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MULTI-AUTHOR REVIEW

New frontiers in primary immunodeficiency disorders: immunology and beyond…

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Primary immunodeficiencies (PIDs) include more than 130 different disorders affecting the development and function of the immune system [1, 2]. PIDs are generally monogenic disorders with a Mendelian inheritance, although a more complex polygenic origin has been described in some cases. PIDs are characterized by a variable clinical spectrum. As for other genetic diseases, phenotypic diversity is often caused by disease penetrance and interactions between genetic and environmental factors. PIDs are rare and have an overall prevalence of approximately 1:10,000 live births, with the exception of IgA deficiency (IgAD). However, a much higher rate is observed among populations with high consanguinity rates or genetically isolated. PIDs are classified according to the component of the immune system that is mostly engaged. Defects in adaptive immune responses include antibody deficiency syndromes and combined immunodeficiencies (CIDs). Conversely, impairment of innate immunity comprises disorders of several cells and molecules involved in the first defense against pathogen (i.e., phagocytes, Toll-like receptors, complement, NK cell). All of these forms are characterized by increased susceptibility to recurrent infections, severe infections, and sometime distinctive susceptibility to various types of pathogens depending on the nature of the immune defect. In addition, there are some forms of PIDs presenting mainly with a phenotype of immune dysregulation rather than immunodeficiency, and there are also syndromes with complex phenotype in which immunodeficiency is only one of multiple components of the disease spectrum.

PIDs affecting the B and T cell compartment are traditionally the "classic" forms of inherited immune deficit widely described and usually recognized also by general practitioners.

Defective antibody production causes increased susceptibility, primarily to bacterial infections with involvement of the upper and lower respiratory tract (otitis, sinusitis, and pneumonia), although recurrent viral infections are also common as well as infections caused by parasites (i.e., Giardia species) [2, 3, 4]. Primary antibody deficiencies (PAD) are the largest group of inherited disorders of the immune system [5] and comprise a variety of defects that interfere with B cell development, maturation, and/or function. They are mainly characterized by a marked reduction or absence of serum immunoglobulins (Ig) due to disturbed B cell differentiation. X-linked agammaglobulinemia (XLA) is a well-described condition affecting males and characterized by mutation of the X-linked Bruton's tyrosine kinase (*BTK*) gene [6, 7], named after Dr. Bruton, who described the first agammaglobulinemia patient in 1952 [8]. BTK, a transduction molecule of both pre-B cell-receptor (pre-BCR) and BCR, is essential for B cell differentiation in bone marrow. Besides XLA, many other gene defects were found as responsible of PAD and most of them have only been identified over the past 6 years. Moreover, the discovery of new PAD has contributed also to improve the clinical care of affected patients. The review by van der Burg et al.

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extensively describes the consequences of genetic defects in the context of B cell differentiation and discusses the technical developments, which can lead to novel insights and potentially to the discovery of unrevealed genetic defects in PAD.

Phagocytes play a key role in the "first" defense against bacteria and fungi. Therefore patients with defects of phagocyte number or function experience recurrent and severe infections of fungal (especially *Candida* and *Aspergillum* species) and bacterial origin. Respiratory tract and cutaneous infections are the majority, but deep-seeded abscesses are also common. Recurrent oral stomatitis is present in most cases. Chronic granulomatous disease (CGD) is caused by an impaired phagocyte function due to a defective NADPH oxidase enzyme complex [8, 9]. This complex enzyme is responsible for the burst of oxygen consumption (respiratory burst), which is associated with phagocytosis by professional phagocytes (i.e., neutrophils, eosinophils, monocytes, and macrophages). The molecular defects in CGD are well described, but the resulting pathophysiology is still not well understood. The key features are the exaggerated inflammatory responses and the formation of granuloma that may in part be explained by insufficient clearance of the causative microorganism [10]. However, inflamed sites are frequently sterile, indicating that this microbial persistence may not be the only explanation. The review of Kuijpers et al. described the latest novelties in the pathogenesis of CGD. In particular, the authors give a nice overview of the dilemma between "inflammation and infection". The role of the enzyme indoleamine 2,3-dioxygenase (IDO) at the border of CGD granulomata is highlighted [11]. This enzyme degrades the amino acid L-tryptophan to L-kynurenine [12]. L-tryptophan is an essential amino acid for most microbial pathogens. On the contrary, L-kynurenine and some of its metabolites halt cell cycle progression and promote apoptosis of T lymphocytes, thereby diminishing the initiation of an immune response [13, 14] and favoring inflammation. Therefore, IDO expression in granulomata may prevent bacterial growth and dampen T cell responses towards the causative pathogen. These findings are certainly pivotal to better understand the boundary of innate and adaptive immunity and the complex interplay between the two defense systems.

