Regulation of ROR γ t in Inflammatory Lymphoid Cell Differentiation

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T-helper 17 (Th17) cells differentiate from naïve CD4⁺ T cells in response to signals from commensal microbiota and produce cytokines critical for the integrity of mucosal barriers. These cells also disseminate throughout the body, and are key participants in numerous inflammatory processes. A key challenge is to elucidate the mechanisms that govern Th17 cell beneficial versus pathogenic functions, characterized by different cytokine profiles. Mucosal Th17 cells require the nuclear hormone receptor ROR γ t for their differentiation in draining lymph nodes. Cytokine expression is enabled in select tissues, to which these cells migrate, by external cues, such as the serum amyloid A proteins produced in response to commensal bacteria by epithelial cells in the small intestine. Additional cell-intrinsic cues contributing to production of Th17 cytokines during both homeostasis and inflammation include the ROR γ t-associated DEAD-box RNA helicase DDX5 and long noncoding RNA (lncRNA) Rmrp. The helicase activity of DDX5 is required for Rmrp-mediated assembly of the complex and colocalization with ROR γ t throughout the genome to regulate key Th17 genes. How these are regulated in diverse microenvironments may provide insights for therapeutic intervention in autoimmune disease.

The vertebrate adaptive immune response has evolved to provide protection from a large variety of both pathogenic and nonpathogenic or commensal microbes. An important aspect of its evolution is the diversification of T-lymphocytes such that different subsets perform distinct functions that are suited to the microbe and the desired outcome. T-helper 17 (Th17) cells exemplify this principle, as they differentiate at mucosal surfaces to perform either homeostatic functions, largely aimed at reinforcing barriers in response to commensal bacteria with defined properties, or protective functions, that limit the growth of subsets of invasive bacteria or fungi. The properties of Th17 cells that render them effective can also cause tissue damage, and hence these cells also participate in a large variety of inflammatory diseases. Th17 cells produce the cytokines IL-17A, IL-17F, and IL-22 (Korn et al. 2009) that regulate central aspects of host immunity, including granulopoiesis, neutrophil recruitment, and the induction of antimicrobial peptides by epithelial cells (Weaver et al. 2007). Under some circumstances, however, after exposure to IL-23, Th17 cells can also contribute to chronic inflammation that results in a number of human diseases. Such responses often involve IL-23-dependent production of the cytokines IFN- γ (interferon- γ) and GM-CSF (granulocyte-macrophage colony-stimulating factor) (CSF2) and result in autoimmune disease pathologies in animal models of inflammatory bowel disease (e.g., Crohn's disease), rheumatoid arthritis (RA), psoriasis, and multiple sclerosis (MS) (Furuzawa-Carballeda et al. 2007). Antibody-mediated blockade of IL-23 or IL-17A is highly effective

in the treatment of patients with psoriasis and psoriatic arthritis, and blockade of IL-23 has also been reported effective in ankylosing spondylitis and Crohn's disease, but, paradoxically, IL-17A blockade exacerbated inflammatory bowel disease (Whibley and Gaffen 2015). This paradox may be explained by the dual nature of Th17 cells, which are most abundant in the gastrointestinal (GI) tract, where mutualism between host and microorganisms is critically dependent on a state of balanced immune activation (Honda and Littman 2012).

Th17 cells are dependent on commensal microbiota for their differentiation. In germ-free mice, there are essentially no IL-17-producing CD4⁺ T cells in the intestine, but colonization with either a diverse commensal flora or with only segmented filamentous bacteria (SFB) results in induction of microbial antigen-specific Th17 cells (Gaboriau-Routhiau et al. 2009; Ivanov et al. 2009; Yang et al. 2014b). Colonization of animals with SFB is linked to exacerbation of Th17-mediated disease in murine autoimmune models of both RA and MS (Wu et al. 2010; Lee et al. 2011). How SFB-induced Th17 cells contribute to systemic autoimmune disease is a major unanswered question.

We recently established a model of acute SFB colonization to investigate the mechanism of Th17 cell induction in a spatiotemporal context (Sano et al. 2015). We found that antigen-specific CD4⁺ T cells are first induced to express ROR γ t (RAR-related orphan receptor γ t), a nuclear hormone receptor described as a “master regulator” for the Th17 program, in the draining mesenteric lymph nodes after SFB colonization. While poised to

be Th17 cells, these differentiated cells migrate to many sites throughout the gut and the rest of the body but express their signature cytokines, including IL-17A, following migration to the lamina propria in the terminal ileum of the small intestine, the site adjacent to epithelial cells to which SFB attaches. This observation suggested that a trigger(s) restricted to the terminal ileum activates a signaling cascade sensed by the poised Th17 cells to turn on transcription of their effector programs.

