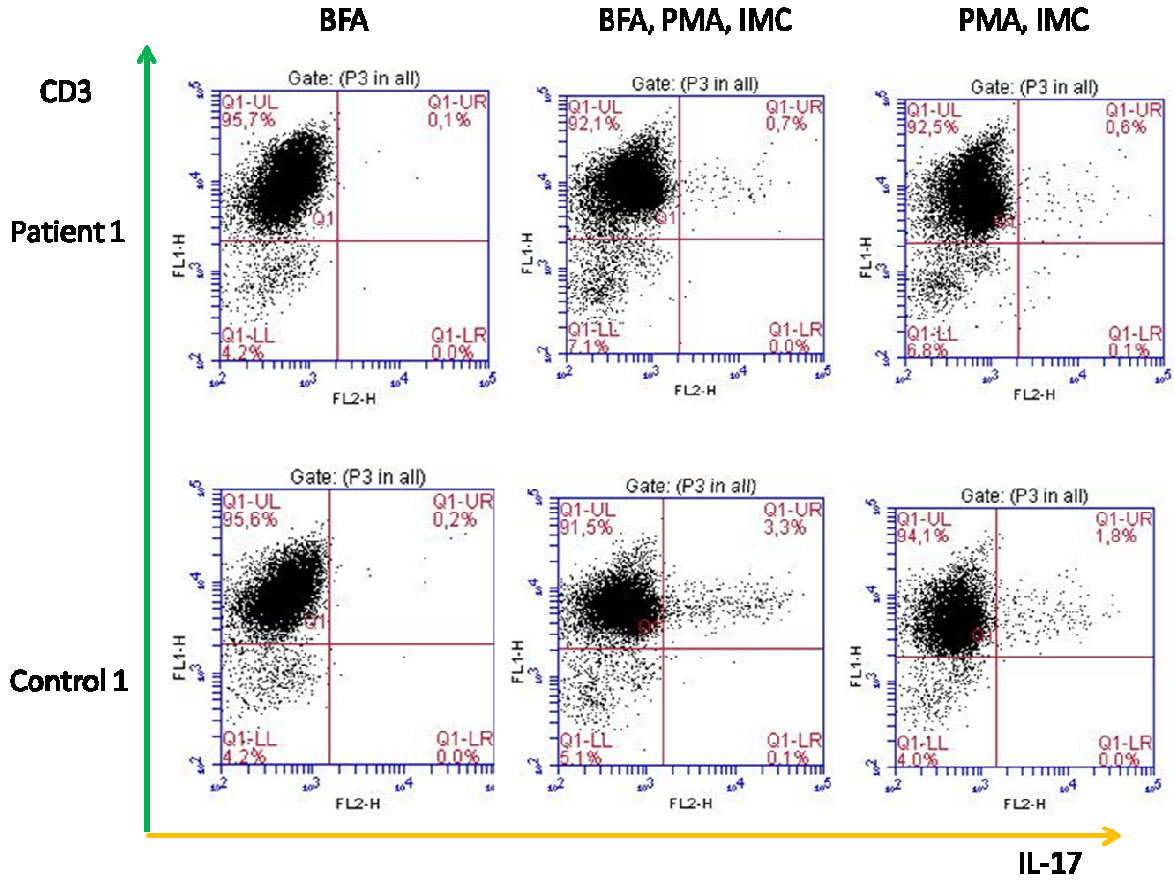


Figure 7. IL-17A, IL-17F, IL-22 and TNF-α secretion of APS I patients. Peripheral blood mononuclear cells were stimulated with heat-killed *Candida* blastoconidia for 5 days at 37^{o}C. Cells were then centrifuged and cytokine concentration was measured in the supernatants by ELISA, as described in Methods. We found impaired secretion of IL-17A, IL-17F, and IL-22 by cells from APS I patients with or without severe candidiasis, as shown by comparison with cells from healthy controls. Secretion of TNF-α was unaffected, and comparable in patients and healthy controls.

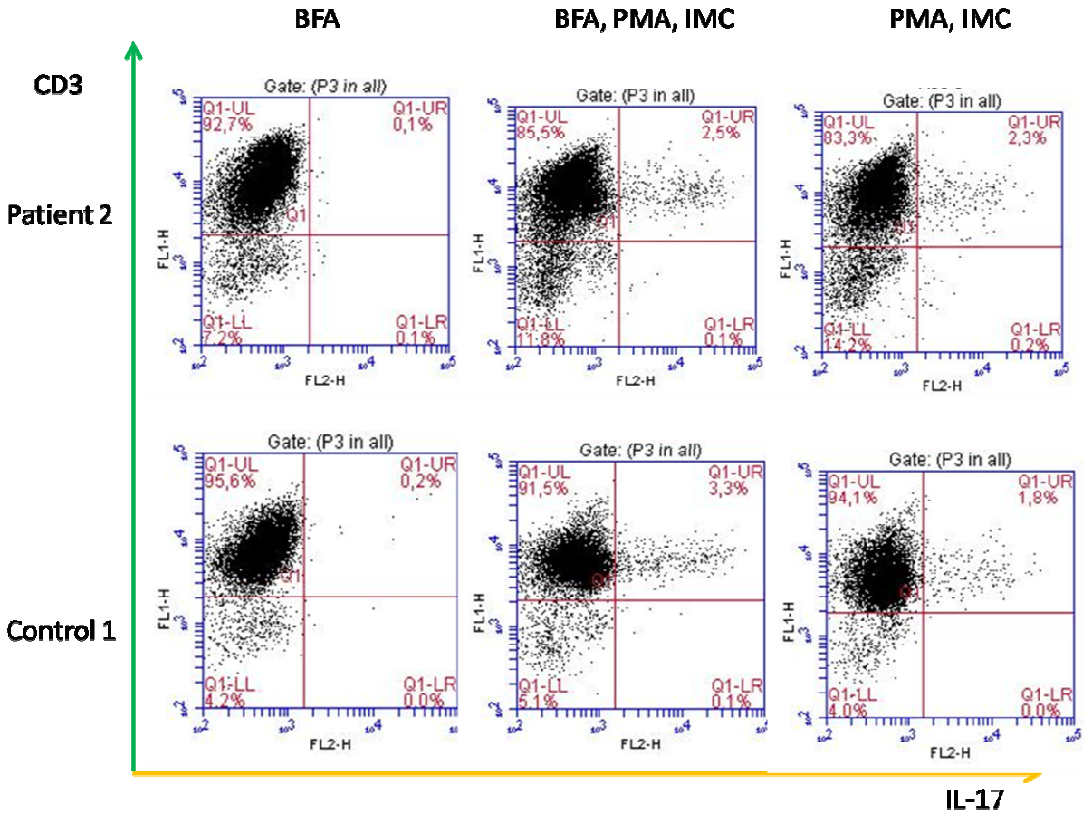
4.5. Interleukin-17+/interleukin-22+ T helper cell differetiation of APS I patients

PBMCs of APS I patients and healthy controls were isolated and treated with IL-17-inducing cytokines as described above. Flow cytometry was used to determine the percentages of CD3+ T cells and CD4+ T cells, producing cytoplasmic IL-17 or IL-22 (Figure 7,8 and Figure 9). Before marker analysis, the cells were treated with phorbol-12-myristate 13-acetat (PMA) -an activating factor of protein kinase C-,ionomycin (IMC) -an ionophore- and Brefeldin A – "Golgi-plug", which inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus- for 6 h. Consistent with the cytokine-release data, APS I patient displayed a mild impaired development of IL-17-producing (Figure 8, 9) and IL-22 (Figure 10) -producing T helper cells, compared with healthy controls. Within Brefeldin A treated, unstimulated cell population; IL-17 and IL-22 secreting cellpercents were similarly low in patients and healthy controls. After PMA stimulation, we found moderately decreased cell persents in APS I patients, involved the CD3+IL-17+ (Figure 8), CD4+IL-17+ (Figure 9) and CD4+IL-22+ (Figure 10) cells, compared with healty controls.

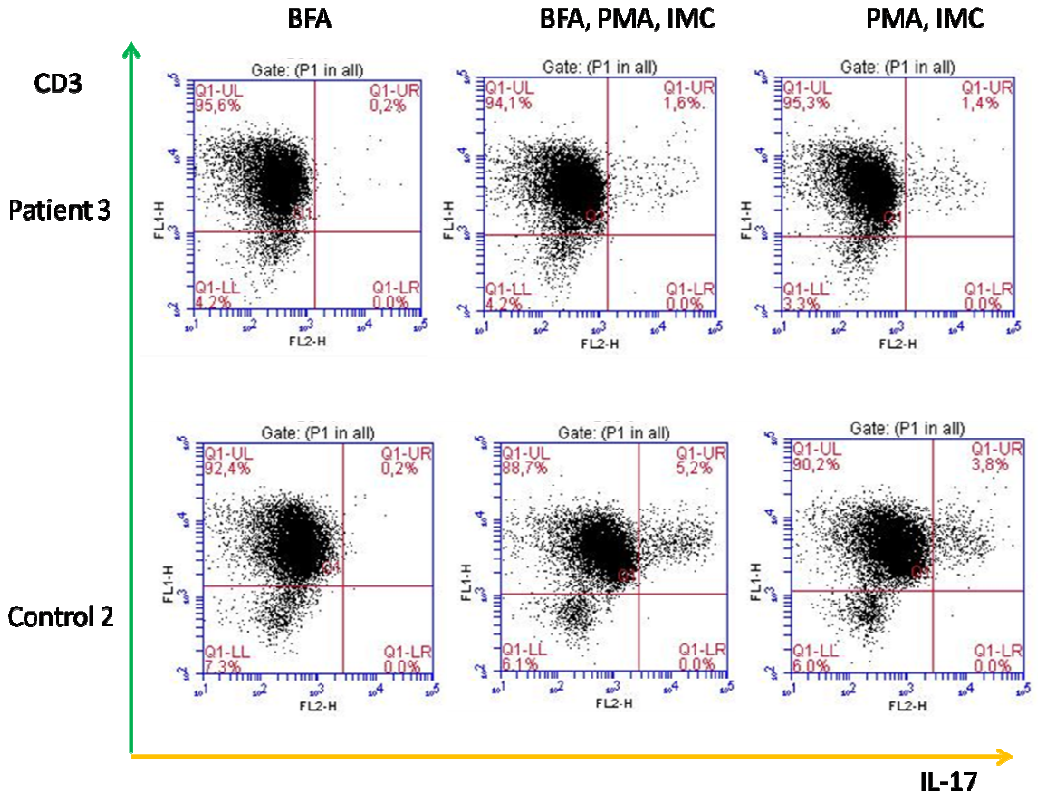
Figure 8A.



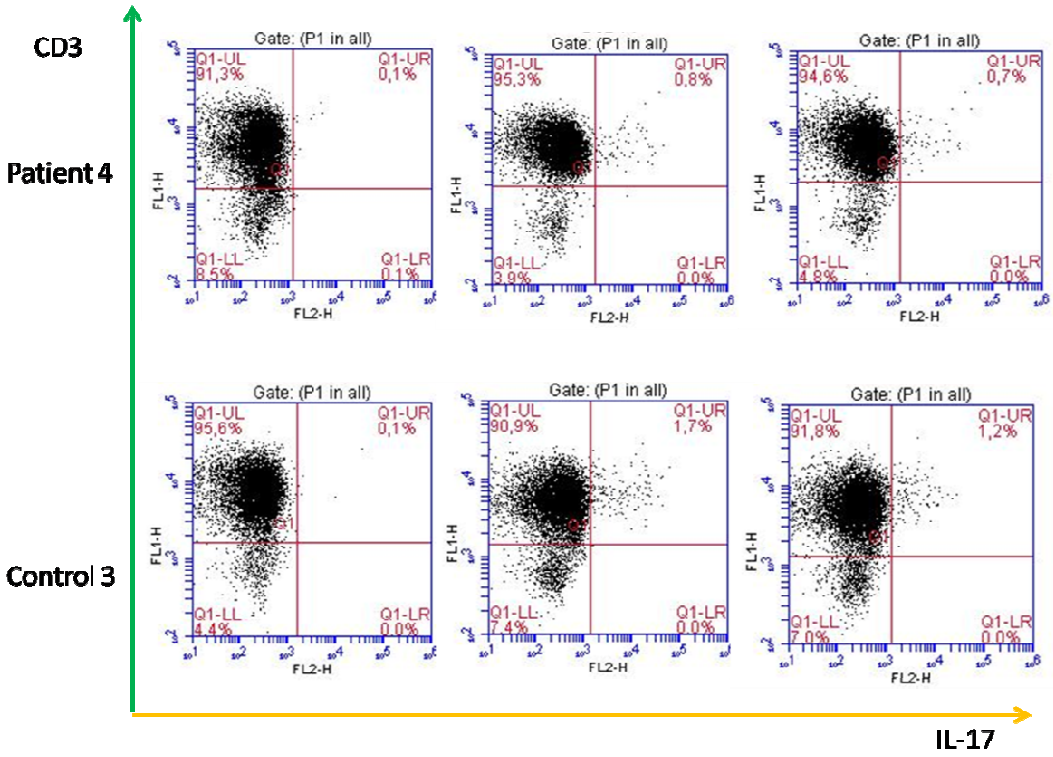
B


BFA
BFA, PMA, IMC
PMA, IMC
CD3
Patient 2
Control 1
IL-17
Gate: (P3 in all)
Q1-UL 92,7%
Q1-UR 0,1%
Q1-LL 7,2%
Q1-LR 0,1%
Q1-UL 85,5%
Q1-UR 2,5%
Q1-LL 11,8%
Q1-LR 0,1%
Q1-UL 83,3%
Q1-UR 2,3%
Q1-LL 14,2%
Q1-LR 0,2%
Q1-UL 95,6%
Q1-UR 0,2%
Q1-LL 4,2%
Q1-LR 0,0%
Q1-UL 91,5%
Q1-UR 3,3%
Q1-LL 5,1%
Q1-LR 0,1%
Q1-UL 94,1%
Q1-UR 1,8%
Q1-LL 4,0%
Q1-LR 0,0%
FL1-H
FL2-H