Over the years, phenotypes relating to aspects other than infections, such as autoimmunity, inflammation, etc., have been associated with PIDs. In this light, the emerging clinical entities caused by impaired cellular cytotoxic activity support the important role of the balance between innate and adaptive immunity. Natural killer (NK) cells act as important sentinels of the immune system and represent a subset of cytotoxic lymphocytes (CTLs) able to recognize and lyse virus-infected cells without previous sensitization [15]. In contrast to B and T cell receptors, NK cell receptors do not undergo somatic recombination; the balance of signals between activating and inhibitory receptors determines the outcome of NK cell function, mainly mediated by secretion of cytokines. This is particularly relevant during the early phases of inflammatory responses when NK cells have a crucial role in connecting with the other cells of the immune system. A complicated network of interactions, similar to a "traffic jam", can take place after the recruitment of these different cells to inflammatory sites in response to tissue damage resulting from invasion by pathogens [16]. Sieni et al. largely described the clinical syndromes caused by impaired NK function and due to mutations of genes involved in the granule-dependent exocytosis pathway. These disorders are genetically heterogeneous, but clinically characterized by hemophagocytic lymphohistiocytosis, a severe syndrome caused by overwhelming inflammation and uncontrolled but ineffective immune response. Cardinal signs and symptoms are prolonged and unexplained fever unresponsive to antibiotics, hepatosplenomegaly, pancytopenia, and hemophagocytosis. Crucial in the pathophysiology of HLH is a defect in cytotoxic activity that prevents efficient removal of antigens and down regulation of immune response resulting in sustained activation and proliferation of CTLs and NK cells [17]. Persistently activated CTLs and NK cells produce large amount of inflammatory cytokines, generating tissue infiltration and inflammation.

Unrestrained inflammation means that somehow a regulatory feedback is lacking. Nevertheless, inflammation involves crosstalk between the innate immune system with the adaptive immune system. The immune balance is maintained by regulatory mechanisms that keep under control immune activation. Virtually all components of the immune system participate in the development or maintenance of immune tolerance; however, immune tolerance mechanisms are generally divided into those that act centrally and those that function peripherally [18]. Central immune tolerance is established through the elimination of auto-reactive T cells during thymic differentiation. Concomitantly, the thymus plays an essential role in the development of naturally arising FOXP3 + regulatory T cells (nTreg) from cells that have intermediate autoreactivity [19]. Immune regulation via peripheral tolerance is mainly achieved by Tregs. These cells can be divided into natural thymic-derived and induced Treg cells that are generated outside the thymus [20]. Peripheral Treg cells probably stem from suboptimal antigen presentation and/or costimulatory signals during T cell activation in the periphery. In contrast, it is thought that natural Treg cells differentiate from thymocytes following high affinity binding to the self-peptide in the thymus. These differentiated T cells are able to suppress potentially autoreactive T cells in the periphery via cytokine secretion or cell–cell

contact. Two mechanisms of suppression have been described: (1) suppression via cytokines, IL-2 consumption and cytolysis targeting T cells, and (2) decreased costimulation or antigen presentation [21]. Defects in immune tolerance mechanisms result in severe autoimmunity. The review of Gambineri and Torgerson summarizes the clinical presentation and molecular basis of a unique group of inherited disorders characterized by immune dysregulation, which have dramatically expanded our understanding of immune tolerance mechanisms in humans. Patients with severe, familial forms of multi-organ autoimmunity have been recognized and clinically described for more than 40 years. Some are characterized primarily by autoimmunity and others by autoimmunity combined with susceptibility to specific infectious organisms. The first mechanistic understanding of these disorders began to emerge approximately 10 years ago with the initial identification of the causative gene of IPEX syndrome (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked), *FOXP3*. This gene encodes for a transcriptional factor that binds to and regulates a large number of target genes and is required for development of the suppressive function of CD4+CD25+FOXP3+ Tregs [22, 23, 24]. The absence of functional FOXP3 leads to a deficiency of functional regulatory T cells and the consequent autoimmunity/immune dysregulation observed in IPEX. The identification of genetic defects in a variety of immune dysregulation syndromes (i.e., APECED, CD25 deficiency, STAT5b deficiency, ITCH deficiency etc.) has yielded important insights into basic mechanisms of autoimmunity and inflammation in humans.

