



Novel monogenic diseases causing human autoimmunity

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Fuelled by the on-going sequencing revolution, the last two years have seen a number of exciting discoveries relating to monogenic disorders predisposing to autoimmunity that provide new insights into the function of the human immune system. Here we discuss a selection of these diseases due to mutations in *PRKCD*, *CTLA4*, *STAT3*, *IFIH1*, *TMEM173* and *COPA*.

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Introduction

Autoimmunity, encompassing both organ specific and systemic forms, can be defined as the occurrence of self antigen-reactive T cells and/or B cells leading to tissue damage. Although autoimmunity is generally considered to be ‘complex’ in pathogenesis, occurring on a background of polygenic predisposing factors and environmental exposures, Mendelian forms of autoimmunity are well recognized, with autoimmune lymphoproliferative syndrome (ALPS), autoimmune polyendocrinopathy candidiasis and ectodermal dysplasia (APECED) and immunodeficiency polyendocrinopathy enteropathy X-linked (IPEX) amongst the most celebrated. The discovery of monogenic disorders that lead to a loss of self-tolerance can provide critical insights into biologic pathways important for the regulation of the human immune response. Very recently, the technologically driven genomic

revolution has resulted in a flurry of exciting discoveries relating to autosomal autoimmunity, and which are the subject of this short review (Table 1).

Protein kinase C delta (PKC δ ; PRKCD) deficiency.

2013 saw the publication of three papers describing the consequences of deficiency of PKC δ inherited on an autosomal recessive basis. Kuehn *et al.* [1^{*}] and Salzer *et al.* [2^{*}] described single patients with a novel ALPS-like disorder associated with non-specific reactive follicular hyperplasia on biopsy. Both patients presented with chronic persistent hepatosplenomegaly and lymphadenopathy. Additionally, they experienced repeated infections in the form of recurrent otitis, sinusitis, gastroenteritis and pneumonia. Autoimmune features were also observed, with the patient described by Salzer and colleagues demonstrating membranous glomerulonephritis, relapsing polychondritis, hypothyroidism and anti-phospholipid syndrome. A third study by Belot *et al.* [3^{*}] emphasized the link to autoimmunity due to loss of function mutations in *PRKCD* when they described three siblings fulfilling American College of Rheumatology criteria for a diagnosis of systemic lupus erythematosus (SLE). In contrast to the other two reports, only one patient in this family showed a transient hepatosplenomegaly and lymphadenopathy, and none experienced significant infections.

At a molecular level, the responsible mutations lead to reduced expression and activity of PKC δ . Mice lacking PKC δ acquire features of SLE characterized by an expansion of the immature B lymphocyte population with the formation of germinal centres, autoreactive antibodies, immune complex-associated glomerulonephritis, and lymphocyte infiltration in multiple organs. Adoptive transfer experiments suggest that this hyperproliferative phenotype is B cell autonomous. Reminiscent of these observations in animals, the human patients demonstrated an increased number of immature B cells, with an expansion of peripheral transitional B cells, increased B-cell proliferation and defective apoptosis. Summarizing, these data indicate that PKC δ is involved in the negative selection of human B cells, and that deficiency of PKC δ can manifest, albeit variably, as both SLE and an ALPS-like disease.

Cytotoxic T lymphocyte associated 4 (CTLA-4) haploinsufficiency.

Three papers published in 2014 have provided exciting new insights into the function of the essential negative regulator of peripheral T cell function CTLA-4, resulting from the identification of heterozygous loss-of-function mutations in *CTLA4*. Kuehn *et al.* [4^{**}]

Table 1

Molecular and cellular features of selected Mendelian causes of autoimmunity

Gene	Inheritance	Molecular pathology	Incomplete penetrance	Phenotypes	Mechanism
<i>PRKCD/PKCδ</i>	AR	LOF	ND	ALPS-like; SLE	B cells
<i>CTLA4</i>	AD	HI	Yes	Lymphocytic infiltration; CVID; multiple AI phenotypes	T cells
<i>STAT3</i>	AD	GOF	Yes	Multiple AI phenotypes; ALPS-like	STAT signalling
<i>IFIH1/MDA5</i>	AD	GOF	Yes	Neuroinflammatory (including AGS); SLE; IgA deficiency	IFN
<i>TMEM173/STING</i>	AD	GOF	Yes	Pulmonary and skin vasculitis; SLE	IFN
<i>COPA</i>	AD	?DN	Yes	Pulmonary haemorrhage; arthritis; renal	ER stress

AD, autosomal dominant; AGS, Aicardi-Goutières syndrome; AI, autoimmunity; ALPS, autoimmune lymphoproliferative syndrome; AR, autosomal recessive; CVID, common variable immunodeficiency; DN, dominant negative; ER, endoplasmic reticulum; GOF, gain of function; HI, haploinsufficiency; IFN, interferon; LOF, loss of function; ND, not described; SLE, systemic lupus erythematosus.