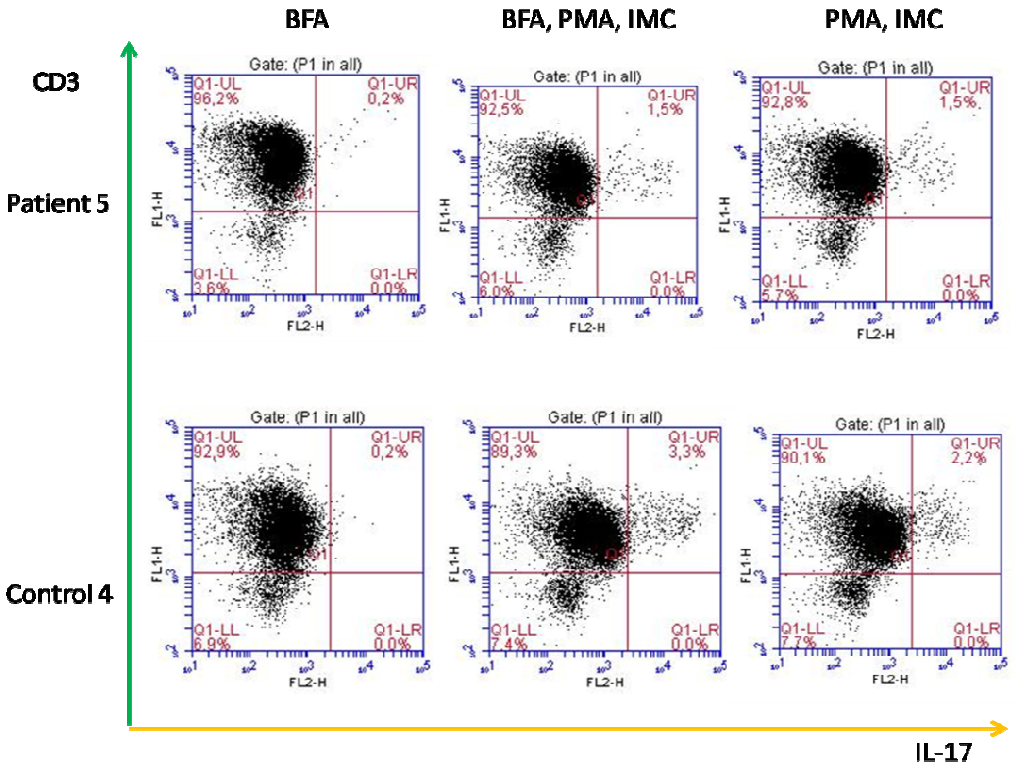
C


BFA
BFA, PMA, IMC
PMA, IMC
CD3
Patient 3
Control 2
IL-17
Gate: (P1 in all)
Q1-UL 95,6%
Q1-UR 0,2%
Q1-LL 4,2%
Q1-LR 0,0%
Q1-UL 94,1%
Q1-UR 1,6%
Q1-LL 4,2%
Q1-LR 0,0%
Q1-UL 95,3%
Q1-UR 1,4%
Q1-LL 3,3%
Q1-LR 0,0%
Q1-UL 92,4%
Q1-UR 0,2%
Q1-LL 7,3%
Q1-LR 0,0%
Q1-UL 88,7%
Q1-UR 5,2%
Q1-LL 6,1%
Q1-LR 0,0%
Q1-UL 90,2%
Q1-UR 3,8%
Q1-LL 6,0%
Q1-LR 0,0%
FL1-H
FL2-H

D


CD3
Patient 4
Control 3
IL-17
Gate: (P1 in all)
Q1-UL 91,3%
Q1-UR 0,1%
Q1-LL 8,5%
Q1-LR 0,1%
Gate: (P1 in all)
Q1-UL 95,3%
Q1-UR 0,8%
Q1-LL 3,9%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 94,6%
Q1-UR 0,7%
Q1-LL 4,8%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 95,6%
Q1-UR 0,1%
Q1-LL 4,4%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 90,9%
Q1-UR 1,7%
Q1-LL 7,4%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 91,8%
Q1-UR 1,2%
Q1-LL 7,0%
Q1-LR 0,0%
FL1-H
FL2-H

E


BFA
BFA, PMA, IMC
PMA, IMC
CD3
Patient 5
Control 4
IL-17
Gate: (P1 in all)
Q1-UL 96,2%
Q1-UR 0,2%
Q1-LL 3,6%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 92,5%
Q1-UR 1,5%
Q1-LL 6,0%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 92,8%
Q1-UR 1,5%
Q1-LL 5,7%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 92,9%
Q1-UR 0,2%
Q1-LL 6,9%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 89,3%
Q1-UR 3,3%
Q1-LL 7,4%
Q1-LR 0,0%
Gate: (P1 in all)
Q1-UL 90,1%
Q1-UR 2,2%
Q1-LL 7,7%
Q1-LR 0,0%
FL1-H
FL2-H

F

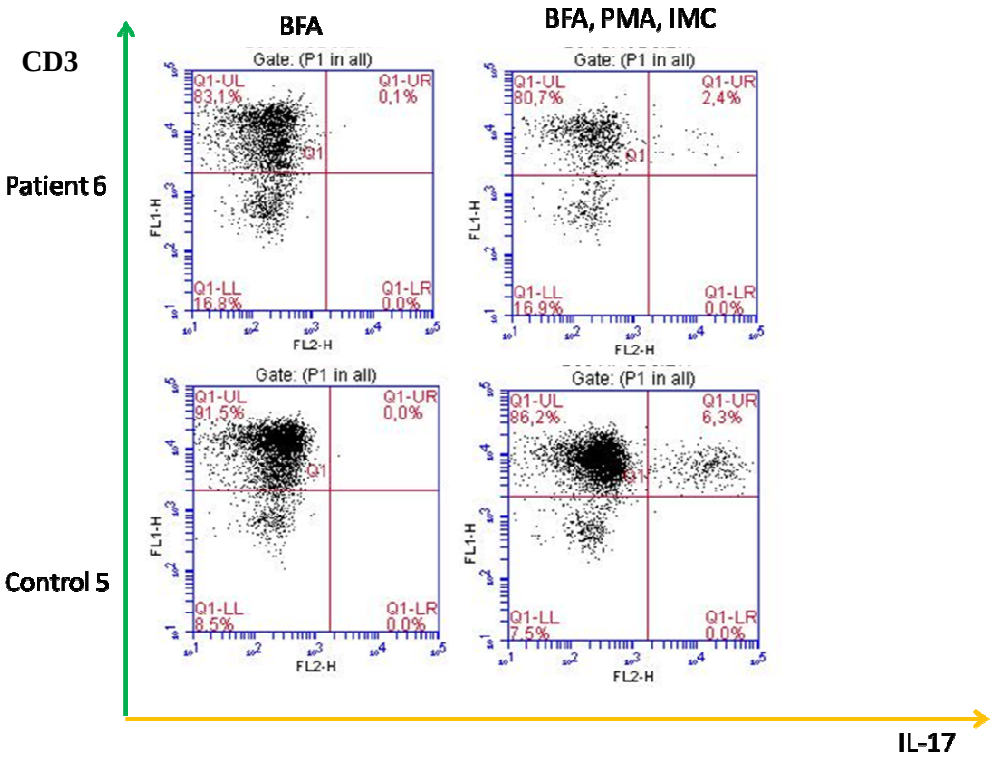


Figure 8A-7F. DevelopmentofCD3+IL-17 producing cells in APS I patients and healthy controls. Percentages of CD3+IL-17+ cells, as determined by flow cytometry, after incubation with combinations of BFA (brefeldin A), PMA (phorbol 12-myristate 13-acetate) and IMC (ionomycin). Patients identification numbers are shown on the Y axis. We found uniformly lower IL-17 and IL-22 producing cell percentages in APS I patients compared with cell percentages of healthy controls (Fig 8A-F).

To investigate the possible impairment of secretion mechanisms, we performed the population analysis without secretion inhibitor (brefeldin A), to. We measured significant difference between results of the brefeldin A-inhibited and the untreated cells of healthy controls. Without secretioninhibitior, healthy controls presented lower IL-17+ and IL-22+ cell persents after PMA stimulation, as a normal consequence of the lower intracellular cytokine concentrations without inhibitor. In contrast, we found similarly percents of CD3+IL-17+ (Figure 8A-E), CD4+IL-17+ (Figure 9A-E) and CD4+IL-22 (Figure 10A-E) cells, with and without secretion inhibitor in patients with APS I. This results suggest that the decreased cytokine secretion of *Candida*-exposed blood cells may based on a functional impairment of the secretion mechanisms of Th17 cells, confirming the possibility of a broader defect of the anti-*Candida* response in APS I patients.