This is also strengthened by the new theories on the pathogenesis of inflammatory bowel disease (IBD), where the immune system plays an important role, as illustrated by Glocker et al. IBD comprises a group of diseases that may be caused by a variety of different irregularities of the immune system. The occurrence of an IBD phenotype in several PIDs (i.e., WAS, IPEX, and in particular CGD) indicates that IBD may be the final stage of several complex immunodeficiencies. The lack of an initial acute inflammation with the reduced elimination of foreign material and bacteria may result in a chronic inflammation with T cell mediated granuloma formation, which are supposed to confine hazardous material and protect the intestine from bacterial spreading [25, 26]. The development of a Chron-like enterocolitis in CGD supports the theory that also the classical form of IBD, as Chron's disease, may be an immunodeficiency due to malfunction of macrophages and impaired pro-inflammatory responses [27]. The continuous secretion of pro-inflammatory cytokines by cells like macrophages that form the granuloma, sustains the chronic inflammation and finally results in extensive tissue damage. Moreover, the role of macrophages in IBD is even more emphasized by IL-10. Several cell types (including monocytes, macrophages, and dendritic cells) [28, 29] can produce IL-10, which is a critical player in maintaining immune homeostasis. IL-10 restricts and terminates immune responses by limiting the secretion of pro-inflammatory cytokines. Due to its unique role in balancing the immune system, IL-10 has always been in the focus of IBD research. Interestingly, the authors have demonstrated pathogenetic mutation within IL-10 and IL-10 receptor genes in certain forms of early onset IBD presenting with enterocolitis and perianal disease, and the formation of multiple abscesses and enterocutaneous fistula [30, 31]. Therefore, these cutting-edge findings support the hypothesis that some forms of IBD may be congenital Mendelian traits in contrast to the established knowledge that IBD is a disorder with a complex multigenic background. The detection of mutations in the IL-10 signaling pathway demonstrates the importance of immune regulatory factors in keeping the immune system in balance to control the chronic stimulation by microbes in the intestine.

In conclusion, the disease that better "summarizes" the diverse clinical manifestations of PIDs is DiGeorge syndrome (DGS), classically defined as a congenital T cell immunodeficiency secondary to aplasia or hypoplasia of the thymus gland associated with congenital heart defects and hypocalcaemia, due to small or absent parathyroid glands. The most common cause of the syndrome is a hemizygous deletion of 22q11.2. This deletion has been linked to a heterogeneous group of disorders with an overlapping phenotype, leading to further expansion of clinical spectrum of DGS [32, 33]. Immunodeficiency is one of the most common manifestations. Gennery extensively reviewed the immunological aspects and the new insights into the pathophysiology of the disorder. The immunological phenotype may vary widely and the degree of immunodeficiency seen in these patients may include defects of T lymphocyte number and function as well as humoral defects [34, 35]. Complete thymic aplasia presents with severe or complete T lymphopenia, like a severe combined immunodeficiency phenotype. In this case, thymic transplantation offers a new approach to treatment, as well as insights into thymic physiology and central tolerance [36]. However, DGS patients are more commonly affected by combined partial immunodeficiency with antibody deficiency, leading to recurrent sino-pulmonary infection in early childhood [37, 38]. Autoimmunity is an increasingly recognized complication in DGS patients, probably linked to the abnormal thymic development (i.e., reduced AIRE expression and Treg development) and resulting in both partial immune-deficiency and immune-dysregulatory features [39, 40].

Big steps have been taken to move forward the field since Bruton's first description of agammaglobulinemia in 1952

and more than 100 different PIDs have been described in the past decades. This increase in the recognition rate of more different types of PIDs is due to advances in our knowledge about the immune system and the novel progress in immunological and molecular techniques. Still, the topic of inborn error of the immune system is far from being fully exhausted. In particular, the understanding of innate and adaptive immunity interaction and regulation remains mostly unrevealed, but also, new fields open with the demonstration that phenotypes classically thought to be caused by an "overreaction" of the immune system (i.e., Chron's disease), have recently been shown to result from PIDs.

Overall, the reviews in this issue of the journal illustrate how novel genetic etiologies of well-known PID sand immunological phenotypes are increasingly being recognized as well as novel phenotypes caused by congenital immune defects, extending well beyond infectious diseases. The elucidation of these will surely constitute our next challenges. So far, we may have seen only a "drop in the ocean"…

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