described seven patients from four families to demonstrate a clinical scenario characterized by organ (most particularly brain, lung and gut) infiltrates, cytopenias, hypogammaglobulinemia and autoantibody production. Meanwhile, Schubert *et al.* [5^{••}] described 14 individuals from six families with an overlap of similar findings, some of whom had received a diagnosis of common variable immunodeficiency and selective IgA deficiency with recurrent infections. Finally, Zeissig and colleagues [6] reported two half-siblings, related through a clinically asymptomatic mother, showing a striking combination of severe inflammatory bowel disease and autoimmune manifestations including type 1 diabetes mellitus, hypothyroidism and haematological cytopenias, as well as suspected primary sclerosing cholangitis, demyelinating encephalopathy, fibrosing interstitial lung disease and chronic sinusitis. The emphasis on different phenotypes in these papers is a reflection of an ascertainment bias according to the interest of the reporting laboratories, and highlights the remarkable associated pleiotropy.

CTLA-4 and the T cell co-stimulatory receptor CD28 share the ligands CD80 and CD86, but CTLA-4 is their high-affinity receptor. CTLA-4 acts as a mediator of regulatory T (Treg) cell suppressive function, as demonstrated by the fatal inflammatory phenotype of *Ctla-4*-null mice, which spontaneously develop T cell expansion with multiorgan lymphocytic infiltration and tissue destruction. Interest in the immunomodulatory role of CTLA-4 has led to the development of antibodies blocking CTLA-4 in patients with advanced malignancies, exemplifying a new treatment paradigm for cancer through harnessing 'antitumor immunity'. In light of the studies mentioned above, it is of particular note that anti-CTLA-4 therapy can be accompanied by autoimmune manifestations in patients with no history of autoimmunity. Indeed, reminiscent of biopsies of inflamed organs in patients receiving anti-CTLA-4 as an immunomodulatory anti-cancer therapy, tissue from CTLA-4 haploinsufficient patients demonstrated mixed lymphoid infiltrates, and wide-ranging effects on circulating lymphocytes, including effector T cells, Treg cells and B cells.

Summarizing, the CTLA-4 deficiency syndrome is remarkable for the wide-ranging aspect of system involvement with multiple types of organ specific autoimmunity, as well as variable age at onset (early infancy to late adulthood), variable expression and, indeed, clinical non-penetrance. The observation of clinically asymptomatic carriers, penetrant at the cellular level, reflects a complex interplay of genetic and environmental factors in determining the balance between homeostasis and disease.

Signal transducer and activator of transcription 3 (STAT3) gain of function. Three papers published in 2014/15 reported novel gain-of-function mutations in *STAT3* as a cause of infantile-onset autoimmunity. Flanagan *et al.* [7^{••}] described five individuals (two of whom were detailed more fully by Haapaniemi *et al.* [8]) with a range of autoimmune phenotypes including infantile-onset diabetes mellitus, enteropathy (coeliac disease), interstitial lung disease, juvenile-onset arthritis and primary hypothyroidism. Meanwhile, Milner *et al.* [9^{••}] highlighted the occurrence of lymphoproliferation and recurrent infections in addition to autoimmunity and short stature: again, the autoimmune dysfunction was remarkably broad, encompassing type 1 diabetes, thyroid disease, enteropathy, liver disease, cytopenias, interstitial pneumonia, hepatitis, atopic dermatitis, alopecia, scleroderma and polyarthritis. Of note, one patient reported by Flanagan *et al.* showed improved growth and complete remission of her autoimmune disease after bone marrow transplant. Whilst the patients in the Flanagan paper all harboured *de novo* mutations, dominant inheritance was observed by Milner and colleagues, with less severe phenotypic expression and, indeed, clinical non-penetrance being observed.

STAT3 encodes a widely expressed transcription factor activated by many cytokines. Tyrosine phosphorylation, mediated by JAK kinases constitutively bound to cytokine receptors, results in the dimerization of STAT3 and its translocation to the nucleus where it drives gene transcription. At a molecular, the particular *STAT3* mutations involved affect conserved residues within the SH2,

transactivation or DNA-binding domains, leading to an increase in STAT3 activity by an undefined mechanism independent of STAT3 phosphorylation status. In contrast, loss-of-function germline mutations affecting the same domains of STAT3 are associated with AD hyper-IgE syndrome, a primary immunodeficiency characterized by high serum IgE levels and recurrent infections. It is possible that STAT3 activation leads to autoimmunity by impairing the development of regulatory T cells and promoting the expansion and activation of T helper type 17 (TH17) cells, although the mechanisms involved remain to be resolved. Summarizing, these recent findings emphasize the critical role of STAT3 in immune homeostasis, and contrast with the germline inactivating *STAT3* mutations that result in hyper IgE syndrome.