G

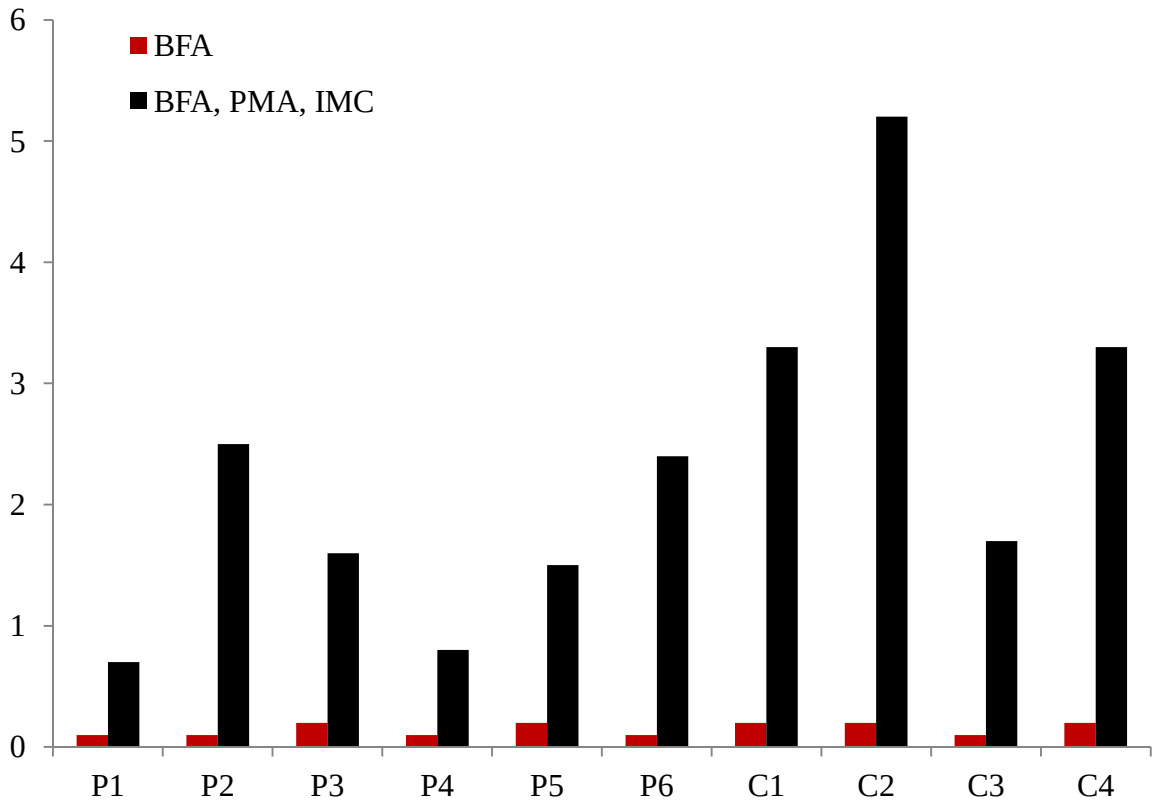
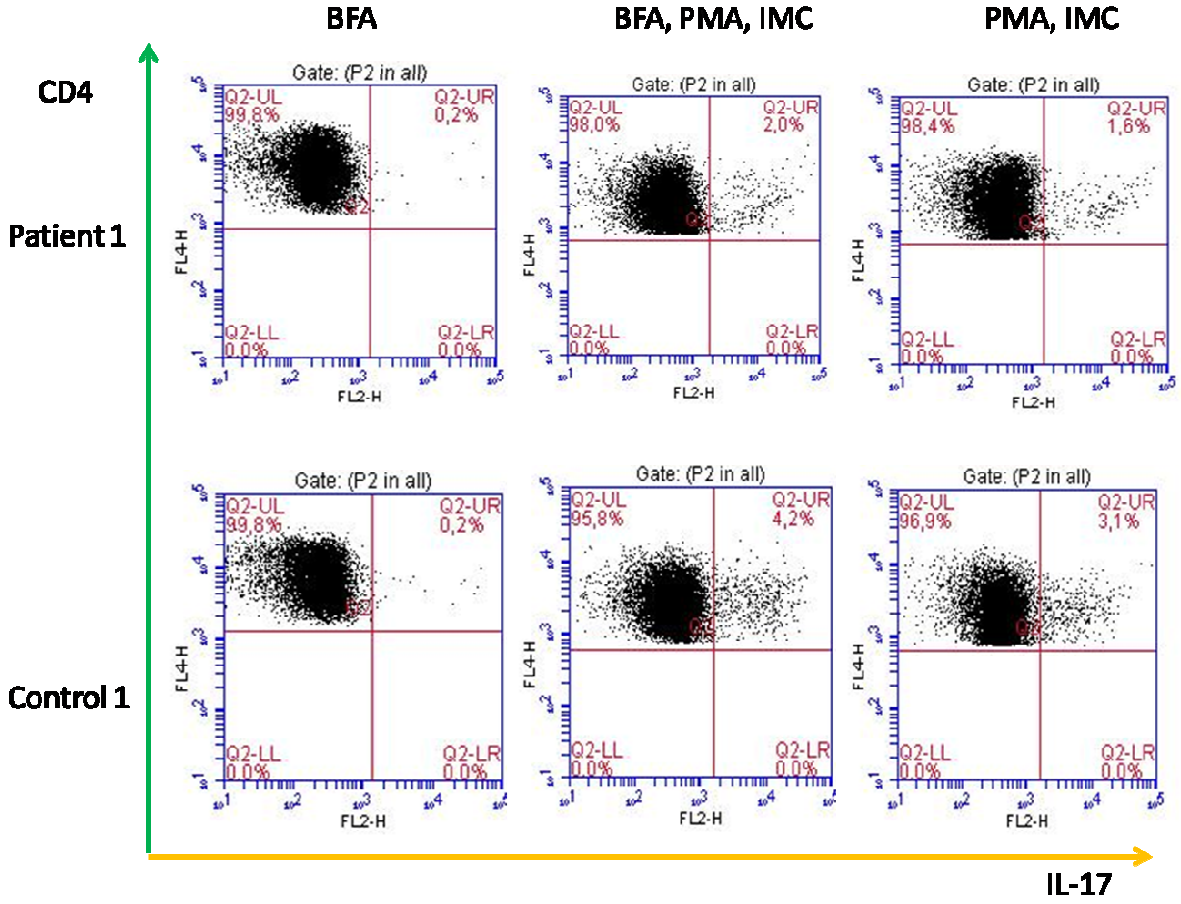
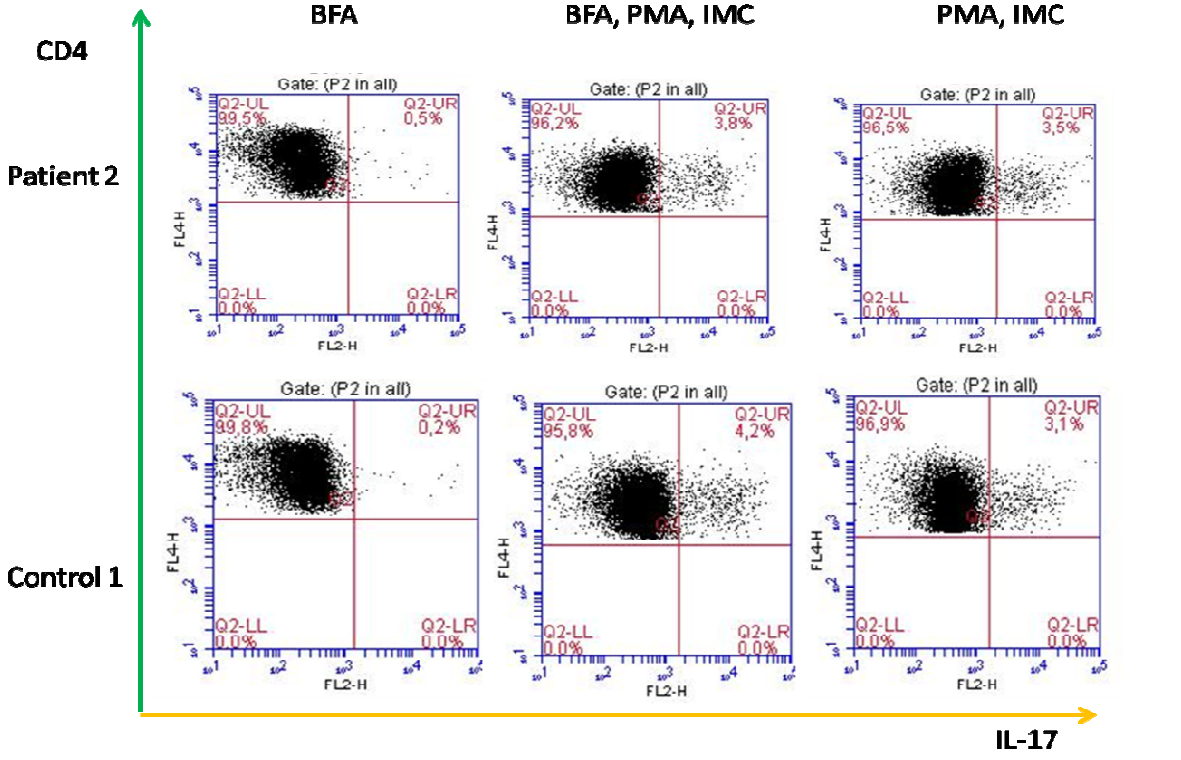


Figure 8G. Summary of CD3+IL-17+ T helper cell percentages of APS I patients and healthy controls. Red columns show CD3+IL-17+ cell percentages of APS I patients and healthy controls after treating with brefeldin A (BFA), without phorbol-myristate.acetate (PMA) stimulation. Black columns show CD3+IL-17+ cell percentages of APS I patients and healthy controls treated with BFA, PMA and ionomycin (IMC). We found decreased percentages of IL-17 secreting CD3+ cells in APS I patients after PMA stimulation, compared with healthy controls.

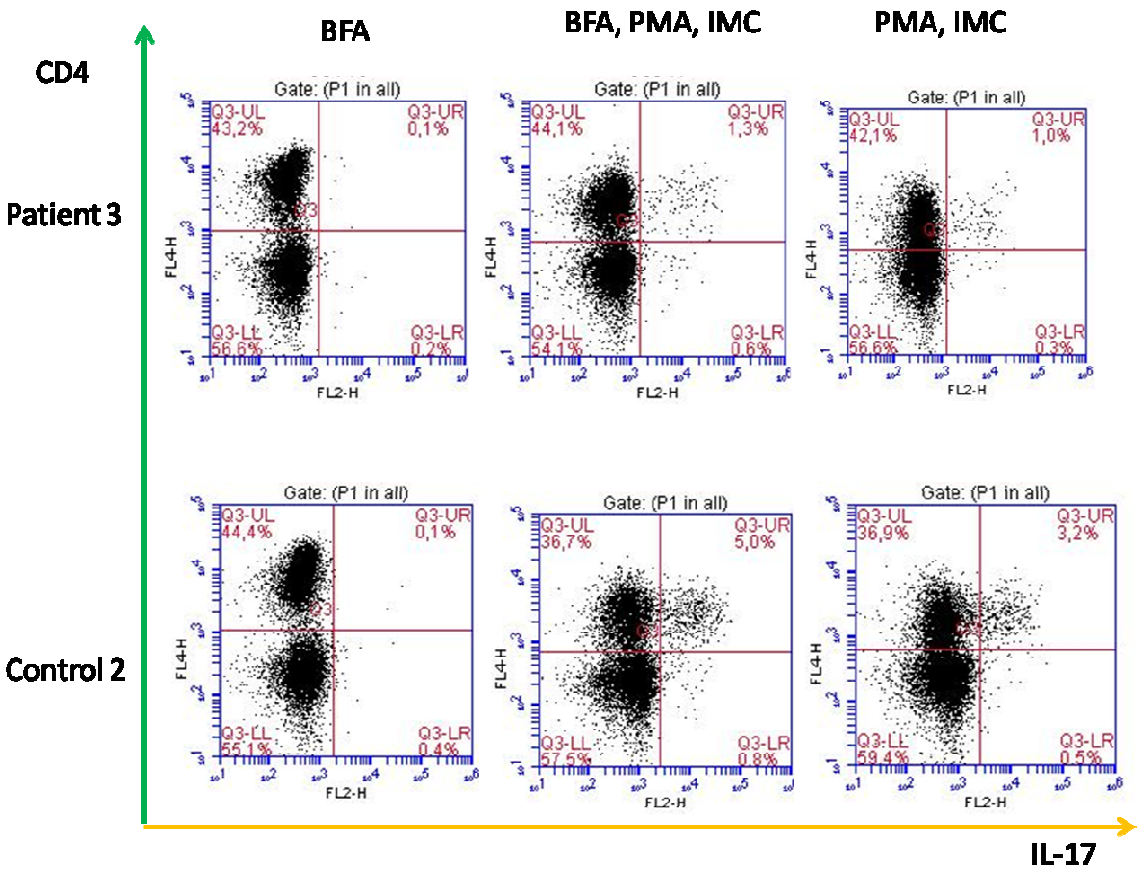
Figure 9A


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 1
Control 1
IL-17
Gate: (P2 in all)
Q2-UL 99,8%
Q2-UR 0,2%
Q2-LL 0,0%
Q2-LR 0,0%
Q2-UL 98,0%
Q2-UR 2,0%
Q2-UL 98,4%
Q2-UR 1,6%
Q2-UL 95,8%
Q2-UR 4,2%
Q2-UL 96,9%
Q2-UR 3,1%
FL4-H
FL2-H

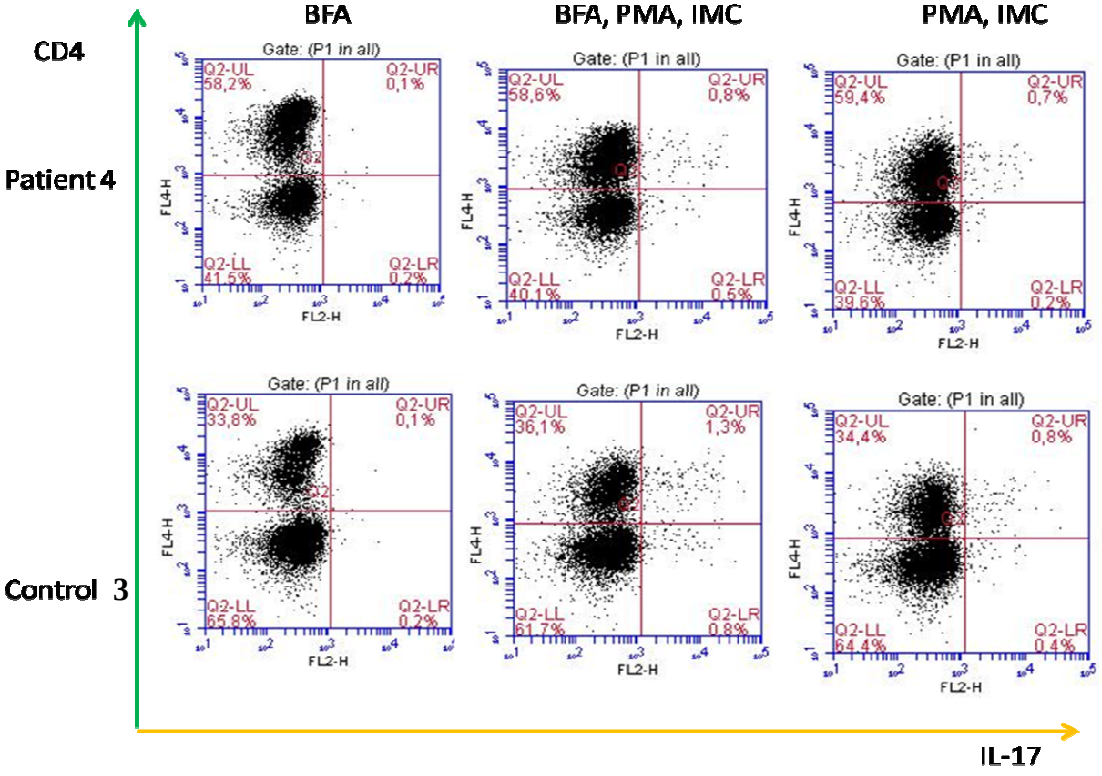
B


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 2
Control 1
IL-17
Gate: (P2 in all)
Q2-UL 99,5%
Q2-UR 0,5%
Q2-LL 0,0%
Q2-LR 0,0%
Q2-UL 96,2%
Q2-UR 3,8%
Q2-UL 96,5%
Q2-UR 3,5%
Q2-UL 99,8%
Q2-UR 0,2%
Q2-UL 95,8%
Q2-UR 4,2%
Q2-UL 96,9%
Q2-UR 3,1%
FL4-H
FL2-H

