

of disease¹³. It is therefore possible that therapeutics that target only the T_H-17 lineage may be effective only at certain times or in certain disease conditions. The most successful treatment strategies will probably target both the T_H1 and T_H-17 cell effector subsets in an autoantigen-specific manner.

Collectively, the data in Du's publication establish a role for miR-326 as a regulator of T_H-17 differentiation and the development of autoimmune disease while identifying a prom-

ising target for use as a diagnostic or therapeutic biomarker. Furthermore, this paper lays a fertile groundwork for future research into the precise roles of miRNAs in helper T cell differentiation, cytokine receptor signaling and transcription factor function.

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T cells need Nod too?

Shahram Salek-Ardakani & Michael Croft

Nucleotide-binding oligomerization domain 2 (Nod2) is required for sensing of intracellular bacteria and subsequent inflammatory responses. Unexpectedly, new evidence suggests that Nod2 influences T helper cell signaling, proliferation and differentiation and effector responses against *Toxoplasma gondii*.

The early immune response to most pathogens is triggered by pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs) and nucleotide binding and oligomerization domain (Nod)-like receptors (NLRs). TLRs can stimulate antigen-presenting cells, thus indirectly allowing the effective induction of T cell immunity. Many TLRs induce activation of the canonical (IKK α -IKK β -IKK γ -dependent) NF- κ B pathway through the adaptor MyD88 and the serine/threonine kinase RIP2 (Fig. 1). Most early studies assumed that any requirement for TLRs and MyD88 in immunity reflected their roles in innate cells, but this view has been modified by recent data demonstrating that MyD88 can directly control T cell function, either through TLRs now known to be expressed on T cells or through IL-1R family members that can also engage MyD88 (refs. 1,2). In this issue of *Nature Immunology*, Shaw *et al.*³ now suggest that this type of innate cell–T cell dual activity is also shown by Nod2, an intracellular PRR. Nod2 can stimulate epithelial cells, macrophages and dendritic cells by sensing muramyl dipeptide (MDP), a component of bacterial peptidoglycan, and can also trigger activation of RIP2 and the canonical NF- κ B pathway in these cells⁴. Shaw *et al.*³ challenge the current dogma that Nod2 is dedicated for innate immune

recognition of bacterial motifs by showing that Nod2 can provide a T cell–intrinsic signal that does not rely on MDP recognition.

The initial focus that led to this conclusion was the finding that Nod2-deficient animals succumb to *T. gondii* infection. Through a series of experiments with this pathogen, Shaw *et al.*³ revealed a specific defect in the ability of T cells from Nod2-deficient mice to produce interferon (IFN)- γ , raising the possibility that Nod2 might exert T cell–intrinsic functions. Consistent with this idea, upon *T. gondii* challenge, wild-type CD4⁺ T cells differentiated into T helper type-1 (T_H1) effector cells in a Nod2^{−/−} host, whereas Nod2^{−/−} CD4⁺ T cells failed to differentiate into T_H1 cells in a Nod2-sufficient environment.

Does Nod2 then have a more general role in T cell function? Shaw *et al.*³ found that Nod2 is required for lymphopenia-induced homeostatic T cell proliferation, a response driven by T cell antigen receptor (TCR)–peptide-MHC interactions and cytokines that signal through the common γ -chain. Nod2^{−/−} donor CD4⁺ T cell populations, if transferred alone, expand normally in a lymphopenic host. However, when transferred with wild-type T cells, Nod2^{−/−} CD4⁺ T cells show impaired population expansion. The authors also used a mouse model of colitis whereby the transfer of naive CD4⁺ T cells into *Rag1*^{−/−} mice leads to gut inflammation. Recipients reconstituted with Nod2^{−/−} T cells remained healthy and did not succumb to wasting disease.