Here, we summarize our recent efforts to characterize cell-extrinsic (“triggers”) and cell-intrinsic (“sensors”) mechanisms that modulate Th17 cell effector functions *in vivo*. We first discuss the role of epithelial serum amyloid A proteins (SAA1 and SAA2) as candidate cell-extrinsic molecules that promote the transcriptional activation of multiple loci that endow Th17 cells with effector functions in the context of SFB colonization in the gut. We then summarize recent studies that revealed critical roles for a ROR γ t-associated RNA helicase and its long noncoding RNA (lnc RNA) substrate in the cell-intrinsic sensing of signals that culminate in differentiation of potentially pathogenic Th17 cells.

AN IL-23R/IL-22 CIRCUIT REGULATES EPITHELIAL SERUM AMYLOID A TO PROMOTE LOCAL EFFECTOR Th17 RESPONSES

SFB colonization of the small intestine results in global transcriptional changes in the host epithelia, including the induction of antimicrobial peptides and stress response genes, such as serum amyloid A (SAA1 and SAA2) (Ivanov et al. 2009). SAAs are typically induced in response to infection and acute injury and can promote inflammation, in part through elicitation of proinflammatory cytokine production and recruitment of granulocytes, monocytes, and T-lymphocytes (Uhlir and Whitehead 1999). Interestingly, the expression of epithelial SAAs was abolished in mice whose type 3 innate lymphoid cells (ILC3) were defective for IL-23R or IL-22. IL-22 produced by ILC3 signals epithelial cell production of SAA1/2. In parallel, ROR γ t⁺ Th17 cells patrolling the ileal lamina propria from mice with IL-22 blockade or deficient for SAA1/2 were unaltered in total numbers but expressed significantly lower levels of IL-17A (Sano et al. 2015). We concluded from these studies that the functional differentiation of intestinal Th17 cells following colonization of mice with SFB occurs in a two-step process: The first step is the priming and polarization of antigen-specific CD4⁺ T cells in the tissue-draining lymph nodes, resulting in a poised state marked by the expression of ROR γ t, and the second is the activation of a cytokine gene expression program in a tissue microenvironment in which epithelial cell-derived factors act on poised cells (Fig. 1). The absence of the second signal, which in the case of SFB-induced Th17 cells is, at least in part, SAA1/2, does not appear to affect the polarization or dissemination of the ROR γ t⁺ Th17 cells induced in the mesenteric lymph nodes (Sano et al. 2015).

Figure 1. Serum amyloid A (SAA) promotes Th17 effector responses. SFB colonizes the ileum in mice. Poised SFB-specific Th17 cells migrate widely, including the mucosal lamina propria. SAAs, produced by epithelial cells in response to SFB, promote poised Th17 cells to become cytokine-producing effector cells.

SAAs are significantly up-regulated in the joints and serum of RA patients (O’Hara et al. 2000) and their levels are correlated with disease progression (Chambers et al. 1983). In mouse models, ROR γ t is needed for Th17 cells to contribute to multiple autoimmune diseases (Leppkes et al. 2009; Huh et al. 2011), and whether SAAs or similar tissue-derived costimulatory molecules mediate induction of pathogenic cytokines remains to be elucidated. As microbiota-specific helper T cells, including SFB-specific Th17 cells, circulate widely following their polarization in the gut-associated lymphoid tissues (Hand et al. 2012; Yang et al. 2014b), it is possible that they contribute to autoimmune disease systemically through induction of their effector functions at sites where inflammatory mediators such as SAAs are elevated. An attractive notion is that SAAs may lower the threshold for activation of Th17 cells in inflammatory microenvironments, such that otherwise harmless self-peptide/MHC-reactive T cells would produce the effector cytokines and thus exacerbate tissue damage. It will be of substantial interest to evaluate whether the multistep process observed with SFB-induced IL-17A-producing Th17 cells in the ileum can be replicated at other sites where there is aberrant SAA production, and whether the resulting cytokine secretion would then contribute to autoimmune pathology. Whether T helper and regulatory lineages that participate in other types of antimicrobial defenses and immune tolerance undergo similar stages of priming/licensing and functional activation is another important question.