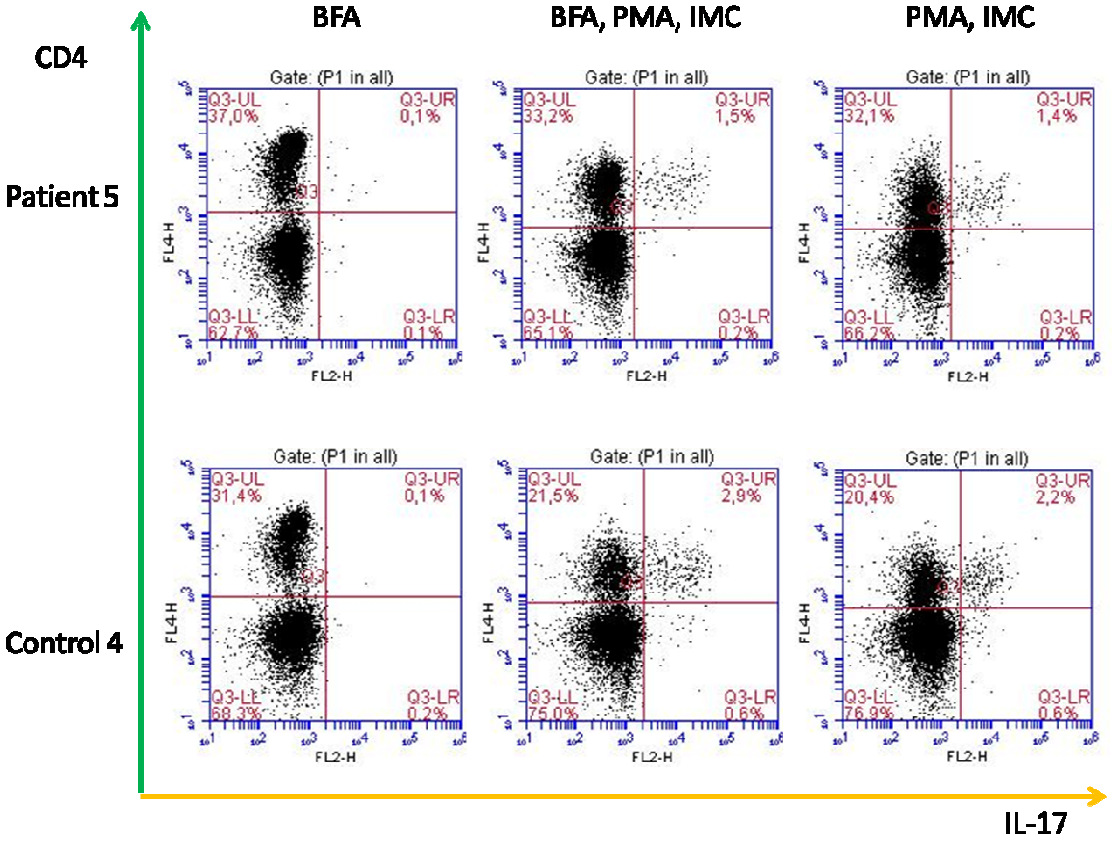
C


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 3
Control 2
Gate: (P1 in all)
Q3-UL 43,2%
Q3-UR 0,1%
Q3-LL 56,6%
Q3-LR 0,2%
Q3-UL 44,1%
Q3-UR 1,3%
Q3-LL 54,1%
Q3-LR 0,6%
Q3-UL 42,1%
Q3-UR 1,0%
Q3-LL 56,6%
Q3-LR 0,3%
Q3-UL 44,4%
Q3-UR 0,1%
Q3-LL 55,1%
Q3-LR 0,4%
Q3-UL 36,7%
Q3-UR 5,0%
Q3-LL 57,5%
Q3-LR 0,8%
Q3-UL 36,9%
Q3-UR 3,2%
Q3-LL 59,4%
Q3-LR 0,5%
FL4-H
FL2-H
IL-17

D


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 4
Control 3
Gate: (P1 in all)
Q2-UL 58,2%
Q2-UR 0,1%
Q2-LL 41,5%
Q2-LR 0,2%
Q2-UL 58,6%
Q2-UR 0,8%
Q2-LL 40,1%
Q2-LR 0,5%
Q2-UL 59,4%
Q2-UR 0,7%
Q2-LL 39,6%
Q2-LR 0,2%
Q2-UL 33,8%
Q2-UR 0,1%
Q2-LL 65,8%
Q2-LR 0,2%
Q2-UL 36,1%
Q2-UR 1,3%
Q2-LL 61,7%
Q2-LR 0,8%
Q2-UL 34,4%
Q2-UR 0,8%
Q2-LL 64,4%
Q2-LR 0,4%
FL4-H
FL2-H
IL-17

E


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 5
Control 4
IL-17
Gate: (P1 in all)
Q3-UL 37,0%
Q3-UR 0,1%
Q3-LL 62,7%
Q3-LR 0,1%
Q3-UL 33,2%
Q3-UR 1,5%
Q3-LL 65,1%
Q3-LR 0,2%
Q3-UL 32,1%
Q3-UR 1,4%
Q3-LL 66,2%
Q3-LR 0,2%
Q3-UL 31,4%
Q3-UR 0,1%
Q3-LL 68,3%
Q3-LR 0,2%
Q3-UL 21,5%
Q3-UR 2,9%
Q3-LL 75,0%
Q3-LR 0,6%
Q3-UL 20,4%
Q3-UR 2,2%
Q3-LL 76,9%
Q3-LR 0,6%
FL4-H
FL2-H

F

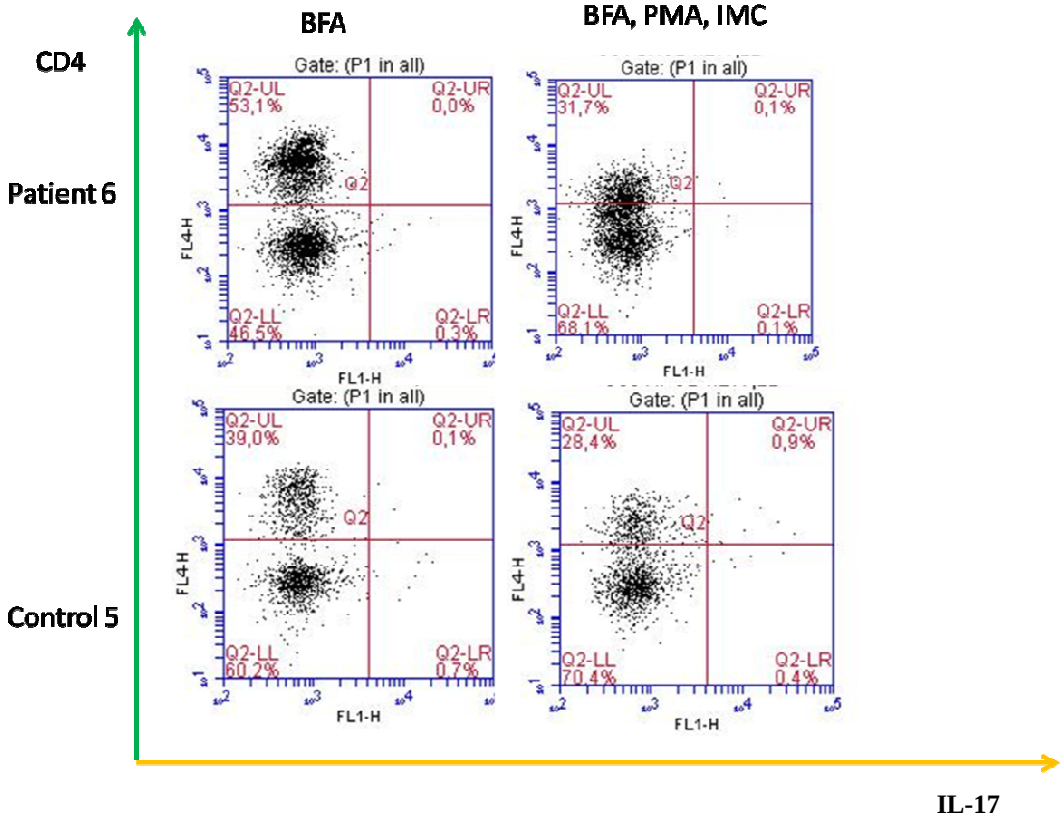

BFA
BFA, PMA, IMC
CD4
Patient 6
Control 5
IL-17
Gate: (P1 in all)
Q2-UL 53,1%
Q2-UR 0,0%
Q2-LL 46,5%
Q2-LR 0,3%
Q2-UL 31,7%
Q2-UR 0,1%
Q2-LL 68,1%
Q2-LR 0,1%
Q2-UL 39,0%
Q2-UR 0,1%
Q2-LL 60,2%
Q2-LR 0,7%
Q2-UL 28,4%
Q2-UR 0,9%
Q2-LL 70,4%
Q2-LR 0,4%
FL4-H
FL1-H

Figure 9A-F. CD4+IL-17 producing cells in APS I patientsand healthy controls. Percentages of CD4+IL-17+ cells, as determined by flow cytometry, after incubation with combinations of BFA (brefeldin A), PMA (phorbol 12-myristate 13-acetate) and IMC (ionomycin). Patients identification numbers are shown on the Y axis.We found uniformly lower IL-17 producing cell percentages in APS I patients compared with cell percentages of healthy controls. In patients, cell percentages were similar with (Fig. 9A-F BFA, PMA, IMC) and without (Fig. 9A-E PMA, IMC) BFA - an effective inhibitor of cytokine secretion and transport -, in contrast with cell percentages of healthy controls, which were significant lower without BFA, as a normal consequence of cytokine secretion and lower intracellular marked cytokine concentration. Results suggest that in patients the development of IL-17 producing T cells and the secretion of IL-17 is also impaired.

