

Patients informing immunobiology: How disorders of IL-21 receptor signaling unravel pathways of CD8 T-cell function

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The study of monogenic diseases of the immune system can help decipher fundamental mechanisms in immunobiology by identifying new immune pathways, clarifying the role of affected molecules in known pathways, and often highlighting differences in gene function between human subjects and nonhuman models. Although extraordinarily rare, clinical entities caused by abnormal cytokine signaling are a group of such disorders that can be highly instructive in this regard.

IL-21 is a cytokine responsible for pleiotropic effects on multiple cell lineages of the immune system. Signal transducer and activator of transcription (STAT) 3 and STAT1 appear to propagate IL-21 signaling through the IL-21 receptor (IL-21R) in a variety of cell types.¹ As such, study of patients with STAT1 deficiency, STAT3 deficiency and autosomal dominant hyper-IgE syndrome (AD-HIES), and the recently identified IL-21R deficiency² all allow for the dissection of the role of this pathway in immune functions.

IL-21 is well known from mouse studies to participate in a variety of CD8 functions, from proliferation and survival to memory formation and cytotoxicity.³ Therefore in this issue of the *Journal*, Ives et al⁴ take advantage of the opportunity presented by these patients to expertly analyze the consequences of defects in each of these molecules in CD8 biology.

The authors made several interesting observations. First, they found that IL-21 requires normal signaling through STAT3, but not STAT1, to induce CD8 cytotoxic machinery. Interestingly, however, patients with STAT1 mutations can have significant viral disease,⁵ whereas impaired responses to primary viral infections are not a common feature of AD-HIES with a STAT3 mutation. To at least partially resolve the conflicting results between *in vitro* cytotoxicity and clinical phenotype, the authors show that T-cell receptor signaling can effectively override the need for maximal STAT3-mediated IL-21 signaling by reducing the threshold, perhaps by using the residual normal STAT3 in these

AD-HIES-derived cells, and rendering them fully cytotoxic. Therefore although it can be observed that IL-21 plays a prominent role in cytotoxicity, human host defenses have intrinsic redundancy that renders such a role less important when normal T-cell receptor engagement and signaling occur. It remains to be seen whether STAT1 and CD8 cytotoxicity truly are independent of the viral infections seen in patients with STAT1 mutations. Similarly, although the 4 patients with IL-21R mutations did have some mild viral infections, they are too few in number and too young in age to draw substantial conclusions about the consequences of IL-21R mutations themselves on host defense against acute or chronic viruses. Of note, it is not very likely that impaired *in vitro* cytotoxicity in IL-21R-deficient patients could explain the most prominent infectious manifestation of their disease, invasive cryptosporidiosis, given the lack of such infection in patients with other diseases that affect CD8 T cells, such as ζ chain-associated protein of 70 kDa (ZAP70) deficiency or disorders of MHC class I.

A second observation made by Ives et al⁴ regards the role of IL-21 in human CD8 homeostasis. IL-21 seems to be required for maximal CD8 proliferation because augmentation of proliferation of CD8 T cells in patients with IL-21R mutations is abolished. Interestingly, however, although IL-21 appears to primarily use STAT1 and STAT3 for signaling, STAT1- or STAT3-mutated CD8 T cells have no such defect. This is consistent with the findings of Siegel et al,⁶ who found that *ex vivo* STAT3 mutant CD8 memory T cells had, if anything, increased cellular turnover rates. These observations are also consistent with the cytotoxicity result, showing that a strong T-cell receptor stimulus can change the threshold for IL-21 activity, arguing either for the requirement of only the small residual amount of STAT3 function seen in patients with AD-HIES or the use of other signaling pathways, such as mitogen-activated protein kinase or Akt,⁷ by IL-21R in the patients with STAT mutations.

Although primary viral infections were not thought to be a significant problem for patients with AD-HIES, Siegel et al⁶ showed that these patients do have widespread abnormalities in the control of chronic latent herpesviruses, namely EBV and varicella zoster virus. It should be noted that although at a substantially lower rate, a subsequent study from Chandresris et al⁸ showed that a number of patients did have complications from primary varicella zoster virus and herpes simplex virus. These clinical studies suggest that a distinct viral phenotype was present in patients with HIES, suggesting a unique pathophysiologic mechanism.

Siegel et al⁶ showed that there appeared to be a specific central memory defect in CD4 and CD8 T cells from patients with AD-HIES and that numbers of varicella zoster virus- and EBV-reactive central memory T cells were substantially lower in patients with AD-HIES. This defect did not appear to be due to increased death or decreased turnover; effector memory

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populations appeared normal, whereas naive cell numbers were increased, arguing for a primary central memory differentiation defect.⁶ In support of this, Siegel et al found that short-term *in vitro* differentiation cultures of naive cells from patients with HIES were less able to acquire memory T-cell surface phenotypes compared with control subjects. The reason for this appeared to be abnormal expression of STAT3 target genes in naive T cells, which are important for the generation of central memory cells, in this case suppressor of cytokine signaling 3 and B-cell lymphoma 6. Those results were consistent with findings by Cui et al,⁹ who showed in mice that the STAT3-targeted genes suppressor of cytokine signaling 3 and B-cell lymphoma 6 were required in CD8 cells specifically for long-lived memory formation.

The third finding by Ives et al⁴ revolved around memory cells. They found defects in both central and effector compartments of CD8 T cells in patients with AD-HIES and patients with IL-21R mutations. The difference between their findings and those of Siegel et al⁶ and Cui et al,⁹ who argued for a specific central memory defect is perhaps due to the different surface marker scheme used for identifying these cells or potentially due to different acute and/or chronic disease states of the 2 groups of patients, who are managed by different clinical teams on different continents. Similar to Siegel et al,⁶ an accumulation of naive cells was noted, and suppressor of cytokine signaling 3 expression was indeed noted to be diminished in stimulated naive cells, arguing in favor of a differentiation defect.

Careful human immunization trials and perhaps mouse models might be needed to definitively determine whether STAT3 mutations lead to focal or more broad memory defects and to determine the precise mechanism. In addition, such studies, along with identifying other diseases with focal impairment in host defense against chronic viral reactivation, will help to establish the extent to which the memory defects observed by Ives et al⁴ and Siegel et al⁶ are directly causal in human subjects.

The observations made in these studies and the questions raised are not just esoteric or academic ones. Pharmacologic inhibition

of cytokines, cytokine signaling, or both, especially of IL-21, STAT1, or STAT3, as therapies for inflammatory and neoplastic disease will require careful attention to the host defense and immunologic consequences of such inhibition, and these patients provide a glimpse into those potential consequences. Similarly, by showing reproducible, concrete *in vitro* observations in these patient groups despite clinical variability, it is possible that other groups of patients with yet unidentified, clinically subtle disruptions in these pathways could be identified more clearly. Taking advantage of the improved understanding of the *ex vivo* cellular consequences of specific molecular defects, consequences that might be measurable more reproducibly even when clinical manifestations are more variable, new genetic diseases of CD8 function might be more easily discovered.

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