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Rare phenotypes in the understanding of autoimmunity

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Abstract

The study of rare phenotypes has a long history in the description of autoimmune disorders. First Mendelian syndromes of idiopathic tissue destruction were defined more than 100 years ago and were later revealed to result from immune-mediated reactivity against self. In the past two decades, continuous advances in sequencing technology and particularly the advent of next-generation sequencing have allowed to define the genetic basis of an ever-growing number of Mendelian forms of autoimmunity. This has provided unique insight into the molecular pathways that govern immunological homeostasis and that are indispensable for the prevention of self-reactive immune-mediated tissue damage and 'horror autotoxicus'. Here we will discuss selected examples of past and recent investigations into rare phenotypes of autoimmunity that have delineated pathways critical for central and peripheral control of the adaptive immune system. We will outline the implications of these findings for rare and common forms of autoimmunity and will discuss the benefits and potential pitfalls of the integration of next-generation sequencing into algorithms for clinical diagnostics. Because of the concise nature of this review, we will focus on syndromes caused by defects in the control of adaptive immunity as innate immune-mediated autoinflammatory disorders have been covered in excellent recent reviews on Mendelian and polygenic forms of autoimmunity.

INTRODUCTION

Tissue injury as a consequence of immunological responses against self is the defining feature of autoimmune and autoinflammatory diseases.^{1–6} These disorders affect about 3–

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CONFLICT OF INTEREST

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5% of the Western population and include common diseases such as rheumatoid arthritis, autoimmune thyroiditis and type 1 diabetes (T1D).^{7,8} Depending on the contribution of the innate and adaptive immune system, clinical conditions of self-reactive tissue damage are distinguished into autoimmune and autoinflammatory disorders.⁶ In this context, autoimmunity is defined to result from adaptive T and B cell responses, whereas autoinflammation is characterized by a predominant role of the innate immune system in the disease process.⁶ Rather than serving as two separate and distinct entities, however, autoimmunity and autoinflammation represent the extremes of a continuum of self-reactive disorders, the vast majority of which are characterized by mixed contributions of the innate and adaptive immune system (Figure 1).⁶

Common autoimmune diseases originate from complex interactions between environmental influences and genetic risk factors. Genetics are a major determinant of the risk to develop autoimmune disorders, with concordance rates being higher for monozygotic compared with dizygotic twins, and, among monozygotic twins, being highest for T1D, psoriasis and Crohn's disease (20–50%) and lower for other disorders such as rheumatoid arthritis (12–15%) and systemic lupus erythematosus (11–40%).⁹ Furthermore, although not reflected in concordance rates, a substantial fraction of 'healthy' relatives of affected individuals develops subclinical signs of autoimmunity or autoimmune-mediated tissue destruction in the absence of clinically overt disease.^{10,11}

Genome-wide association studies (GWAS) of more than 20 autoimmune disorders have identified hundreds of disease-associated genetic loci and revealed an astonishing complexity of the genetic architecture of immune-mediated diseases.^{2,3} Although the majority of genetic polymorphisms identified by GWAS are associated with only a modest increase or decrease in disease risk, clustering of polymorphisms in specific cellular pathways has highlighted potential novel mechanisms involved in disease pathogenesis.^{2,3} Consistent with this notion, biological targeting of pathways enriched in disease-associated polymorphisms, such as the IL-12/IL-23/Janus kinase pathway, has demonstrated efficacy in the treatment of autoimmune disorders, including inflammatory bowel disease and psoriasis.^{12–14}

Although GWAS have provided significant insight into the genetic basis of autoimmune diseases, associations identified in these studies have rarely been tracked down to single causal alleles.^{2,15} Furthermore, an estimated 90% of causal alleles are noncoding and may predominantly exert their effects through modulation of gene expression.¹⁶ As a consequence, the specific pathways linking individual single-nucleotide polymorphisms (SNPs) to disease pathogenesis have remained unknown for the vast majority of genetic associations in autoimmune diseases. In contrast, as outlined in the following section, the study of patients with familial and/or extreme phenotypes of autoimmunity has revealed genetic variants of high effect size with direct functional implications for the affected biological pathways. Although such deleterious variants often give rise to rare Mendelian forms of autoimmunity, the occurrence of common SNPs in the same genes suggests shared pathways in rare and common forms of autoimmunity.