G

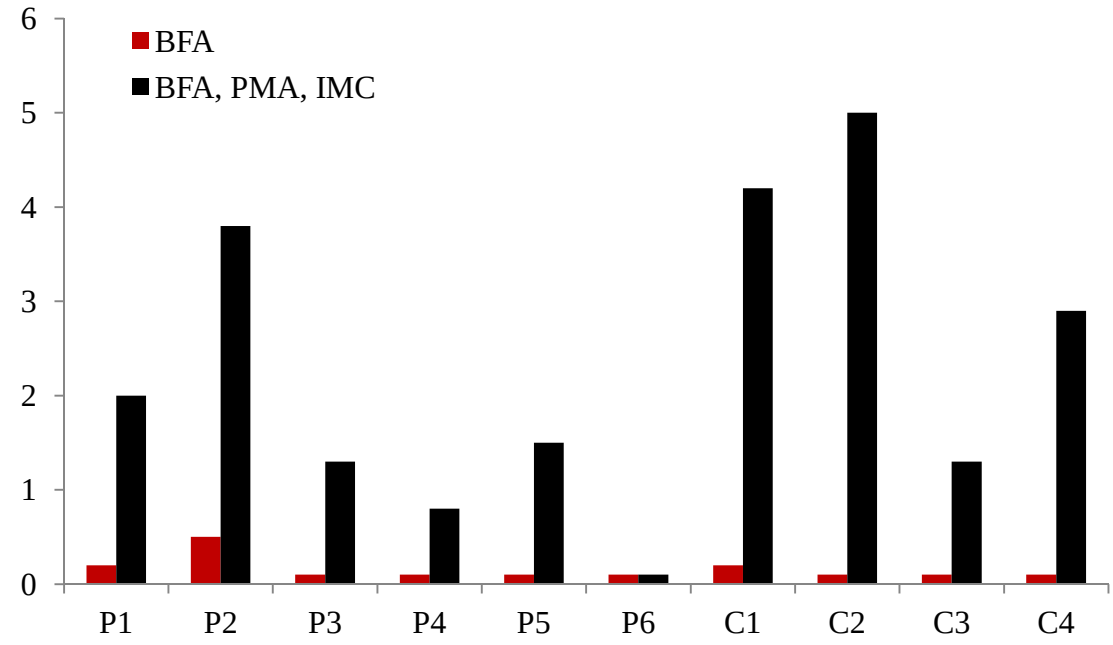
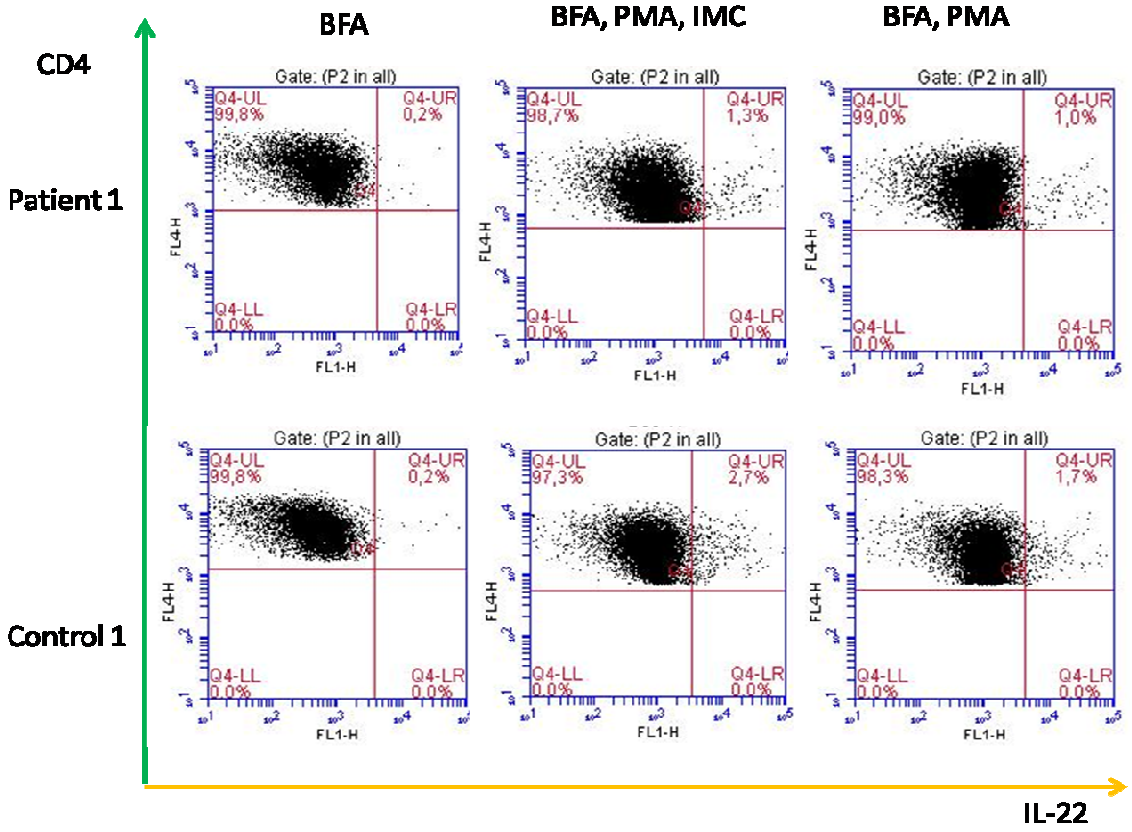
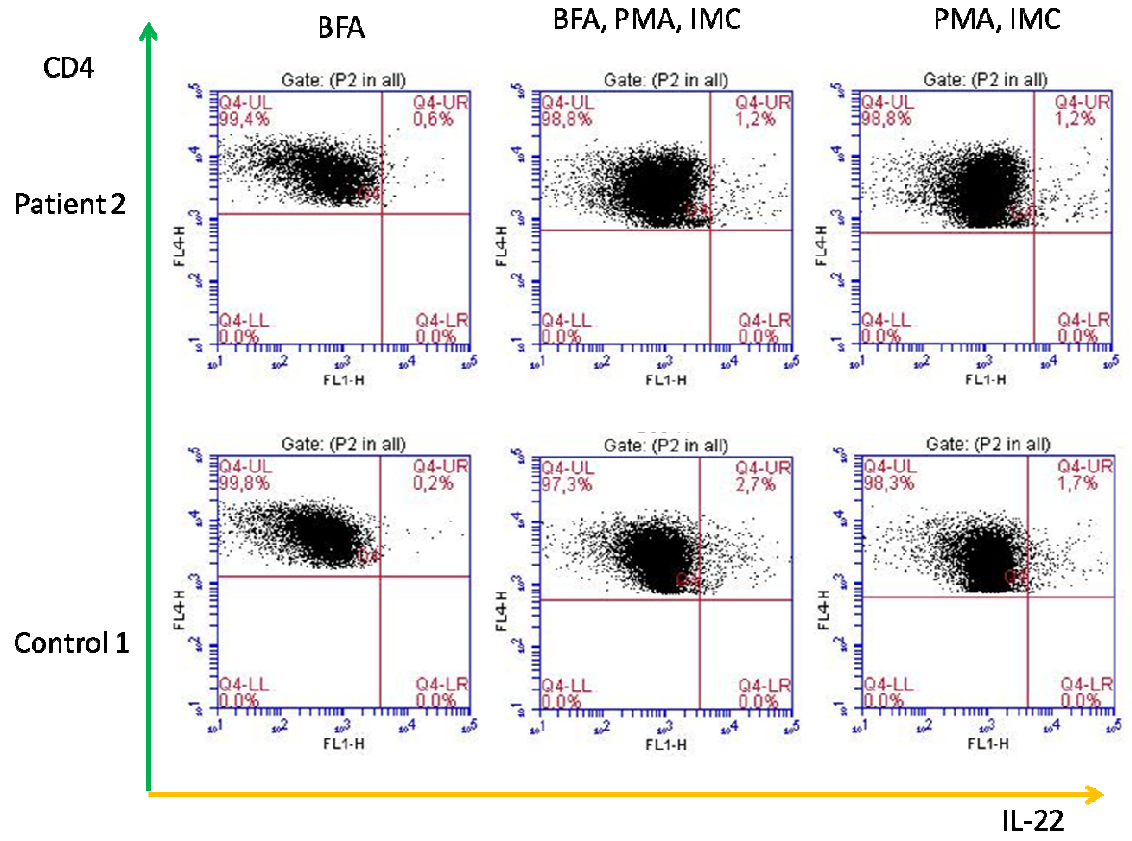


Figure 9G. Summary of CD4+IL-17+ T helper cell percentages of APS I patients and healthy controls. Red columns show CD4+IL-17+ cell percentages of APS I patients and healthy controls after treating with brefeldin A (BFA), without phorbol-myristate.acetate (PMA) stimulation. Black columns show CD4+IL-17+ cell percentages of APS I patients and healthy controls treated with BFA, PMA and ionomycin (IMC).We found decreased percentages of IL-17 secreting CD4+ cells in APS I patients after PMA stimulation, compared with healthy controls.

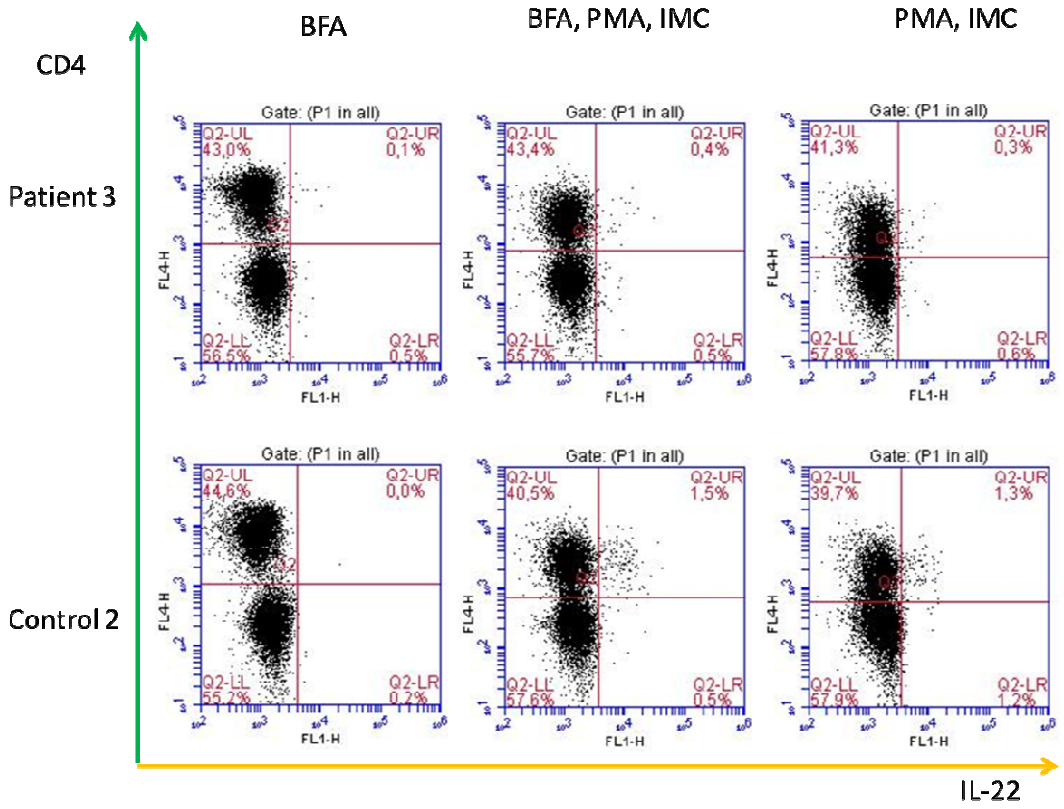
Figure 10A


BFA
BFA, PMA, IMC
BFA, PMA
CD4
Patient 1
Control 1
IL-22
Gate: (P2 in all)
Q4-UL 99,8%
Q4-UR 0,2%
Q4-LL 0,0%
Q4-LR 0,0%
Q4-UL 98,7%
Q4-UR 1,3%
Q4-UL 99,0%
Q4-UR 1,0%
Q4-UL 97,3%
Q4-UR 2,7%
Q4-UL 98,3%
Q4-UR 1,7%
FL1-H
FL4-H

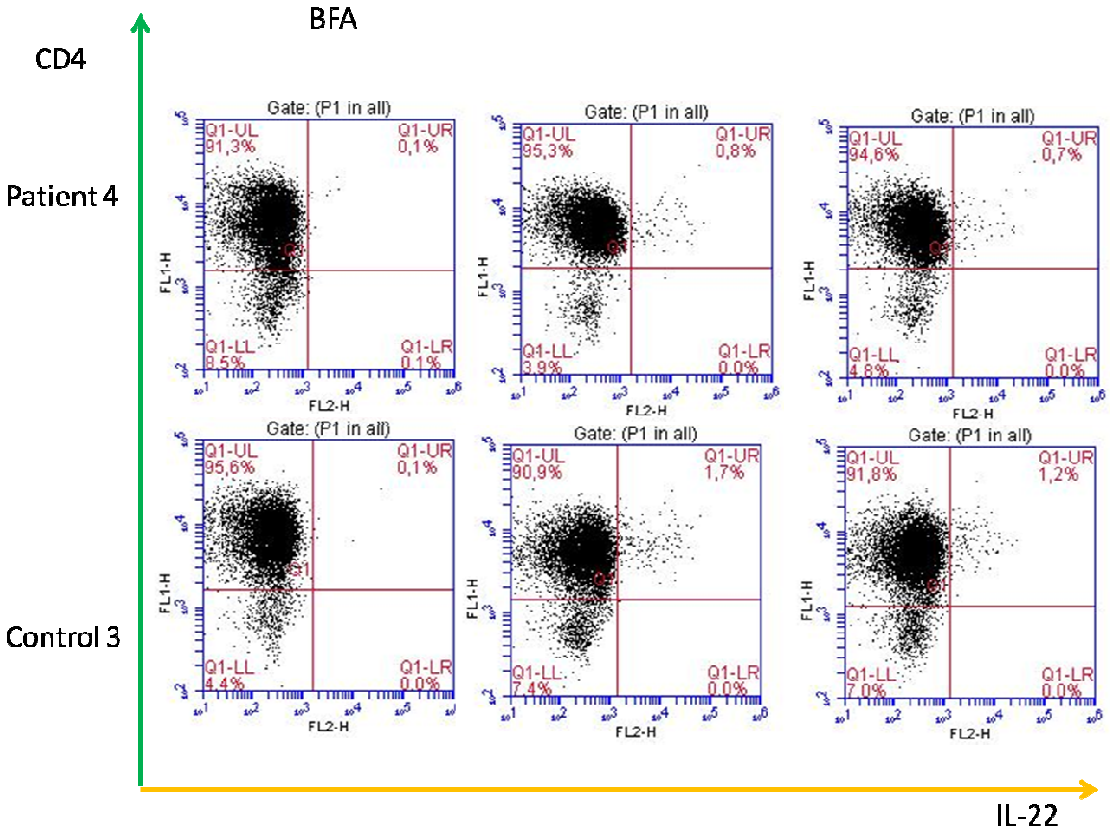
B


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 2
Control 1
IL-22
Gate: (P2 in all)
Q4-UL 99,4%
Q4-UR 0,6%
Q4-LL 0,0%
Q4-LR 0,0%
Q4-UL 98,8%
Q4-UR 1,2%
Q4-UL 99,8%
Q4-UR 0,2%
Q4-UL 97,3%
Q4-UR 2,7%
Q4-UL 98,3%
Q4-UR 1,7%
FL1-H
FL4-H