Using sorted mouse or human CD4⁺ T cells cultured under suboptimal Th17 polarizing conditions with limited TGF- β (transforming growth factor- β) availability,

recombinant SAAs substantially enhanced production of IL-17A and IL-17F in ROR γ t⁺ Th17 cells (Sano et al. 2015; J.-Y. Lee, unpubl.). Thus, SAA1/2 appears to act directly on poised T cells, through yet to be identified receptor(s). A specific subset of the Th17 gene expression program was modulated by rmSAA1 (recombinant mouse SAA1) treatment, as assessed by RNA sequencing (RNA-seq), and Ingenuity Pathway Analysis predicted ROR γ t as the top upstream regulatory factor sensitive to SAA stimulation. The DNA-binding domains of RORs recognize ROR response DNA elements (ROREs) within promoters and other regulatory regions of their target genes (Jetten et al. 2001). RORE-driven luciferase assays in Th17 cells confirmed that ROR γ t transcriptional activity was potentiated during rmSAA1 stimulation in culture. It should be noted that recent work from Kenya Honda's group (Atarashi et al. 2015), as well as our previous work in collaboration with Honda (Ivanov et al. 2009), failed to show direct stimulation of T cells with recombinant SAA and, instead, found that IL-17 induction required activation of an intermediary myeloid cell. We believe that the discrepancy can be attributed to different preparations of recombinant mouse SAA1, as we can now obtain stimulation of T cells with high confidence.

ROR γ t and ROR γ , the products of the *Rorc* locus, belong to the nuclear receptor (NR) superfamily (Huang et al. 2010). Although ROR γ is expressed widely, particularly in liver, muscle, and adipose tissue, ROR γ t expression is restricted to lymphoid cells. Among lymphocytes, ROR γ t has multiple other functions in addition to guiding differentiation of Th17 cells and other IL-17-producing T cells, such as $\gamma\delta$ 17 cells. These include mediating survival of thymocytes and regulating the development of lymphoid tissue inducer (LTi) cells that guide the formation of secondary and tertiary lymphoid tissues, and of type 3 innate lymphoid cells (ILC3s) that protect epithelial barriers, largely through production of IL-22 (Sun et al. 2000; Spits and Di Santo 2011). How ROR γ and ROR γ t function in different cell types to direct distinct transcriptional and functional programs is not understood. As with other members of the family, the ligand-binding domain (LBD) at the carboxyl terminus of ROR γ /t makes these ideal cell-intrinsic "sensors" for environmental cues. Ligand binding to ROR γ /t results in recruitment of coactivators, but, as shown with other NRs, there are likely a variety of cofactors and posttranslational modifications (PTMs) that modulate transcriptional activity in different cells or tissues and under diverse conditions (for review, see Huang and Glass 2010). We had found that cholesterol biosynthetic intermediates are required for endogenous production of ROR γ /t agonistic ligands (Santori et al. 2015), and it is possible that different ligands can also contribute to tissue-selective activities of ROR γ /t.

There are multiple pharmaceutical and biotechnology company programs targeting ROR γ t for treating diseases such as psoriasis, rheumatoid arthritis, and inflammatory bowel diseases, all of which are thought to be mediated in large part by Th17 cells (Chang et al. 2014). We and

others have searched for small molecule antagonists of ROR γ /t transcriptional activity, which allowed for identification of digoxin family glycosides and several other classes of molecules as highly specific ROR γ /t ligand-binding domain-interacting antagonists or inverse agonists (Huh et al. 2011, 2013). Although ROR γ t is indeed an attractive therapeutic target for multiple autoimmune diseases (Huh et al. 2011; Yang et al. 2014a), particularly in light of effective antibody targeting of this pathway, interfering with the shared ligand-binding pocket of ROR γ may result in undesired side effects in peripheral organs and tissues. Next, we will summarize our most recent efforts at identifying and examining Th17 cell-intrinsic factors that contribute to ROR γ t functions in a tissue-specific manner, providing a path toward targeting not only Th17 cells but also other cell types dependent on RNA helicase-dependent programs.