RARE PHENOTYPES IN THE UNDERSTANDING OF PATHWAYS TO AUTOIMMUNITY

Families with multiple members affected by autoimmune disorders as well as extreme traits of autoimmunity, including very early-onset disease, severe treatment-refractory disease and syndromes with affection of multiple organs, often exhibit an increased genetic contribution to disease pathogenesis.^{17–21} Accordingly, genetic studies in such entities have revealed rare Mendelian forms of autoimmunity in which defects in single genes are associated with breakdown of immunological tolerance and severe self-reactive tissue destruction.^{1,3,4,18} The investigation of rare disease phenotypes in the characterization of autoimmunity is, however, not a novel concept. Prototypical autoimmune and autoinflammatory disorders such as autoimmune polyendocrine syndrome type 1 (APS1) and periodic fever syndromes were clinically defined decades ago, although their molecular etiology was not solved until many years later.^{6,22–26} Recent advances in sequencing technology and in particular the advent of next-generation sequencing have revolutionized genetic studies and have led to the discovery of numerous novel Mendelian disorders of autoimmunity in the recent past.⁴ Indeed, despite formidable challenges in the interpretation of genome-wide data and their relation to a given phenotype, whole-exome and whole-genome sequencing continue to transform diagnostic approaches in medicine and are increasingly recognized as an integral part of diagnostic clinical algorithms, particularly for extreme traits or familial disease.^{17,19,27–29} The large number of non-synonymous variants detected by exome sequencing in each individual—whether affected or not—is still a major hurdle in linking genetic variants to disease.¹⁵ However, advances in bioinformatic processing of exome and genome information, improvements in *in silico* analysis of genetic variants, and particularly recent advances in genome engineering associated with the ability to rapidly investigate the functional impact of candidate variants in cell lines and model organisms have greatly facilitated the study of genetic associations in autoimmunity. As such, the study of extreme phenotypes and familial disorders of autoimmunity by linkage analysis and later by exome and genome sequencing has led to the description of a rapidly growing number of Mendelian syndromes of autoimmunity in the past two decades. The striking clinical phenotypes observed in these syndromes reflect the critical role of individual pathways in control of immunological homeostasis. In the following section, we will highlight past and recent examples of monogenic autoimmune disorders that have provided unique insight into the central and peripheral pathways required for the regulation of immunity and the prevention of self-reactive tissue damage.

LESSONS FROM MENDELIAN DISORDERS OF AUTOIMMUNITY

Central immune tolerance and AIRE

In the early 20th century, a clinical syndrome with onset in childhood or adolescence was described, which was characterized by mucocutaneous candidiasis and endocrine dysfunction, most commonly consisting of autoimmune hypoparathyroidism, autoimmune adrenal failure, ovarian failure, and less commonly T1D, autoimmune hypothyroidism, gastritis and hepatitis.^{1,24} This Mendelian syndrome of multi-organ autoimmunity was termed APS1 or autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy and

was demonstrated to follow an autosomal recessive pattern of inheritance. In 1997 and thus about 80 years after the first clinical description of APS1, two groups identified mutations in a gene termed the autoimmune regulator (*AIRE*) underlying APS1.^{25,26} Subsequent fascinating work demonstrated that *AIRE* can drive the expression of a multitude of otherwise tissue-specific antigens in thymic medullary epithelial cells, which leads to the presentation of these self antigens in the thymus and negative selection of autoreactive T cells.^{30,31} Accordingly, murine deficiency in *AIRE* was shown to lead to autoimmunity resembling human APS1.^{30,31} The description of *AIRE* explained previous, surprising observations of thymic expression of tissue-specific antigens^{32–34} and confirmed a critical role of this pathway in central tolerance and the prevention of organ-specific autoimmunity.