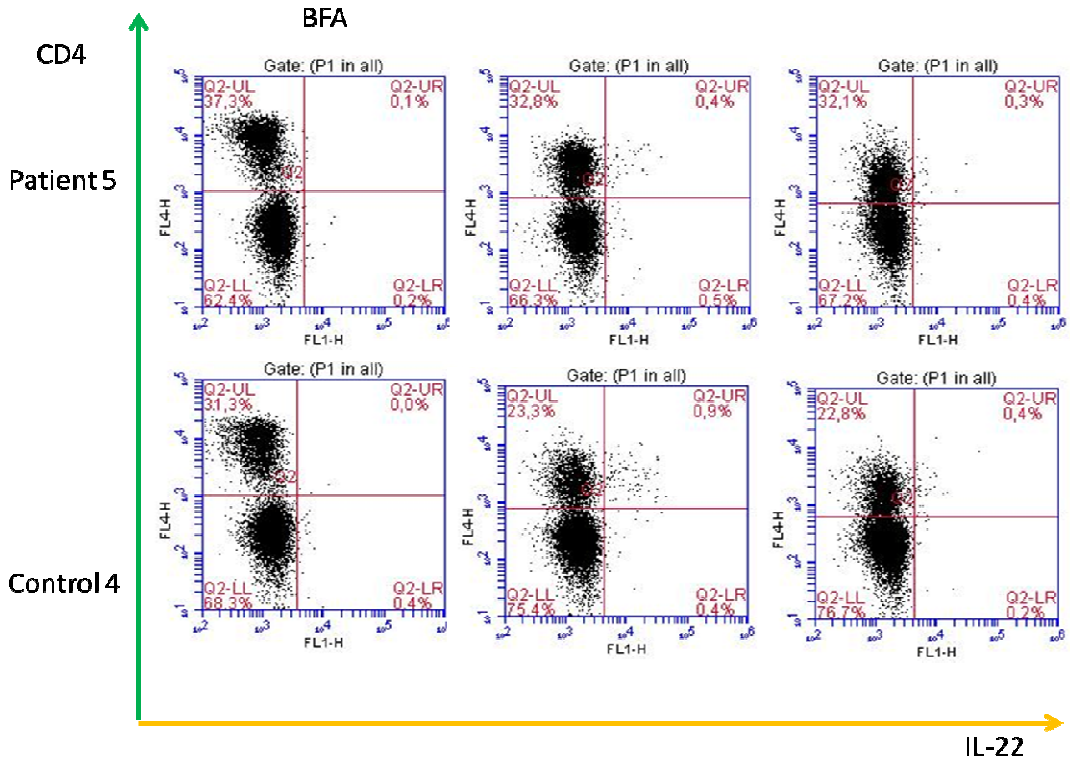
C


BFA
BFA, PMA, IMC
PMA, IMC
CD4
Patient 3
Control 2
IL-22
Gate: (P1 in all)
Q2-UL 43,0%
Q2-UR 0,1%
Q2-LL 56,5%
Q2-LR 0,5%
Q2-UL 43,4%
Q2-UR 0,4%
Q2-LL 55,7%
Q2-LR 0,5%
Q2-UL 41,3%
Q2-UR 0,3%
Q2-LL 57,8%
Q2-LR 0,6%
Q2-UL 44,6%
Q2-UR 0,0%
Q2-LL 55,2%
Q2-LR 0,2%
Q2-UL 40,5%
Q2-UR 1,5%
Q2-LL 57,6%
Q2-LR 0,5%
Q2-UL 39,7%
Q2-UR 1,3%
Q2-LL 57,9%
Q2-LR 1,2%
FL1-H
FL4-H

D


BFA
CD4
Patient 4
Control 3
IL-22
Gate: (P1 in all)
Q1-UL 91,3%
Q1-UR 0,1%
Q1-LL 8,5%
Q1-LR 0,1%
Q1-UL 95,3%
Q1-UR 0,8%
Q1-LL 3,9%
Q1-LR 0,0%
Q1-UL 94,6%
Q1-UR 0,7%
Q1-LL 4,8%
Q1-LR 0,0%
Q1-UL 95,6%
Q1-UR 0,1%
Q1-LL 4,4%
Q1-LR 0,0%
Q1-UL 90,9%
Q1-UR 1,7%
Q1-LL 7,4%
Q1-LR 0,0%
Q1-UL 91,8%
Q1-UR 1,2%
Q1-LL 7,0%
Q1-LR 0,0%
FL1-H
FL2-H

E


BFA
CD4
Patient 5
Control 4
IL-22
Gate: (P1 in all)
Q2-UL 37,3%
Q2-UR 0,1%
Q2-LL 62,4%
Q2-LR 0,2%
Q2-UL 32,8%
Q2-UR 0,4%
Q2-LL 66,3%
Q2-LR 0,5%
Q2-UL 32,1%
Q2-UR 0,3%
Q2-LL 67,2%
Q2-LR 0,4%
Q2-UL 31,3%
Q2-UR 0,0%
Q2-LL 68,3%
Q2-LR 0,4%
Q2-UL 23,3%
Q2-UR 0,9%
Q2-LL 75,4%
Q2-LR 0,4%
Q2-UL 22,8%
Q2-UR 0,4%
Q2-LL 76,7%
Q2-LR 0,2%
FL4-H
FL1-H

F

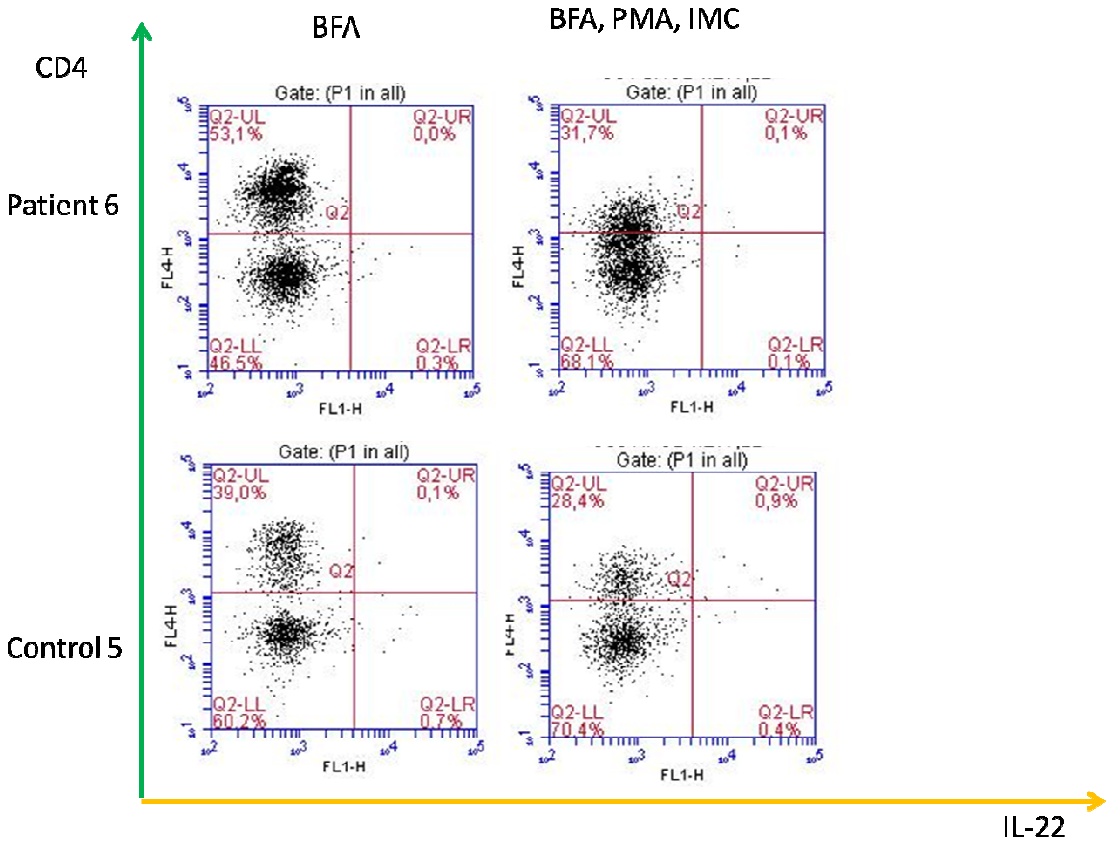

BFA
BFA, PMA, IMC
CD4
Patient 6
Control 5
IL-22
Gate: (P1 in all)
Q2-UL 53,1%
Q2-UR 0,0%
Q2-LL 46,5%
Q2-LR 0,3%
Q2-UL 31,7%
Q2-UR 0,1%
Q2-LL 68,1%
Q2-LR 0,1%
Q2-UL 39,0%
Q2-UR 0,1%
Q2-LL 60,2%
Q2-LR 0,7%
Q2-UL 28,4%
Q2-UR 0,9%
Q2-LL 70,4%
Q2-LR 0,4%
FL4-H
FL1-H

Figure 10A-F. Development of CD4+IL-22-producing T cells in APS I patients and healthy controls. Percentages of CD4+IL-22+ cells, as determined by flow cytometry, after incubation with combinations of BFA (brefeldin A), PMA (phorbol 12-myristate 13-acetate) and IMC (ionomycin). Patients identification numbers are shown on the Y axis. We found uniformly lower IL-22 producing cell percentages in APS I patients compared with cell percentages of healthy controls. In patients, cell percentages were similar with (Fig. 10A-F BFA, PMA, IMC) and without (Fig. 10A-E PMA, IMC) BFA - an effective inhibitor of cytokine secretion and transport -, in contrast with cell percentages of healthy controls, which were significant lower without BFA; as a normal consequence of cytokine secretion and lower intracellular marked cytokine concentration. Results suggest that in patients the development of IL-22 producing T cells and the secretion of IL-22 is also impaired.

Table 5. IL-17 and IL-22 producing T cells in APS I patients and healthy controls, after PMA stimulation, with and without secretion inhibitor.

	CD3+ IL-17+			CD4+ IL-17+			CD4+ IL-22+		
Patients	**BFA+**	**BFA-**	**-/+**	**BFA+**	**BFA-**	**-/+**	**BFA+**	**BFA-**	**-/+**
P1	0,7	0,6	**85%**	2,0	1,6	**80**	1,3	1,0	**76,9%**
P2	2,5	2,3	**92%**	3,8	3,5	**92,1**	1,2	1,2	**100%**
P3	1,6	1,4	**87,5%**	1,3	1,0	**76,9**	0,4	0,3	**75%**
P4	0,8	0,7	**87,5%**	0,8	0,7	**87,5**	0,8	0,7	**87,5%**
P5	1,5	1,5	**100%**	1,5	1,4	**93,3**	0,4	0,3	**75%**
C1	3,3	1,8	54,5%	4,2	3,1	73,8%	2,7	1,7	62,9%
C2	5,2	3,8	73%	5,0	3,2	64%	1,5	1,3	86,4%
C3	1,7	1,2	70,5%	1,3	0,8	61,5%	1,7	1,2	70,5%
C4	3,3	2,2	66,6%	2,9	2,2	64%	0,9	0,4	44,4%

Without secretion inhibitior, healthy controls presented lower IL-17+ and IL-22+ cell percentages after PMA stimulation (44,4-86,4% of the result with BFA, mean value is 66%), as a normal consequence of the lower intracellular cytokine concentrations and lower concentrations of marked intracellular components without inhibitor. In contrast, we found

comparable percentages of CD3+IL-17+, CD4+ IL-17+ and CD4+IL-22 cells, with and without secretion inhibitor in patients with APS I. Without inhibitor, IL-17 and IL-22 producing cells were 75-100% (mean value is 86,4%) of the result with inhibitor, what suggest that APS I patients secreted the marked cytokines in lower proportion, comparing with healthy controls.

