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INSIGHTS

The nature of human IL-6

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Countless functions have been attributed experimentally to IL-6. In this issue of *JEM*, Spencer et al. (https://doi.org/10.1084/jem.20190344) reveal essential, nonredundant functions of human IL-6. Patients with genetic deficiencies of the IL-6 receptor suffer from "hyper IgE syndrome."

Human cytokines have never been shown to do in natura what they were predicted to do by in vitro studies, and even in vivo studies in mice. Inherited deficiencies of human IFN-α/β, IFN-γ, IL-2, IL-7, IL-10, IL-11, IL-12, IL-17, IL-21, IL-23, and GM-CSF, or of their receptors, have all been associated with unexpected clinical phenotypes (Picard et al., 2018). Some inborn errors of immunity disrupting cellular responses to multiple cytokines have proved even more surprising, as illustrated by IRAK4 and MyD88 deficiencies, which abolish responses not only to most TLR agonists, but also to all IL-1 cytokines, including IL-1β, IL-18, and IL-33, while conferring a selective predisposition to staphylococcal and pneumococcal infections with weak inflammation (Casanova et al., 2011). More detailed immunological phenotypes of inborn errors of cytokines also frequently turn up surprises and provide new insight. However, from a genetic perspective, these observations are not actually as surprising as they might initially appear, because outbred humans living in a village are not really equivalent to inbred mice tested in a cage. Admittedly, some patients lacking a cytokine or its receptor are the offspring of first-cousin parents, but only ∼6.25% of their genome is homozygous, a proportion much smaller than the 100% homozygosity of inbred mice (with their many loss-of-function alleles). Human genetic studies are robust, especially when consistent phenotypes are found in unrelated patients of various ancestries. How many mouse cytokine knockouts have been tested in multiple genetic backgrounds? When rigorously conducted, human genetic studies of single patients are also robust (Casanova et al., 2014). Could there be a better way to delineate essential and redundant functions of any gene than by defining the natural, as opposed to experimental, phenotypes of mutant organisms? Should physiology and pathology not ultimately aim to define the ecologically relevant and evolutionarily selected functions of genes at both the whole-organism and whole-population levels? Is this goal not best achieved in humans (Quach and Quintana-Murci, 2017; Davis and Brodin, 2018)?

The time has come for IL-6 to begin to reveal its true nature. IL-6, first identified as B cell stimulatory factor 2, or BSF2, was cloned in 1986 and has been one of the most intensively studied cytokines ever since (Kishimoto, 2005). Its pleiotropy is legendary. It is produced by and acts on many cell types by binding to a receptor composed of the transmembrane IL-6R (or its soluble form, sIL-6R) and the transmembrane GP130 protein. The result is a tremendous diversity of effects, in cis and trans, on the development and function of many leukocyte subsets and various other cell types. IL-6–producing cells include bone marrow stromal cells, T cells, macrophages, dendritic cells, fibroblasts, synovial cells, endothelial cells, glia cells, and keratinocytes, whereas IL-6–responsive cells include B cells, T cells, hepatocytes, monocytes, vascular endothelial cells, and synoviocytes. Studies of mouse IL-6 at the whole-organism level have revealed multiple roles, the most frequently studied of which relates to inflammation, host defense, metabolism, bone homeostasis, and tissue regeneration (Kishimoto, 2005). Mice with knockouts of IL-6 or IL-6R display a wide range of immunological and nonimmunological phenotypes, including impaired inflammatory responses to localized tissue damage, impaired induction of acute-phase proteins, and impaired responses to or enhanced susceptibility to various microbes (e.g., vesicular stomatitis virus, vaccinia virus, and *Listeria monocytogenes*). Following these studies, therapeutic agents blocking IL-6 activity have been introduced into clinical practice for various inflammatory conditions. These studies raised questions about the essential immunological functions of human IL-6 and the most likely clinical phenotype of humans with genetic defects resulting in a lack of IL-6 immunity.

Insights from Anne Puel and Jean-Laurent Casanova.

In this issue of *JEM*, Spencer et al. provide the first description of humans lacking the

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Table 1. **Impaired IL-6 immunity and HIES**

Gene defect	High IgE	Eczema	Staphylococcal disease	Inflammation	Extra-hematopoietic features	IL-6 response	References
TYK2$^{-/-}$ (Japanese patient)	+	+	+	ND	–	–	Minegishi et al., 2006
TYK2$^{-/-}$ (other patients)	–	–	–	+	–	+	Kreins et al., 2015
TYK2$^{P1104A/P1104A}$	–	–	–	+	–	+	Boisson-Dupuis et al., 2018
STAT3$^{+/-}$	+	+	+	–	+	–	Minegishi et al., 2007
ZNF341$^{-/-}$	+	+	+	+/–	+/–	–	Béziat et al., 2018; Frey-Jakobs et al., 2018
IL6ST$^{-/-a}$	+	+	+	–	+	–	Schwerd et al., 2017
IL6R$^{-/-}$	+	+	+	–	–	–	Spencer et al., 2019
Anti–IL-6 autoantibodies	–	–	+	–	–	–	Puel et al., 2008

[a]The *IL6ST* mutant allele is hypomorphic.