Autoimmunity due to loss of functional *AIRE* also contributes to immunodeficiency and serves as an interesting and mechanistically well-understood example of the close association between auto-immunity and immunodeficiency. As such, self-reactive humoral immune responses with the production of antibodies directed against cytokines of the IL-17/IL-22 pathway contribute to defects in IL-17-dependent antifungal immunity, which explains the predisposition to mucocutaneous candidiasis observed in essentially all patients with APS1.^{35,36} In conclusion, APS1 demonstrates how the description of an unusual phenotype of multi-organ autoimmunity has led to the discovery of a pathway essential for central thymic control of immunity and immunological homeostasis.

Cell death in peripheral control of immunity

In 1967, Canale and Smith³⁷ described the cases of five children who, early in life, developed lymphadenopathy, hepatosplenomegaly and signs of autoimmunity, including hemolytic anemia. The authors concluded, with remarkable prescience, that ‘The changes in the lymph nodes might be conceived as an immune response to a recognized auto-antigen’. Other autoimmune cytopenias as well as solid tissue affection, including hepatitis, nephritis, uveitis, dermatitis and involvement of the nervous system, were later described as part of the same disorder, subsequently termed the autoimmune lymphoproliferative syndrome (ALPS).¹ Intriguingly, a related phenotype was observed in the *lpr* and *gld* mouse strains and, following the discovery of mutations in *Fas* and *Fas* ligand (*FasL*) in these mouse models, mutations in the human orthologues were described in patients with ALPS.^{38–42} Furthermore, mutations in other genes in the same pathway, such as those encoding caspase-10 and -8, can give rise to similar or related clinical syndromes in humans.¹

FasL-induced ligation of Fas on T cells, B cells and antigen-presenting cells (APCs) such as dendritic cells (DCs) leads to the activation of caspase-8 and -10, which results in the activation of effector caspases and cell death.¹ The Fas–FasL pathway thus plays a critical role in immune cell death and the cessation of immune responses, whereas interference with this pathway results in the breakdown of immunological homeostasis. Consistent with the concept of a central role of Fas signaling in T cells, B cells and DCs, conditional deletion of *Fas* in either of these cell types in mice is sufficient to elicit autoimmunity.^{43,44} Although Fas-mediated cell death seems largely negligible for curbing lymphocyte responses to strong antigenic stimulation^{43,45}, it is of particular importance in limiting reactivity to weak

antigens,⁴³ thus serving as a mechanistic basis to explain autoimmunity in individuals with defects in this pathway.

Regulatory T cells and cytokines in peripheral tolerance

Powell *et al.*⁴⁶, in 1982, described a large kindred with severe and often fatal multi-organ autoimmunity, consisting of diarrhea, diabetes, eczema, hemolytic anemia and thyroiditis. Similar cases of severe autoimmune enteropathy, skin disease and autoimmune polyendocrinopathy were subsequently described by other groups with the lethality of this syndrome, termed Immune Dysregulation, Polyendocrinopathy, Enteropathy, and X-Linked (IPEX), considerably exceeding that found for APS1.¹ A related phenotype was described in the *Scurfy* mouse, an X-linked recessive mutant, which showed early postnatal lethality due to multi-organ lymphocytic infiltration and excessive cytokine production.⁴⁷ In 2001, the genetic defect underlying pathology in *Scurfy* mice was demonstrated to be a frameshift mutation in *Foxp3*, a forkhead/winged-helix family of transcriptional regulators that is highly conserved in humans.⁴⁷ Consistent with the phenotypic resemblance between pathology in the *Scurfy* mouse and that observed in IPEX, mutations in *FOXP3* were subsequently identified in human IPEX patients.^{48,49} Moreover, FoxP3 was demonstrated to be highly expressed by regulatory T cells (Tregs) and to be critical for Treg-mediated immune suppression^{50–52} The remarkable phenotype observed in IPEX patients and *Scurfy* mice thus documents the central role of Tregs in peripheral immune regulation and the prevention of severe and fatal autoimmunity. Indeed, this pathway of immunoregulation is required throughout life as interference with Treg function, even in adult mice, is associated with severe autoimmunity.⁵³ In accordance with the mechanistic basis of disease in IPEX, allogeneic stem cell transplantation can provide a causal treatment for IPEX patients and lead to long-term disease remission.^{54,55} As such, the description of IPEX and *Scurfy* has not only provided critical insight into the pathways required for immunological homeostasis but has also demonstrated the value of genetic studies in the development of strategies for personalized medicine.