G

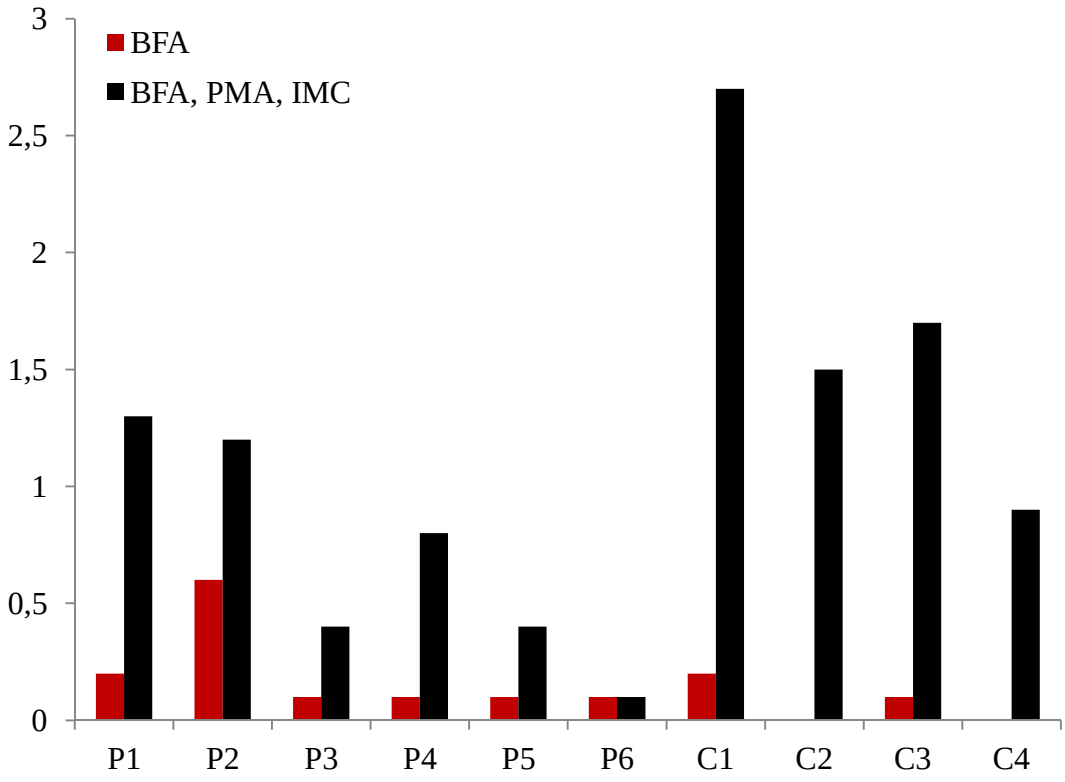
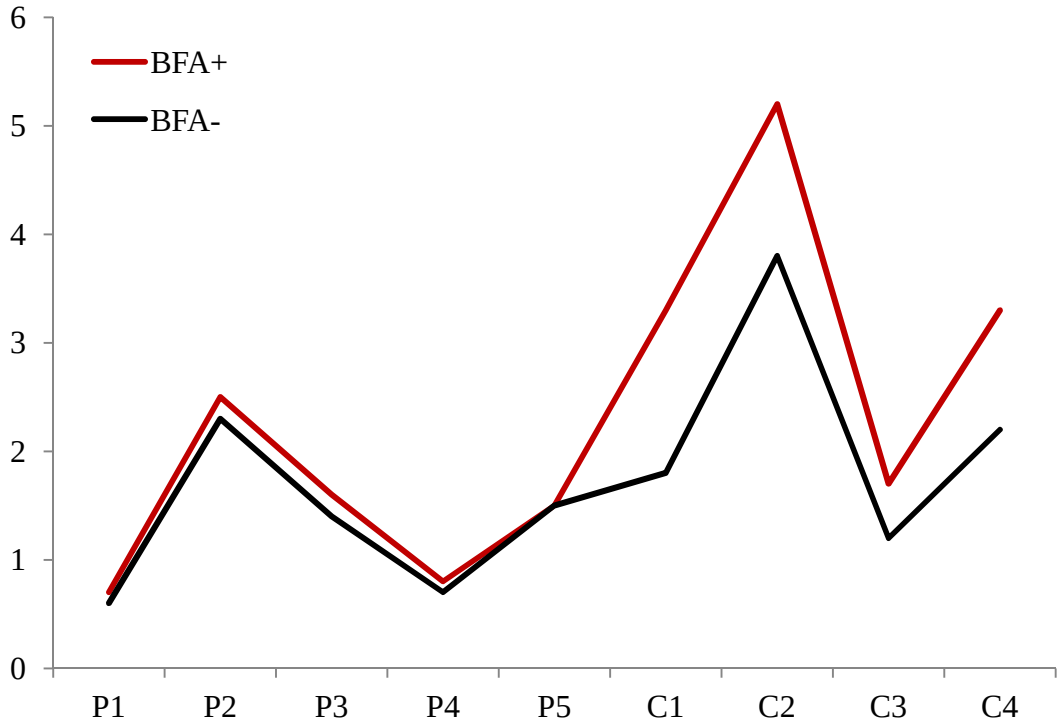
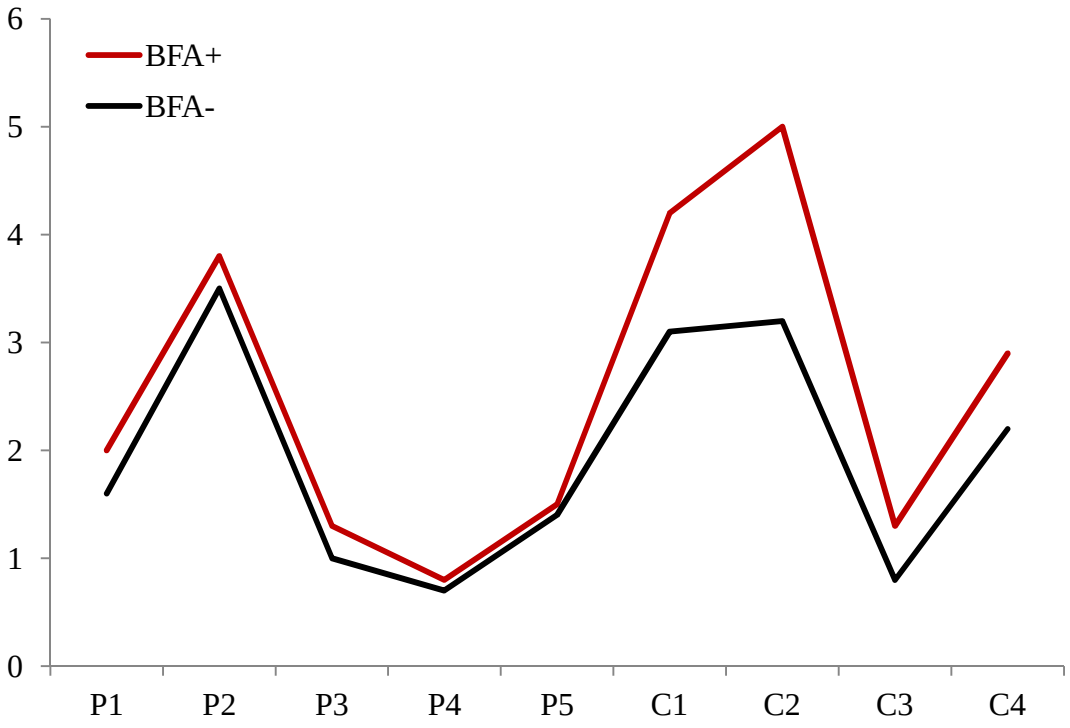


Figure 10G. Summary of CD4+IL-22+ T helper cell percentages of APS I patients and healthy controls. Red columns show CD4+IL-22+ cell percentages of APS I patients and healthy controls after treating with brefeldin A (BFA), without phorbol-myristate-acetate (PMA) stimulation. Black columns show CD4+IL-22+ cell percentages of APS I patients and healthy controls treated with BFA, PMA and ionomycin (IMC). We found decreased percentages of IL-22 secreting CD4+ cells in APS I patients after PMA stimulation, compared with healthy controls.

Figure 11A


6
5
4
3
2
1
0
BFA+
BFA-
P1
P2
P3
P4
P5
C1
C2
C3
C4

B


6
5
4
3
2
1
0
BFA+
BFA-
P1
P2
P3
P4
P5
C1
C2
C3
C4

C

Figure 11A-C. Comparison of CD3/4+IL-17/IL-22+ cell percentages of APS I patients and healthy controls with and without secretion inhibitor. Without secretion inhibitior, healthy controls presented lower IL-17+ and IL-22+ cell percentages after PMA stimulation, as a normal consequence of the lower intracellular cytokine concentrations and lower concentrations of marked intracellular components without inhibitor. In contrast, we found comparable percentages of CD3+IL-17+, CD4+ IL-17+ and CD4+IL-22 cells, with and without secretion inhibitor in patients with APS I. This results suggest that secretion of IL-17 and IL-22 by CD3/4+IL-17/IL-22+ T cells of APS I patients is decreased compared with healthy controls.

5. DISCUSSION

CMC is the most common infectious manifestation in APS I, which is one of three diagnostic criteria of the syndrome (Ahonen et al. 1990, Husebye et al. 2009, Myhre et al. 2001, Perheentupa 2006). In this study, we found CMC with variable severity (mild angular cheilitis, isolated nail candidiasis, recurrent severe oropharyngeal candidiasis) in almost 80 % of the 19 patients tested. Hypoparathyreosis, the second most common manifestation and diagnostic criteria was present in 73,6% of patients, consistent with the published literature (Weiler et al. 2006, Gylling et al 2003, Myhre et al. 2001). Close to sixty percent of our patients presented with adrenal insufficiency, and 78,9% of patients fulfilled the classical diagnostic criteria i.e. at least two of the CMC, HP and AI, moreover one patient had CMC and an affected member in the family. The most common mutation, called “Finnish major mutation” were found in 42,1% of patients, including the two Finnish patient. In this study, the second most common genetic condition was the compound heterosigosity for R257/13 bp deletion.The 13 bp deletion is the second most common *AIRE* mutation worldwide. The classical diagnostic triad were present in 36,8%, especially in patients with the Finnish major mutation and the R257X/13 bp deletion. Both Sardinian patients presented with autoimmune hepatitis and carried the R139X mutation. P1, despite homozygosity for the R257X *AIRE* mutation, has had only dental enamel dysplasia, alopecia universalis and nail dystrophy at age 10, whereas his sister (P2) had developed both CMC and HP by the age of 18 months. P3, with the R257X mutation is till symptomless now, despite the severe clinical phenotype of his sister. These results confirmed the clinical and genetical heterogeneity of APS I, in accordance with data from others (Myhre et al. 2011, Tóth et al. 2010, Wolff et al. 2007, Meloni et al 2012, Orlova et al. 2010).

We provide the first description of the possible effect of IVIG as a treatment option in patients with severe CMC in APS I patients, and the transient improvement of mucosal candidiasis following monthly IVIG infusions in Patient 2 was intriguing. IVIG replacement therapy is widely used to prevent infectious complications in patients with primary immunodeficiency disorders (Sibérli et al. 2007, Negi et al. 2007). In addition to prophylaxis, IVIG concentrates may also be helpful for the treatment of various inflammatory conditions (Kreuz et al. 2010, Orange et al. 2006, Sami et al. 2002). However, the mode of action of this therapy in autoimmune diseases remains poorly understood. IVIG contains anti-idiotype antibodies against disease-associated autoantibodies. These anti-idiotype antibodies may be able to bind

and neutralize the pathogenic anticytokine antibodies typically produced in patients with APS I, consistent with the decrease in autoantibody levels observed in other conditions following IVIG therapy (Orange et al. 2006). We believe that IVIG treatment may increase the elimination of *Candida* by opsonophagocytosis, given the clinical observation that patients with X-linked agammaglobulinemia characterized by an almost complete lack of serum immunoglobulins typically do not suffer from CMC or invasive candidiasis. We observed no detectable decrease in anti-IL-17A and anti-IL-22 antibody levels during IVIG therapy in Patient 2 (data not shown), but we believe that further studies of the possible therapeutic effects of IVIG in APS I patients with severe CMC are warranted, based on our observation.

The importance of the IL-17 familycytokines in immunity to *Candida* is well established (Cypowyj et al. 2012, Eyerich et al. 2008, Kisand et al. 2010, 2011, Maródi et al. 2012, Puel et al. 2012). Previous studies suggested that anti-cytokine autoantibodies are involved in the pathogenesis of CMC in APS I patients. It has been suggested that autoantibodies against cytokines, including IL-17A, IL-17F and IL-22, may underlie CMC in patients with APS I. Recent findings have suggested that the presence of autoantibodies against IL-22 may be a useful predictor of CMC (Puel et al. 2010, 2012, Kisand et al. 2010, Kisand et al. 2011, Oftedal et al. 2011). It has also been suggested that IL-17 plays a key role in protection against *Candida* infection, and some patients with CMC disease have smaller than normal proportions of IL-17-producing T cells, and produce low levels of IL-17 (Eyerich et al. 2008, Soltész et al. 2013, Puel et al. 2010, Puel et al. 2012).In this study, Patient 2, with severe and prolonged CMC, presented with high levels of autoantibodies against IL-17A. The other five patients with the same genotype or heterozygous *AIRE* mutation had low or undetectable anti-IL-17A antibody levels and were asymptomatic or presented only mild and transient signs of mucocutaneous candidiasis. Previous studies have suggested that IL-22 plays an important role in the protection of skin and epithelial surfaces against *Candida* (Zelante et al. 2011, De Luca et al. 2010, Eyerich et al. 2011). It has been reported that IL-22-producing cells help to protect mucosal surfaces against fungal infection in conditions of defective adaptive immunity, through a primitive antifungal effector mechanism (Zelante et al. 2011). Furthermore, IL-22 has been reported to play a role in candidiasis, by controlling fungal invasion and epithelial homeostasis. All six patients included in this study had high levels of anti-IL-22 autoantibodies, but only Patient 2, with high levels of anti-IL-17A antibodies, displayed a predisposition to severe CMC. Furthermore, OD values for anti-IL-17F antibodies

were high in all patients, but those in Patient 2 they were no higher than those in the other patients. These findings argue against a primary role of anti-IL-17F and anti-IL-22 antibodies in the susceptibility of APS I patients to CMC, instead favoring the concept that anti-IL17A antibodies are the primary components of impaired anti-candidial immunity in APS I patients. Measuring functional activity of these antibodies could have confirmed further our findings but such assays are not currently available in our lab.