Interferon-induced helicase C domain-containing protein 1 (IFIH1; melanoma differentiation associated gene 5 — MDA5) gain of function. In 2014, Rice *et al.* [10^{••}] described gain of function mutations in a variety of neuro-inflammatory phenotypes including classical Aicardi-Goutières syndrome (AGS), later onset subacute neurological regression and apparently non-syndromic spastic paraparesis with normal neuroimaging. Of the eight patients described, in addition to major neurological deficits, two demonstrated features of a severe inflammatory lupus-like disease (including ulcerative photosensitive skin lesions, hepatosplenomegaly, lymphadenopathy, arthritis, pericardial effusion, haemolytic anaemia, and raised ESR, CRP and autoantibodies). This link to autoimmunity was highlighted more recently by van Eyck and colleagues [11] who reported a single patient with selective IgA-deficiency and mild lower limb spasticity diagnosed by the third year of life, followed by the occurrence of severe SLE with increased autoantibodies at 8 years of age. Of note, so far, two *IFIH1* mutation-positive subjects, aged 49 and 80 years, have been described demonstrating biochemical penetrance for an interferon signature, in the absence of any clinical features, thus demonstrating that pathological interferon signalling is not itself sufficient to cause/predict clinical disease.

IFIH1 encodes the cytosolic dsRNA sensor *IFIH1*/MDA5. The heterozygous gain of function mutations observed in these cases leads to marked type I interferon induction, which is considered as likely directly relevant to disease pathogenesis.

Transmembrane protein 173 — TMEM173 (stimulator of interferon genes — STING) gain of function. Two papers were published in 2014 describing gain of function mutations in *TMEM173*, encoding the cytosolic DNA adaptor molecule STING. Lui *et al.* [12^{••}] reported six children, all with disease due to *de novo* mutations, demonstrating a remarkably stereotyped clinical syndrome which the authors called STING-Associated Vasculopathy with onset in Infancy (SAVI), characterized

by neonatal-onset systemic inflammation, elevated erythrocyte sedimentation rate and levels of C-reactive protein, a severe cutaneous vasculopathy leading to extensive tissue loss and major interstitial lung disease. As for the majority of patients with gain of function mutations in *IFIH1*, none of these patients demonstrated features of autoimmunity, despite marked upregulation of type I interferon signalling. In contrast, Jeremiah and colleagues [13[•]] reported dominant inheritance in a three-generation family segregating a systemic inflammatory state with antinuclear autoantibody production, where cutaneous involvement was not prominent, and where the oldest mutation carrier in the family experienced no lung or skin disease to the age of 65 years.

STING is an essential adaptor molecule in the signalling of cytosolic DNA via the receptor cGAS, thus inducing type I interferon production. *TMEM173* gain of function mutations appear to cause constitutive activation of STING independent of ligand binding, with translocation to the Golgi indicative of spontaneous dimerization of the protein.

Despite marked upregulation of type I interferon signalling, the data published so far indicate that gain of function mutations in *IFIH1* and *TMEM173* are inconsistently linked to autoimmunity. Considering the well documented association of lupus with type I interferon, this observation is perhaps surprising, and presumably reflects variable engagement of the adaptive immune system secondary to an initial innate immune stimulus.

Coatamer subunit alpha (COPA) heterozygosity. In 2015, Watkin *et al.* [14^{••}] reported 21 patients from five families demonstrating a novel autoimmune phenotype characterized by childhood onset acute pulmonary haemorrhage with interstitial infiltration by lymphocytes, increased autoantibody production, inflammatory arthritis and immune-mediated renal disease. These authors identified autosomal dominant transmission of deleterious mutations in the Coatamer subunit alpha (*COPA*) gene all located within the same functional domain. Of note, there were a significant number of non-penetrant obligate carriers. The authors showed that *COPA* variants impair binding of proteins targeted for retrograde Golgi to ER transport. Overexpression studies suggested that these mutations might be acting in a dominant negative manner. Expression of mutant *COPA* led to ER stress and the upregulation of Th17 priming cytokines, and affected patients demonstrated a significant skewing of CD4⁺ T cells towards a T helper 17 (Th17) phenotype.

Summarizing, these findings implicate a new driver of autoimmunity due to alterations in cellular homeostasis caused by a defect in the intracellular trafficking pathway.

Conclusion

The six novel disorders described above highlight the already appreciated co-occurrence of autoimmunity and immunodeficiency in the same context [15]. Moreover, these newly recognized entities provide further examples of the value of monogenic disease characterization in the understanding of human autoimmunity. In that regard, we also draw the reader's attention to the recent work of Oftedal and colleagues indicating that specific heterozygous mutations in *AIRE* may contribute to a wide variety of autoimmune phenotypes [16,17]. The number of patients so far ascertained for each of the genotypes detailed here is small; however, major themes relating to variable expression and penetrance are already obvious, begging interesting questions as to the mediators of such differences [18[•],19–21]. The identification of somatic second-hits, polymorphisms/mutations in other genes and contributory environmental factors represent new frontiers in genetic research, whilst these novel phenotypes confirm what we already knew — that even monogenic disorders should be considered as ‘complex’.

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