α chain of the IL-6 receptor. These patients present skin allergies, high serum IgE concentrations, and cold staphylococcal lesions—the core triad defining hyper IgE syndrome (HIES). This finding is probably surprising for the vast majority of scientists and physicians familiar with IL-6, but it is much less so—indeed, it is even confirmatory—for those familiar with human inborn errors of immunity. Indeed, the first evidence for a causal role of IL-6 deficiency in the HIES triad was provided by Minegishi's discovery of a Japanese patient with inherited TYK2 deficiency and impaired IL-6, IL-10, IL-12, IL-23, and IFN-α/β responses (Minegishi et al., 2006). It was suggested that impaired responses to IL-6, IL-12, and IFN-α/β accounted for the patient's HIES, mycobacterial infections, and viral infections, respectively (Table 1). This discovery led Minegishi to another important finding: that of dominant-negative *STAT3* mutations impairing responses to IL-6 and other cytokines (e.g., IL-10) causing the autosomal dominant form of full-blown HIES, which also includes a number of nonhematopoietic features (Minegishi et al., 2007). Interestingly, human IL-6 responses were later shown not to be dependent on TYK2 when the lack of response to IL-6 of the TYK2-deficient Japanese patient was shown to be unrelated to TYK2 deficiency (Kreins et al., 2015). Cells from other TYK2-deficient patients, of various ancestries and without HIES, responded normally to IL-6. They had viral disease because of impaired IFN-α/β signaling, and mycobacterial disease because of impaired IL-12 and IL-23 signaling. Moreover, homozygosity for the P1104A TYK2 allele, which selectively disrupts IL-23 responses, underlies mycobacterial disease without HIES and viral infections (Boisson-Dupuis et al., 2018). The Japanese TYK2-deficient patient's poor response to IL-6 thus remains unexplained, but probably underlies his core HIES features, which are not seen in other TYK2-deficient patients, and also, by inference, HIES in patients heterozygous for dominant-negative *STAT3* mutations.

Patients with an autosomal recessive deficiency of ZNF341, a transcription factor that governs the production and activity of STAT3, also display most of the features of HIES (Béziat et al., 2018; Frey-Jakobs et al., 2018). Their inflammatory responses are stronger and their extra-hematopoietic features are milder than those of patients with STAT3 deficiency. Cells from ZNF341-deficient patients display impaired STAT3-dependent responses to IL-6 and other cytokines (e.g., IL-21). These findings further suggest that impaired IL-6 immunity underlies at least some of the features of the HIES triad. Moreover, homozygous deleterious mutations of *IL6ST*, encoding the GP130 common chain (Schwerd et al., 2017), have been found in patients with HIES and extra-hematopoietic features reminiscent of STAT3 heterozygotes (e.g., dental abnormalities or skeletal abnormalities, such as craniosynostosis and scoliosis). GP130 is shared by the receptors for IL-6, IL-11, IL-27, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotrophin 1, and cardiotrophin-like cytokine. These *IL6ST* mutations apparently impair responses to IL-6, IL-11, IL-27, and oncostatin M, whereas responses to leukemia inhibitory factor seem to be preserved, and other responses were not studied. Biallelic null mutations of *IL1RA* were found to underlie some of the bone lesions seen in patients with *STAT3* and *IL6ST* mutations, such as craniosynostosis and dental abnormalities. Based on comparisons with patients presenting *TYK2*, *STAT3*, and *ZNF341* mutations, the disruption of IL-6 responses probably underlies the HIES triad in IL-6ST-deficient patients. Collectively, the discoveries of inherited TYK2, STAT3, ZNF341, and IL-6ST deficiencies suggest that the disruption of cellular responses to IL-6 may underlie some, perhaps even all, features of the HIES triad: cold staphylococcal lesions, severe cutaneous allergy, and elevated serum IgE levels.

In this context, the findings of Spencer et al. (2019) in two unrelated kindreds provide compelling genetic evidence that IL-6 is essential for host inflammatory defense against staphylococci and for the regulation of IgE-mediated allergy, including severe eczema in particular. Patients treated with IL-6 blockers for long periods should be carefully monitored for these side effects. Remarkably, patients with neutralizing autoantibodies against IL-6 have also been reported (Puel et al., 2008). They suffer from cold cutaneous staphylococcal lesions

with no detectable increase in serum C-reactive protein concentration. Collectively, inborn errors of IL-6R and their autoimmune phenocopies suggest that the mildly symptomatic staphylococcal infections seen in patients with IRAK4 or MyD88 deficiency may result in part from the poor production of IL-6 in response to IL-1 cytokines and TLR agonists. Intriguingly, patients with autoantibodies against IL-6 have no eczema or high serum IgE concentrations. Perhaps IL-6 is neutralized incompletely or too late in life in these patients? Alternatively, the alleles at modifier loci may differ between IL-6R–deficient patients and those with autoantibodies against IL-6. The remote but finite possibility that IL-6 and allergy are not related, and that IL-6R–deficient patients suffer from eczema and high IgE concentrations for another reason (e.g., another, undetected causal mutation), is very low, but cannot yet be completely excluded. It is not only the features displayed by patients with impaired IL-6 immunity that are interesting, but also those that are absent. Many facets of human IL-6 seem to be redundant. Indeed, many of the infectious phenotypes of IL-6/IL-6R–deficient mice were not found in the corresponding human patients. Moreover, IL-6R–deficient patients, like TYK2- and IL-6ST–deficient patients and unlike STAT3- and ZNF341-deficient patients, apparently display normal IL-17 immunity and do not suffer from chronic mucocutaneous candidiasis. Finally, the discovery of IL-6R–deficient patients and the essential functions of human IL-6 in natura raises new and exciting questions. For example, what is the molecular and cellular basis of cold staphylococcal lesions, skin allergy, and high IgE levels in IL-6R–deficient patients? It may be possible to address such questions in mice, provided that these phenotypes can be modeled properly. However, we are more likely to find the answers to these questions in the pursuit of the genetic and immunological dissection of these phenotypes in humans, as already illustrated by the considerable progress made since the discovery of TYK2 deficiency, in which this work on IL-6R is an important, but not the final, milestone.

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