The role of anti-IFN antibodies in the pathogenesis of disease signs in APS I patients is not fully understood. Recent studies have shown that high levels of autoantibodies against type I IFNs, including IFN-α and IFN-ω in particular, are found in most patients with APS I, suggesting that anti-IFN autoantibodies are hallmarks of the disease (Meager et al. 2003, Meager et al. 2006, Meager et al. 2008, Meloni et al. 2008, Meloni et al. 2012, Meloni et al 2008). These studies showed that autoantibodies against IFN-ω were clearly present in all patients but their levels were not clearly correlated with the number or duration of disease manifestations (Meloni et al. 2008). We found high levels of autoantibodies against IFN-α in the sera of four patients studied, and of autoantibodies against IFN-ω in Patients 1 and 3. The levels of anti-IFN-ω autoantibodies were low in Patient 2, 4 and 5. These data confirm the lack of relationship between severity of APS I and anti-IFN autoantibody level. The role of the variable levels of anti-IFN-ω antibodies in this multiorgan primary immunodeficiency disorder therefore remains to be elucidated. Moreover, the almost 100% presence of this autoantibodies in APS I patients propose the role of this antibodies as a new diagnostic criterion (Meloni et al. 2008). In sibling P3, the antibodies against IFN-ω appeared before those against IL17 type cytokines. It would be interesting to test more pre-symptomatic infants in APS-I families serially, starting with cord blood, both for autoantibodies and for Th17 and Th22 cell function such long term studies may provide evidence on the precise timing of the various responses and Th-cell deficiencies and on their prognostic value. Our results show strikingly high neutralizing autoantibody titers against IFN-ω, IFN-α2 and/or IL-22 already at 6-7 months of age -well before any sign of APS-I- in an unaffected *AIRE*-mutant patient (P3). We also found endocrine organ-specific autoantibodies, especially to adrenal cortex, surprisingly early in 2 in two cases. Since several weeks, at least, are needed to generate an antibody response, even in adults —and still longer for organ damage sufficient to cause disease—the process must have begun soon after birth in some of the infants studied here, or even earlier. The well-known delays, sometimes for several years, between

appearance of tissue-specific autoantibodies and the corresponding autoimmune feature are often taken to implicate T-cells as the pathogenic effectors, whereas the autoantibodies behave as useful diagnostic markers. Autoimmunity in APSI appears to result primarily from a failure to tolerize T-cells developing in the absence of *AIRE*, leading stochastically to autoimmunization against a wide range of *AIRE*-regulated targets. That may be compounded by loss of tolerogenic *AIRE*-expressing cells in peripheral lymphoid tissues (Eldershaw et al. 2011). However, both the highly precocious, sharply focused autoantibody responses shown here and the consistent early onset of such unusual autoimmune features as CMC, HP and AD argue for more selective autoimmunization in *AIRE*-deficient thymus.

Recent studies suggested that impairment of Th17 cell population and development, or IL-17F and IL-22 secretion by PBMC's is CMC patients may be an important factor in predisposition to candidiasis. The role of IL-17A secretion seems somewhat controversialand poorly defined in studies in APS I patients. Reports on increased numbers of *C. albicans*-induced IL-17A-producing cells in PBMC from APS I patients, measured by flow cytometry by intracellular staining of IL-17A, contrasting other studies, which consistently found impaired IL-17A responses in APS I patients (Ahlgren et al. 2011). *Candida*-exposed PBMCs from our patients released small amounts of IL-17A and much smaller, or negligible amounts of IL-17F and IL-22 compared to cells from healthy controls. Recent studies suggestthat in patients with APS I, the pools of CD16+ monocytes and regulatory T cells (Tregs) were also lower compared with healthy individuals (Wolff et al. 2010), and propose that this difference may influence the inflammatory processes in APSI patients. Th17 cell proliferation and IL-17 production were normal unless exposed to APS I plasma, which inhibited both functions in both APS I and normal PBMCs (Ng et al. 2010). *Candida* species–stimulated IL-22 production was impaired in all patients with CMC, including APS I patients, whereas IL-6 and IL-23 responses were also normal (Ng et al 2010). We found impaired secretion of IL-17A, IL-17F and IL-22 by mononuclear cells from APS patients, with, or without severe candidiasis, in contrast to release of TNF-α. Moreover, patients with APS I had a decreased percentages of CD3+IL-17+, CD4+IL-17+ and CD4+IL-22+, IL-17 and IL-22 producing T cells, after differentiation, and stimulation by phorbole myrystate-acetate, compared with healthy controls. The percentages of the IL-17 and IL-22 producing T cells are nearly constant with and without the inhibition of cytokine secretion in APS I, in contrast with the results of healthy controls. Recent findings suggest, that the wild type (WT) *AIRE* gene product has the ability to be

targeted into the nucleus, where it is found associated with distinct speckled domains in the nucleoplasm (Eldershaw et al. 2011, Rinderle et al. 1999). These findings showed with immunocytofluorescence detection, that the normal AIRE protein co-lokalizes with the cytoskeletal filaments, especially with the vimentin, and the truncated AIRE protein showed altered cytoskeletal interactions and subcellular localization (Rinderle et al. 1999). We suggest that these differences between the wild type and the mutant subcellular distribution, may effect as a disturbing factor the subcellular processes of the mechanisms of cytokine secretion and transport.

6. CONCLUSIONS

1. Based on our results, we conclude that organ specific autoantibodies play very important role in the endocrine organ hypofunctions, but autoantibody production may precedes and presages endocrine manifestations and hormone levels may fluctuate for years.

2. Our genetic results, genotype-phenotype correlations, especially the occurrence of chronic mucocutaneous candidiasis and major criteria, were in conjuction with data of published literature. We report here a correlation between anti-IL-17A autoantibody level and predisposition to chronic mucocutaneous candidiasis in APS I patients.

3. We suggest that anti-IL-17A autoantibodies may play a more important role than anti-IL-22 and anti-IL-17F antibodies in rendering APS I patients susceptible to candidiasis. We suggest that there are no strong chronological relationship between the production of anti-cytokine autoantibodies and the development of clinical signs.The almost 100% presence of type I interferon autoantibodies in APS I patients propose the role of this antibodies as a new diagnostic criterion.

4. We present data suggesting that the release of IL-17A, IL-17F and IL-22 by peripheral blood mononuclear cells after *Candida* stimulation is impaired in APS I patients, and this impairment affects only IL-17 and IL-22 secretion, and does not influence secretion of other cytokines. This result is confirmed that predisposition to candidiasis of APS I patients is a multifactorial defect of the secretion and signalization of IL-17 and IL-22, and queries the obligate role of anti-cytokine autoantibodies.

5. We suggest, that the development of IL-17+IL-22+ T helper cells is impaired in APS I patients, nevertheless, secretion mechanisms of these cells are also affected.

6. We present here the follow-up a young APS I patient (P3) with the classical and most common mutation of the *AIRE* gene, with a severe case in the family history and high

titers of anti-cytokine autoantibodies to IL-17F, IL-22 and type I interferons, but normal endocrine organ functions, laboratory and clinical parameters till now. This case confirmed the clinical heterogenity of APS I and underlies that there are no strong chronological relationship between the autoantibody production and clinical manifestations of APS I.

7. SUMMARY

Autoimmune polyendocrine syndrome type I is a rare recessively inherited disorder, caused by mutations in the autoimmune regulator (AIRE) gene, characterized by high titers of autoantibodies against a wide variety of endocrine organs and cytokines, that could partly be responsible for the clinical symptoms, mainly endocrine organ hypofunctions and chronic mucocutaneous candidiasis, but the relative roles of these autoantibodies in susceptibility to candidiasis remain poorly defined.

In summary, we report here a correlation between anti-IL-17A autoantibody level and predisposition to chronic mucocutaneous candidiasis in APS I patients. We suggest that anti-IL-17A autoantibodies may play a more important role than anti-IL-22 and anti-IL-17F antibodies in rendering APS I patients susceptible to candidiasis. We suggest that high levels of anti-IL-22 and anti-IL-17F autoantibodies may not be correlated with the occurrence of candidiasis in APS I patients. We present data suggesting that the release of IL-17A, IL-17F and IL-22 by peripheral blood mononuclear cells after *Candida* stimulation is impaired in APS I patients, and this impairment affects only IL-17 and IL-22 secretion, and does not influence secretion of other cytokines. This result is confirmed by the normal secretion of TNF-α of APS I patients, after *Candida* stimulation. We found, that patients with APS I have a decreased persent of CD3+IL-17+, CD4+IL-17+ and CD4+IL-22+, Il-17 and IL-22 producing T cells, after in vitro differentiation, compared with healthy controls. Moreover, the percents of the IL-17 and IL-22 producing T cells are nearly constant with and without the inhibition of cytokine secretion in APS I, in contrast with the results of healthy controls. Based on this results, we suggest, that the development of IL-17+, IL-22+ T helper cells is impaired in APS I patients, nevertheless, secretion mechanisms of these cells are also affected.

We propose that the predisposition to candidiasis of APS I patients is based on a complicated conjuction of diversified factors, included the antifungal cytokine-neutralization by autoantibodies, the impaired development of IL-17 and IL-22 producing T cells, and decreased cytokine secretion capacity of these cells.

ÖSSZEFOGLALÁS

Az I-es típusú autoimmune poliendokrin szindróma egy ritka, autoszomális recesszív módon öröklődő kórkép, melynek hátterében az autoimmune regulator (*AIRE*) gén mutációja áll. A betegség jellegzetessége specifikus anutoantitestek termelése számos szerv, szövet, citokin valamint hormonok szintézisében részt vevő enzimek ellen.

Munkánk során az I-es típusú autoimmun poliendokrin szindrómában szenvedő betegek antifungális védekező mechanizmusainak vizsgálatával foglalkoztunk. Az irodalmi adatokban fellelhető eredményekkel ellentétben korrelációt találtunk a betegek IL-17A ellenes autoantitestek mennyisége és a krónikus mucocutan candidiasis előfordulása között. Eredményeinkből arra következtettünk, hogy az IL-17A ellenes antitestek kiemelt szerepet játszanak az APS I-es betegek candidiasisra való hajlamosságában, és a tünetek súlyossága az antitestek mennyiségével korrelál. Emellett az IL-17F és IL-22 ellenes autoantitestek mennyisége és a tünetek előfordulása között nem találtunk összefüggést. További vizsgálatokkal igazoltuk, hogy az APS I-es betegek candidiasisra való fogékonyságának hátterében további defektusok is megfigyelhetőek. APS I-es betegeink mintáiból jelentősen csökkent antifungális citokin szekréciót figyeltünk meg, *Candida* stimulációt követően, míg egy Th17 vonaltól függetlennek tekinthető citokin, a TNF-α szekréciója normálisnak bizonyult. A sejtes vizsgálatok felvetették, hogy az APS I-es betegek IL-17 illetve IL-22 termelő sejtjeinek aránya csökkent az egészséges kontrollokéhoz viszonyítva, és a sejtek citokin szekréciója is elmarad az egészséges kontrollok eredményeihez képest. Eredményeink alátámasztják, hogy az APS I-es betegek kónikus mukokután candidiasisának hátterében az eddig gondoltnál sokkal szélesebb körű defektus áll, amely nem merül ki az antifungális citokinek elleni autoantitest termelésben, de érinti a testfelszíni *Candida* ellenes védekezésben kiemelt szerepet játszó Th17 sejtek differenciációját és citokin szekréciós mechanizmusait is.

8. KEYWORDS

Autoimmune polyendocrine syndrome type I, IL-17, IL-22

KULCSSZAVAK

Autoimmun poliendokrin szindróma I-es típus, IL-17, IL-22

9. ABBREVATIONS

AD	-	Autosomal dominant
AH	-	Autoimmune hepatitis
AhR	-	Aryl hydrocarbon receptor
AI	-	Adrenal insufficiency
AIRE	-	Autoimmune regulator
Alo	-	Alopecia
AP	-	Anaemia perniciosa
APECED	-	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
APS	-	Autoimmune polyendocrine syndrome
AR	-	Autosomal recessive
CARD9	-	Caspase recruitment domain 9
CLR	-	C-type lectin receptor
CMC	-	Chronic mucocutanous candidiasis
CMCD	-	CMC disease
DC	-	Dendritic cell
DOCK8	-	Dedicator of cytokinesis 8
EAP	-	Enhanced adherence to polystyrens
FcRγ	-	Fc receptor γ
FH	-	Factor H
GAD	-	Glutamic acid decarboxylase
GM-CSF	-	Granulocyte-monocyte colony-stimulating factor
HIES	-	Hyper-IgE syndrome
HLA	-	Human leukocyte antigen
HP	-	Hypoparathyreosis
Ig	-	Immunoglobulin
IL	-	Interleukin
IL-10Rβ	-	Interleukin -10 receptor β

IL-22R	-	Interleukin-22 receptor
IMD	-	Immune mediated diabetes
Mal	-	Malabsorption
MBL	-	Mannose binding receptor
MYD88	-	Myeloid differentiation primary response 88
NF-κB	-	Nuclear factor-κB
NK	-	Natural killer
NLR	-	NOD-like receptor
NLRP3	-	NOD-, LRR- and pyrin domain-conatining 3
PAG	-	Poliglandular autoimmune syndrome
PAMP	-	Pathogen-associated molecular pattern
PAR	-	Protease activated receptor
PBMC	-	Perapheral blood mononuclear cell
PPARγ	-	Peroxisome proliferator activated receptor-γ
RAF	-	Rapidly Accelerated Fibrosarcoma
PRR	-	Pattern recognition receptor
RAGE	-	Receptor for advanced glycation and-products
STAT 1	-	Signal transducer and activator of transcription 1
STAT3	-	Signal transducer and activator of transcription 3
SYK	-	Spleen-tyrosine kinase
Th	-	T helper
TIR	-	Toll/IL-1 receptor
Treg	-	T regulatory
WT	-	Wild type

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