Published in final edited form as:

J Allergy Clin Immunol. 2013 August ; 132(2): 412–413. doi:10.1016/j.jaci.2013.06.024.

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

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Keywords

Stat1; stat3; il-21; cd8; Antiviral immunity

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 humans and non-human models. While extraordinarily rare, clinical entities caused by abnormal cytokine signaling are a group of such disorders, which can be highly instructive in this regard.

IL-21 is a cytokine responsible for pleiotropic effects on multiple cell lineages of the immune system. STAT3 and STAT1 appear to propagate IL-21 signaling via the IL-21r in a variety of cell types¹. As such, study of patients with STAT1 deficiency, STAT3 deficiency—autosomal dominant hyper-IgE syndrome (AD-HIES), and the recently identified IL-21r deficiency all allows 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 cytotoxicity². In this issue, Ives et al therefore take advantage of the opportunity presented by these patients by utilizing primary tissue from these patients and expertly analyzing the consequences of defects in each of these molecules in CD8 biology.

The authors made several interesting observations. First, they find that IL-21 requires normal signaling through STAT3, but not STAT1, to induce CD8 cytotoxic machinery. Interestingly, though, STAT1 mutant patients can develop significant viral disease³, while impaired responses to primary viral infections is not a common feature of STAT3-mutant AD-HIES. To at least partially resolve the conflicting results between in vitro cytotoxicity and clinical phenotype, the authors show that TCR signaling can effectively override the need for maximal STAT3-mediated IL-21 signaling, by reducing the threshold—perhaps utilizing the residual normal STAT3 in these AD-HIES derived cells—and rendering them fully cytotoxic. Therefore while it can be observed that IL-21 plays a prominent role in cytotoxicity, human host defenses have intrinsic redundancy which renders such a role less important when normal TCR engagement and signaling occurs. It remains to be seen whether STAT1 and CD8 cytotoxicity truly are independent of the viral infections seen in

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STAT1 mutant patients; similarly while the four 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 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 other diseases which affect CD8 T-cells such as ZAP70 deficiency or disorders of MHC Class I.

A second observation made was regarding the role of IL-21 in human CD8 homeostasis. IL-21 seems to be required for maximal CD8 proliferation, as augmentation of proliferation of CD8 T-cells in IL-21r mutant patients is abolished. Interestingly, however, while IL-21 appears to primarily utilize STAT1 and STAT3 for signaling, STAT1 or STAT3 mutated CD8 T-cells have no such defect. That is consistent with the findings of Siegel et al⁴ who found that ex vivo STAT3mut CD8 memory T-cells had, if anything, increased cellular turnover rates. These observations are also consistent with the cytotoxicity result, showing that a strong TCR 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 AD-HIES, or the utilization of other signaling pathways, such as MAPK or Akt⁵ by IL-21r in the STAT mutant patients.

While indeed primary viral infections were not thought to be a significant problem for AD-HIES patients, Siegel et al showed that these patients do have widespread abnormalities in the control of chronic latent herpesviruses, namely EBV and VZV. It should be noted that while at a substantially lower rate, a subsequent study from Chandesris et al showed that a number of patients did have complications from primary VZV and HSV⁶. These clinical studies suggest that a distinct viral phenotype was present in HIES, suggesting a unique pathophysiologic mechanism.

Siegel et al had shown that there appeared to be a specific central memory defect in CD4 and CD8 T-cells from AD-HIES patients, and that VZV and EBV-reactive central memory T-cells were substantially lower in AD-HIES patients. This defect did not appear to be due to increased death or decreased turnover, effector memory populations appeared normal, while naïve cells were increased, arguing for a primary central memory differentiation defect⁴. In support of that, Siegel et al found that short term in vitro differentiation cultures of naïve HIES were less able to acquire memory T-cell surface phenotypes compared to controls. The reason for this appeared to be abnormal expression of STAT3 target genes in naïve T-cells which are important for generation of central memory cells, in this case SOCS3 and BCL6. Those results were consistent with findings by Cui et al, who had shown in mice that the STAT3 targeted genes SOCS3 and BCL6 were required in CD8 cells specifically for long-lived memory formation⁷.

The third finding by Ives et al revolved around memory cells. They found defects in both central and effector compartments of CD8 T-cells in AD-HIES patients and IL-21r mutant patients. The difference between their findings and those of Siegel and Cui et al 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 two groups of patients, who are managed by different clinical teams on different continents. Similar to Siegel et al, an accumulation of naïve cells was noted, and SOCS3 expression was indeed noted to be diminished in stimulated naïve cells, arguing in favor of a differentiation defect.

To definitively determine whether STAT3 mutations lead to focal or more broad memory defects, and to determine the precise mechanism, careful human immunization trials and perhaps mouse models may be needed. 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 al and Siegel et al are indeed directly causal in humans.

The observations made in these studies and the questions raised are not just esoteric or academic ones. Pharmacologic inhibition of cytokines and/or cytokine signaling, 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 more be identified. Taking advantage of the improved understanding of the ex vivo cellular consequences of specific molecular defects, consequences which might be measurable more reproducibly even when clinical manifestations are more variable, new genetic diseases of CD8 function may be more easily discovered.

Acknowledgments

This work was supported by the intramural research program of the NIAID, NIH.

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