Tregs express high levels of CD25, the alpha chain of the heterotrimeric IL-2 receptor (encoded by *IL2RA*), and are dependent on IL-2 for their maintenance and function.^{56,57} Accordingly, mice deficient in IL-2⁵⁸ as well as those deficient in the IL-2 receptor alpha⁵⁹ or beta chain⁶⁰ develop fatal autoimmunity characterized by peripheral accumulation of activated T and B cells and organ damage. Consistent with these observations in mice, patients with *IL2RA* mutations were described, who developed a severe IPEX-like syndrome with enteropathy, lymphadenopathy, hepatosplenomegaly, hemolytic anemia, eczema and endocrinopathy in the absence of mutations in *FOXP3* but with compound heterozygous⁶¹ or homozygous⁶² mutations in *IL2RA*. Analysis of one of these patients revealed normal numbers of Tregs but lack of their production of the regulatory cytokine IL-10.⁶¹ Thus, similar to defects in FoxP3, alterations in IL-2 signaling are associated with breakdown of peripheral immune tolerance.

Tregs and many other cells can regulate immune responses through the secretion of IL-10. Studies in mice have revealed that Treg-specific ablation of *Il10*, in contrast to loss of FoxP3, is not associated with signs of systemic autoimmunity such as splenomegaly or

lymphadenopathy but leads to organ-specific lymphocytic infiltration and spontaneous inflammation, particularly in the intestine and the lung.⁶³ This is consistent with observations in mice with CD4⁺ T cell-specific⁶⁴ or constitutive deletion of *Il10*⁶⁵, which develop spontaneous intestinal inflammation and enhanced skin contact hypersensitivity in the absence of systemic autoimmunity. These murine defects thus document a specific role of IL-10 in the prevention of inflammation at mucosal surfaces that is distinct from immunological defects observed upon interference with IL-2 signaling or general alterations in Treg function.

In 2009 and thus 16 years after the original description of *Il10*-knockout mice⁶⁵, the groups of Christoph Klein and Bodo Grimbacher reported a remarkably similar phenotype in two families with severe early-onset colitis, folliculitis and arthritis, which resulted from mutations in the IL-10 receptor subunits IL10R1 (*IL10RA*) and IL10R2 (*IL10RB*).⁶⁶ Similar observations were subsequently made in additional patients harboring mutations in *IL10*, *IL10RA* or *IL10RB*, all of whom showed very-early-onset intestinal inflammation, whereas allogeneic stem cell transplantation led to long-term clinical remission in accordance with a defect in bone marrow-derived cells^{66–71}. As such, while broad defects in Treg function due to loss of FoxP3 are associated with multi-organ autoimmunity, genetic perturbation of IL-10 signaling leads to inflammation predominantly affecting the intestine and the skin. Mendelian defects in IL-10 and the IL-10 receptor subunits have thus revealed an indispensable role of IL-10 in the prevention of inflammation at environmental surfaces.

Co-inhibitory signaling in peripheral tolerance

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is a central negative regulator of peripheral T cell responses. CTLA-4 is constitutively expressed by Tregs and involved in Treg-dependent immune suppression.⁷² Furthermore, CTLA-4 expression is induced upon activation of conventional T cells, which contributes to termination of T cell-dependent immune responses.⁷² CTLA-4 is a homolog of the costimulatory molecule CD28 and acts through high-affinity binding to the CD28 ligands CD80 and CD86. As such, CTLA-4 prevents CD28 from binding to CD80/86 and, in addition, provides direct, cell-intrinsic inhibitory signals and can also reduce CD80/86 expression on APCs through transendocytosis.⁷²