REGULATION OF ROR γ t FUNCTIONS IN Th17 CELL DIFFERENTIATION BY AN RNA HELICASE PAIRED WITH AN lncRNA

To identify cell type-specific partners for ROR γ t, we applied a combination of proteomics and next-generation RNA sequencing to in vitro polarized mouse Th17 cells. We first identified the DEAD-box RNA helicase DDX5 in a screen for ROR γ t-associated proteins (Huang et al. 2015). DDX5 functions in multiple cellular processes (Huang and Liu 2002), including transcription and ribosome biogenesis (Lin et al. 2005; Caretti et al. 2006; Jalal et al. 2007; Clark et al. 2008; Fuller-Pace and Moore 2011; Linder and Jankowsky 2011; Arun et al. 2012) in both a helicase activity-dependent and -independent manner. In mice deficient for DDX5 in T cells, expression of multiple ROR γ t target genes was attenuated and the animals were resistant to disease in models of Th17 cell-mediated autoimmunity, including colitis and multiple sclerosis. Remarkably, conditional inactivation of DDX5 in TCR $\alpha\beta$ (T-cell receptor $\alpha\beta$) T cells rendered ROR γ t⁺ cells defective for induction of not only IL-17A but also IFN- γ , a cytokine associated with autoinflammatory functions of Th17 cells, in the T-cell transfer colitis disease model. Change in IFN- γ expression was not observed in classical Th1 cells, which express T-bet but not ROR γ t. At steady state, the number of ROR γ t⁺ T cells in the intestinal lamina propria was similar in mice with T cells sufficient or deficient for DDX5, but there was substantial reduction in the proportion of these cells producing IL-17A and IL-17F in the small intestine. DDX5 therefore appears to have a key role in regulating both homeostatic expression of IL-17 in mice colonized with appropriate microbiota and production of additional cytokines by the same cells under inflammatory conditions. The inflammatory functions of Th17 cells are promoted by IL-23, and it is possible that signaling by way of IL-23R may influence additional functions of ROR γ t-associated DDX5. Intriguingly, in human studies, a functional distinction has been made between two types of circulating CD4⁺ T cells that are both dependent on

ROR γ t and express either IFN- γ and the chemokine receptors CXCR3 and CCR6 or IL-17 and only CCR6. The former have been designated Th1* cells and are the predominant respondents to mycobacterial antigen, whereas the latter are Th17 cells (Becattini et al. 2015). Remarkably, patients with homozygous null mutations in *RORC* (which encodes both isoforms of the nuclear receptor) have defects in both Th17 and Th1* cells and are susceptible to both mucocutaneous candidiasis (controlled by Th17 cells) and dissemination of bacillus Calmette–Guérin (BCG) after its use as a vaccine (Okada et al. 2015). The Th1* cells may be the human equivalent of the pathogenic IFN- γ -producing Th17 cells in mice, and it will therefore be of considerable interest to determine the role of DDX5 in their differentiation.

In our structure–function analysis of DDX5, we found that it can enhance IL-17A expression only if its RNA helicase function is intact (Huang et al. 2015). We therefore searched for RNAs associated with DDX5 in primary Th17 cell lysates. By depleting ribosomes and polysome-associated translated RNAs, we enriched for noncoding RNAs in DDX5 and ROR γ t immunoprecipitates, allowing us to identify an associated lncRNA, Rmrp, by RNA-seq analysis. Rmrp, RNA component of the mitochondrial RNA-processing endoribonuclease (RNase MRP), is highly conserved between mouse and human and is essential for early murine development (Rosenbluh et al. 2011). Mutations in human *RMRP* result in the autosomal-recessive disease cartilage hair hypoplasia (CHH), characterized by skeletal dysplasia, immune system defects, predisposition to lymphoma, and neuronal dysplasia of the intestine (Makitie et al. 1998; Bonafé et al. 2005). *RMRP* mutations in humans are never null but either biallelic point mutations or compound heterozygous mutations retaining one transcribed mutant allele, suggesting that, as in mouse, the gene is essential for early development, although several hypomorphic forms of the RNA can be tolerated. We found that, in Th17 cells, Rmrp localizes exclusively to the nucleus, promotes ROR γ t-DDX5 assembly both in vitro and in vivo, and is recruited to ROR γ t-occupied genomic loci of critical genes implicated specifically in the Th17 effector program (Huang et al. 2015). The formation of the complex requires ATP-dependent helicase activity and likely involves a conformational change in the lncRNA. Expression of wild-type, but not a CHH mutant, Rmrp promoted Th17 cell differentiation in a DDX5-dependent manner. T cells from mice carrying a single-nucleotide change (270 G>T) in *Rmrp*, corresponding to one found in CHH patients (262 G>T), had a compromised Th17 cell effector program and lost association of DDX5 with ROR γ t. Rmrp thus acts with DDX5 and ROR γ t⁺ to confer target locus-specific activity, enabling the T-cell effector programs, including those involved in autoimmune disease (Fig. 2). We speculate that defective T-cell-dependent immunity in CHH patients may reflect, at least in part, reduced Rmrp-dependent activity at ROR γ t target genes. It will be of interest to learn whether a subset of CHH patients have *RMRP* mutations resulting in defective Th1* differ-