These results support the idea that, in some situations, Nod2 expressed in T cells can affect T cell activity independently of sensing a

bacterial product. In support of this argument, Shaw *et al.*³ also found that in the apparent absence of any Nod2 ligand, Nod2-deficient CD4⁺ TCR-transgenic T cells exposed to antigen *in vitro* produced less interleukin (IL)-2 and showed impaired differentiation into T_H1 or T_H2 cells. Addition of exogenous IL-2 restored the defective T_H cell differentiation, leading the authors to speculate that the primary requirement for Nod2 is to regulate IL-2 secretion. In light of the *T. gondii* studies, this might be a reasonable conclusion as *Il2*^{−/−} mice succumb to this pathogen in a time frame similar to that reported for Nod2^{−/−} mice⁵. However, it is not clear that defective production of IL-2 by Nod2^{−/−} T cells can explain the defects in homeostatic proliferation, as competition for cytokines and a requirement for Nod2 in common γ -chain cytokine receptor signaling is just as feasible. In the colitis experiments, Shaw *et al.*³ found reduced IFN- γ production by Nod2^{−/−} CD4⁺ T cells, but this might also be unrelated to a lack of IL-2 production. IL-2 produced by CD4⁺ T cells can induce peripheral conversion of CD25[−] T cells into CD25⁺Foxp3⁺ inducible regulatory (iT_{reg}) cells, leading one to predict that if the primary defect of Nod2^{−/−} T cells in this situation was in producing IL-2, a more severe disease would ensue as a result of impaired iT_{reg} cell generation.

The present model of Nod-mediated signaling in non-T cells holds that after detection of MDP, Nod2 recruits RIP2, leading to a common pathway shared by TLRs, the IL-1R family and members of the tumor necrosis factor receptor (TNFR) superfamily, wherein Tab-TAK1-mediated activation

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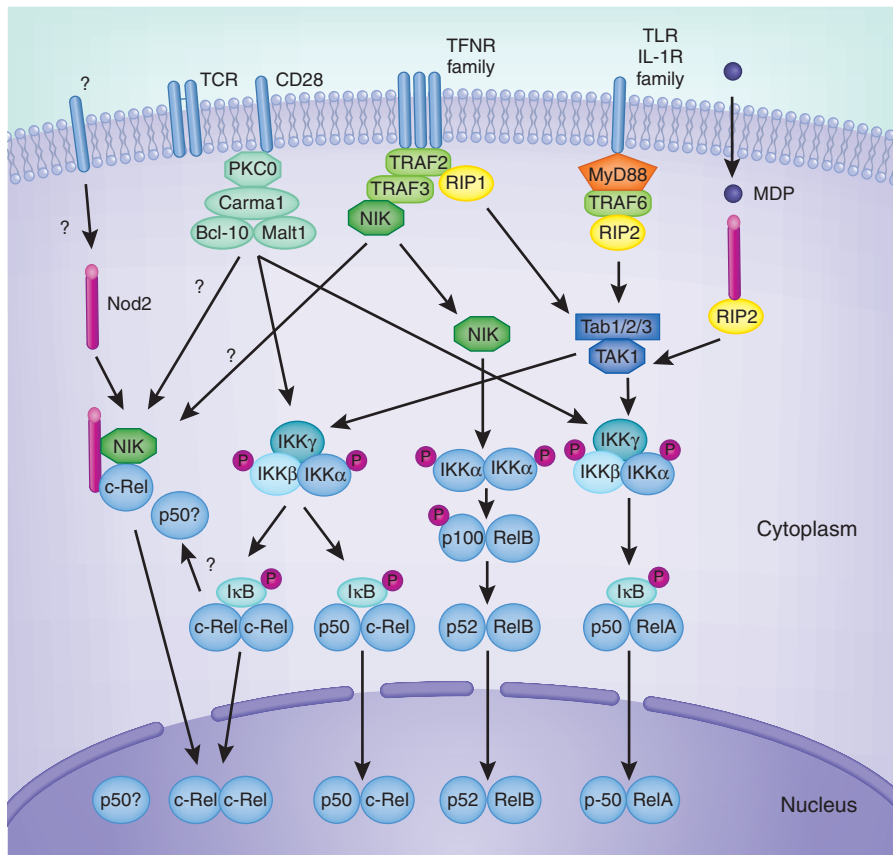