The critical role of CTLA-4 in immune regulation was revealed by the generation of mice with constitutive deletion of the murine homolog *Ctla4*, which demonstrate early lethality at 3–4 weeks of age and severe systemic autoimmunity with massive splenomegaly and lymphadenopathy as well as infiltration of the heart, pancreas, liver and lung by activated lymphocytes.^{73,74} Accordingly, therapeutic antibody-mediated blockade of CTLA-4 in human cancer immunotherapy, while highly efficacious, is associated with immune-related adverse events in the majority of patients, most frequently characterized by inflammation of the intestine, skin and endocrine glands.⁷⁵ In 2014, the groups of Uzel, Grimbacher and our groups independently described a severe syndrome of systemic autoimmunity as well as immunodeficiency associated with mutations in *CTLA4*.^{76–78} In remarkable similarity with the phenotype described for *Ctla4*^{−/−} mice almost 20 years earlier, individuals with *CTLA4* mutations exhibited lymphocytic tissue infiltration predominantly of the intestine, lung and the central nervous system, autoimmune enteropathy and lung disease, hepatosplenomegaly,

lymphadenopathy, autoimmune cytopenia, hypogammaglobulinemia, skin disease, thyroiditis, T1D, demyelinating encephalopathy, arthritis, and significant lethality.^{76–78} Functional studies revealed normal Treg numbers but hyperproliferation of activated T cells in part owing to defects in Treg-dependent inhibition of T cell proliferation.^{76–78} CTLA-4 thus plays a critical role in the inhibition and termination of T cell responses, whereas its deficiency results in severe systemic autoimmunity.

Together, defects in Fas, Fas ligand, caspase-8/10, FoxP3, CTLA-4 as well as alterations in IL-10 and IL-2 signaling highlight the importance of these pathways in different aspects of peripheral control of immunity, while mutations in *AIRE* serve as an intriguing example for autoimmunity resulting from defects in central negative selection of T cells in the thymus.

MENDELIAN DISEASES AND IMPLICATIONS FOR COMMON AUTOIMMUNE DISORDERS

Mendelian forms of autoimmunity are rare disorders. However, genetic studies in prevalent autoimmune diseases have revealed that common SNPs often reside in the same genetic loci as rare deleterious variants. As such, genetic pathways to self-reactive inflammation discovered through the study of Mendelian diseases also contribute to complex polygenic forms of autoimmunity and provide critical insight into the pathophysiology of these disorders.

A remarkable example for the convergence of rare and common variants in autoimmunity is *CTLA4*. As outlined above, mutations in *CTLA4* were recently described to be associated with severe multi-organ autoimmunity characterized by enteropathy, skin disease, thyroiditis, T1D and arthritis.^{76–78} In accordance with a broader role of CTLA-4 in autoimmunity, common SNPs in *CTLA4* were also found to be associated with more prevalent diseases such as rheumatoid arthritis, Hashimoto thyroiditis, T1D, IBD and celiac disease. These observations suggest that alterations in CTLA-4-dependent regulation of immunity indeed contribute to the pathophysiology of common autoimmune diseases. Consistent with this notion, recombinant CTLA-4 has recently entered the clinics as an efficacious biological treatment for rheumatoid arthritis.

Examples of genes containing both common variants associated with complex traits as well as rare coding variants associated with Mendelian autoimmunity are not limited to CTLA-4. For example, although mutations that disrupt IL-10 signaling are associated with severe, Mendelian forms of intestinal and skin inflammation as well as arthritis, SNPs in the *IL10* region were shown to be associated with IBD and the progression of rheumatoid arthritis.^{79,80} Similarly, SNPs in the *IL2* and *IL2RA* loci are associated with IBD, rheumatoid arthritis, T1D, vitiligo and multiple sclerosis.