Figure 2. Examples of RNA helicase and long noncoding RNA (lncRNA) functions in transcriptional regulation. (Left) DDX5 is required for the chromatin localization of the lncRNA Rmrp at select ROR γ t targets genome-wide in the regulation of ROR γ t-dependent homeostatic and pathogenic Th17 programs in vivo. (Right) In *Drosophila* male cells, MLE (maleless) mediates ncRNA roX1/2 remodeling and interaction with the MSL (male specific lethal) dosage compensation complex (DCC), which consists of MSL2 and three other proteins, allowing for histone acetylation and transcriptional up-regulation of genes throughout the X chromosome.

entiation with associated candidiasis and susceptibility to BCG vaccination.

Our results have several implications and raise a number of interesting questions. First, they show that an lncRNA acts in *trans* across the genome, regulating ROR γ t-dependent genes upon interacting with enzymatically active DDX5 helicase. The concept of RNA helicase/lncRNA function in *trans*-regulation of transcription was first elegantly shown with the lncRNAs roX1 and roX2, substrates of the RNA helicase MLE, that enhance *Drosophila* male X-chromosome gene expression to achieve dosage compensation (Fig. 2; Ilik et al. 2013; Maenner et al. 2013). A second example is the role of the DEAD-box helicase DDX21, which acts on the noncoding RNA 7SK to regulate ribosomal gene expression (Calo et al. 2015). Although the roX RNAs act broadly on one chromosome, by way of the MSL dosage compensation complex, and 7SK acts at ribosomal gene loci, releasing p-TEFb to allow for transcriptional elongation, Rmrp acts on genes distributed throughout the genome, and thus participates in conferring a distinct differentiation program to T cells (Fig. 2). Together, these findings suggest that the role of DDX5 in regulating Th17 cell differentiation may be but one example of how pairing of diverse DEAD-box RNA helicases with distinct lncRNAs allows for cell-specific targeting of DNA or chromatin-binding transcriptional regulators to direct differentiation pathways. Indeed, DDX5 was shown to also associate with MyoD, a key regulatory transcription factor that directs muscle cell differentiation, and coexpression of these factors along with a DDX5-binding putative lncRNA, SRA, resulted in synergistic induction of muscle-specific genes in the myogenic cell line C2C12 (Fig. 3; Caretti et al. 2006). Whether these interactions occur in vivo and whether SRA is required for DDX5 recruitment to MyoD-occupied chromatin during skeletal muscle development awaits further investigation.

We do not know whether Rmrp and DDX5 act on genes other than ROR γ t targets to regulate the Th17

Figure 3. Model for diverse DEAD-box RNA helicase interactions with distinct lncRNAs. Functional pairing of RNA helicases and their substrates may allow for cell-specific targeting of DNA or chromatin-binding transcriptional regulators to direct differentiation and physiological pathways. TF, transcription factor.

cell program, but it appears that they each influence the expression of a set of genes independently of ROR γ t. This raises the possibility that DDX5 and Rmrp pair with other partners, perhaps targeting other transcriptional complexes (Fig. 3). How the association of DDX5 and Rmrp is regulated remains a very interesting puzzle, because both molecules are widely expressed at relatively high levels. For example, DDX5 and Rmrp are abundant in developing thymocytes and in peripheral naïve and differentiated T-helper subsets. However, loss of DDX5 in immature thymocytes or replacement of Rmrp with a mutant sequence that impaired Th17 cell differentiation had no apparent effect on thymocyte development, which requires ROR γ t for cell survival. Development of lymph nodes also appeared normal in mice with mutant Rmrp or with a lymphoid cell-specific inactivation of DDX5, suggesting that lymphoid tissue inducer cells and ILC3, whose development is wholly dependent on ROR γ t, are intact in such mice. Intriguingly, in contrast to Th17 cell lysates, there was very little Rmrp coprecipitated with DDX5 from thymocyte lysates. If, indeed, DDX5-Rmrp contribute to ROR γ t-dependent functions only in Th17 cells, there would need to be tissue- or cell type-specific mechanisms that regulate assembly with the transcriptional complexes. There may be Th17 cell-specific chaperones that guide interaction of DDX5 with Rmrp or there may be competition from other lncRNAs or helicases that would govern specific pairing in each cell type. Further characterization of DDX5-associated RNAs in different tissues, including thymus and liver (where ROR γ / γ t is expressed) and developing muscle (where MyoD is expressed), may provide more insight into this problem.