Mutations in *AIRE* are associated with autosomal recessive and highly penetrant forms of multi-organ autoimmunity. In contrast, previous attempts to link genetic variation in *AIRE* to common autoimmune diseases such as T1D, Addison's disease and vitiligo have largely failed.¹ Oftedal *et al.*⁸¹ recently re-investigated the *AIRE* locus and could describe a number of dominant negative variants, all of which were located in the first plant homeodomain

(PHD1) of *AIRE*. These variants were inherited in a dominant manner and associated with milder and less penetrant forms of autoimmunity, later onset of disease, and often involvement of only one or two organs, most frequently vitiligo and pernicious anemia.⁸¹ Of note, some of these autosomal dominant variants showed a frequency of one to two per thousand in the general population, suggesting that they may indeed contribute to the pathogenesis of common forms of autoimmunity.⁸¹ Thus, although Mendelian disorders of autoimmunity are exceedingly rare, common polymorphisms in the same genetic loci suggest shared pathways to inflammation in common and rare forms of autoimmunity.

COINCIDENCE AND CAUSALITY OF GENETIC VARIANTS IN AUTOIMMUNITY

Because of improved accessibility of advanced sequencing technology as well as a considerable decrease in sequencing cost, whole-exome and -genome sequencing have increasingly become part of clinical diagnostic routines, particularly for extreme and familial cases of autoimmunity.^{17,19,28} However, the interpretation of such large genetic datasets is complicated by the wealth of variants discovered in each individual and limitations in *in silico* prediction of their functional effects. Even if the functional impact of a genetic variant is experimentally confirmed, it remains both critical as well as challenging to confirm the causality of discovered variants. This is further complicated by the observation that even within the group of Mendelian disorders incomplete penetrance is commonly observed.^{18,76,77} As false assignment of causality may have major detrimental consequences, various approaches for confirmation of causality have been suggested. MacArthur *et al.*¹⁵ emphasized the primacy of robust statistical analysis of genetic data, requesting that, among other measures, causality should only be suggested when variants in the same gene are associated with a similar clinical presentation in multiple unrelated individuals. Although the recommended strategy is well-suited to prevent type I errors and false assignment of causality, Casanova *et al.*⁸² noted that more than 20% of primary immunodeficiencies had initially been reported in single cases and thus, at that time, did not fulfill the requirements suggested by MacArthur. Casanova *et al.*⁸² therefore emphasized the critical role of experimental support in determining causality of genetic variants and encouraged the report of single patient-based discoveries as a first step towards potential confirmation by other groups. In line with the recommendation of robust experimental support, particularly through animal models, it is worthy to note that the description of spontaneous autoimmune phenotypes in knockout mice in the past not only often preceded, but also tremendously facilitated, the subsequent identification of similar disorders in man. As such, the genetic etiology of a number of autoimmune syndromes, including those associated with defects in Fas, FasL, FoxP3, CTLA-4, as well as IL-10 and IL-2 signaling was first identified in mice, with the subsequent description of mutations in similar pathways in human autoimmunity.

CONCLUSIONS AND FUTURE PERSPECTIVE

Technological advances in next-generation sequencing as well as continuous progress in the analysis of genetic data have dramatically facilitated the discovery of Mendelian disorders of

autoimmunity in recent years. This work has confirmed that rare phenotypes of autoimmunity, characterized by extraordinary severity, an early onset of disease and/or multi-organ involvement are often associated with a strong genetic component and may result from monogenic diseases. Mendelian disorders of autoimmunity, in particular, have delineated pathways essential for immunological homeostasis and the prevention of ‘horror autotoxicus’ and have highlighted potential novel targets for drug therapy and personalized medicine. Although Mendelian autoimmunity is of low prevalence, common polymorphisms are often found in similar genes, suggesting shared pathways in the pathogenesis of rare and common forms of autoimmunity.

Future work should focus on further implementation of next-generation sequencing in diagnostic algorithms in clinical medicine. This will require better strategies to delineate potential causal variants among hundreds or thousands of other non-synonymous variants found in the same individual and includes a need for refined tools for *in silico* prediction of functional outcomes of genetic variants as well as a requirement for high-throughput pipelines for experimental testing of variants in cell culture or animal models. The latter will be facilitated by recent advances in genetic engineering, such as CRISPR/Cas9-based approaches, which allow for rapid and inexpensive engineering of genetic variants in cell lines and model systems.

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Figure 1.

Examples of Mendelian forms of autoinflammation and autoimmunity. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.