IMPLICATIONS FOR TARGETING Th17 CELLS IN AUTOIMMUNE DISEASE

The contribution of DDX5-Rmrp to expression of ROR γ t-dependent genes in T cells suggests that it provides a cell-intrinsic function in response to environmental cues. Indeed, SFB-colonized mice had similar numbers of ileal lamina propria ROR γ t⁺ Th17 cells regardless of whether or not the cells expressed DDX5 or wild-type Rmrp. However, deficiencies in these cofactors resulted in reduced proportions of these cells producing IL-17A. This leads us to speculate that epithelium-derived SAAs in the lamina propria act on ROR γ t⁺ cells to enable DDX5-Rmrp assembly and/or interaction with ROR γ t at relevant sites throughout the genome. A critical question is how the homeostatic and pathogenic functions of Th17 cells are differentially regulated by external cues and by cell-intrinsic components, such as the ROR γ t/DDX5/Rmrp complex. Each molecule in the complex is required for both homeostatic function in response to SFB colonization and pathogenicity in autoimmune disease models. Additional signaling pathways may provide either/or digital signals to direct Th17 effector programs, or there may be analog-like differential tuning through ROR γ t to achieve diverse outcomes. It has been suggested that lipid biosynthetic pathways differ in pathogenic and nonpathogenic Th17 cells and that ROR γ t binds to different genomic targets under divergent conditions (Wang et al. 2015), but it is not yet known whether the DDX5-Rmrp axis has a role in this process.

A better understanding of how ROR γ t contributes to Th17 cell programs in different settings is critical for designing effective therapies for the numerous autoin-

flammatory conditions mediated by these cells. Ideally, it will be possible to target transcriptional pathways that attenuate pathogenic Th17 cells or Th1* cells, without affecting the homeostatic process by which Th17 cells maintain barrier integrity. The discovery of the role of DDX5 and Rmrp in Th17 cell differentiation is a first step on the way to better discriminate ROR γ t functions in Th17 cells versus other cell types in which this nuclear receptor also performs critical tasks. Elucidation of the mechanisms by which DDX5 alters the conformation of Rmrp to permit subsequent binding to ROR γ t may reveal features that could be vulnerable to therapeutic intervention. High-throughput screens designed to identify compounds that disrupt the complex may be valuable for developing therapies that selectively inhibit Th17-mediated inflammation, sparing thymocyte and ILC3 development.

CONCLUSION

The discovery of the roles of DDX5 and Rmrp in T-cell differentiation offers multiple opportunities to enhance our understanding of how Th17 cells are regulated and of whether there is a broader role for RNA helicases and their noncoding RNA substrates in directing developmental/differentiation programs (Fig. 3). Future investigation of the structural basis of the DDX5-Rmrp-ROR γ t interactions, of whether DDX5 performs lncRNA-dependent transcriptional functions in other cell types, and of whether related DEAD-box helicases have analogous functions in other cell lineages will likely yield important insights that will guide the development of novel cell type-specific therapies. These studies may also provide a better understanding of the developmental defects observed in CHH patients with different RMRP allelic variants and may reveal new components of the pathway that contribute to either immune deficiency or to autoimmune disease susceptibility.

ACKNOWLEDGMENTS

We thank members of our laboratory and our many collaborators for their contributions to the studies described in this overview. The work was supported by National Institutes of Health (NIH) grant R01AI080885 (D.R.L.), NIH training grant T32 CA009161 (W.H.), and the Howard Hughes Medical Institute (D.R.L.).

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Cold Spring Harbor Symposia on Quantitative Biology

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Cold Spring Harb Symp Quant Biol 2015 80: 257-263 originally published online March 11, 2016
Access the most recent version at doi:[10.1101/sqb.2015.80.027615](https://doi.org/10.1101/sqb.2015.80.027615)

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