Figure 1 Possible cross-talk between Nod2, TCR, CD28, TLR and TNFR family members in regulating NF- κ B. In non-T cells, muramyl dipeptide (MDP) can initiate a signaling cascade that requires Nod2 and RIP2, and results in nuclear accumulation of p50 and RelA as a result of Tab-TAK1-mediated activation of the IKK α -IKK β -IKK γ complex and subsequent degradation of I κ B. This pathway is also activated by TLR and IL-1R family members through MyD88, TRAF6 and RIP2, and by TNFR family members through TRAF2 and RIP1. Whereas TLR, IL-1R and TNFR members can promote p50-RelA activation in T cells, it is not clear whether MDP, through Nod2, can also affect this pathway in T cells or whether T cells can take up MDP through specific transporters or phagocytosis. Shaw *et al.*³ propose that Nod2, independent of MDP recognition, influences T cell activity by binding NIK and c-Rel and by promoting c-Rel nuclear accumulation (left). Whether c-Rel is activated as a homodimer or as a heterodimer with p50 was not investigated. It is unclear whether this putative NIK-c-Rel-Nod2 complex is downstream of the TCR, CD28, cytokine receptors or other receptors, and whether it is associated with other molecules. Cooperation between the TCR and CD28 in T cells can also promote p50-RelA nuclear translocation through PKC θ -Carma1-Bcl10-Malt1. TCR, CD28, TNFRs and TLRs can also trigger nuclear translocation of c-Rel (in the form of c-Rel-c-Rel or p50-c-Rel dimers) by targeting I κ B. TNFR family members, by recruiting TRAF3 and thus inhibiting TRAF3-mediated degradation of NIK, can also activate the IKK α -dependent noncanonical NF- κ B pathway leading to p52-RelB nuclear translocation. This may also promote cross-talk with Nod2 by stabilizing and freeing NIK to allow formation of the NIK-c-Rel-Nod2 complex.

of the IKK α -IKK β -IKK γ complex promotes nuclear translocation of the NF- κ B subunits p50 and RelA (ref. 4) (Fig. 1). In line with a ligand-independent activity of Nod2 in T cells, Shaw *et al.*³ found that mice lacking RIP2 were resistant to *T. gondii* infection; these findings also agree with previous reports that T cell proliferation and differentiation are normal in the absence of RIP2 (ref. 6). In contrast, overexpression systems and reporter assays indicate that the function of Nod2 in T cells may be related to its ability to interact with the NF- κ B family member c-Rel, as well as with

NF- κ B-inducing kinase (NIK), a downstream target of TNFR family members and a trigger of the noncanonical (IKK α -dependent) NF- κ B pathway and nuclear translocation of p52-RelB heterodimers. T cell production of IL-2 in response to TCR and CD28 costimulation can depend on c-Rel, which can interact with NF- κ B binding sites as well as the CD28-response element (CD28RE) in the proximal *Il2* promoter region^{7,8}. Additionally, it has been proposed that the ability of c-Rel to interact with CD28RE can be regulated by NIK⁹. Certainly, the *in vitro* data from Shaw *et*

*al.*³ support the notion of a cooperative action of c-Rel and NIK in promoting *Il2* transcription, and the notion that this can be boosted by Nod2. This conclusion also agrees with their finding of weaker nuclear accumulation of c-Rel in the absence of Nod2. In contrast, they found no effect of Nod2 deficiency on nuclear accumulation of RelA, suggesting that Nod2 does not affect the canonical NF- κ B pathway, which induces nuclear translocation of p50-RelA heterodimers, in T cells.

However, several caveats warrant further investigation. First, how strongly c-Rel or NIK, compared to p50-RelA, contribute to IL-2 production by T cells in most physiological conditions is unknown. Additionally, although initial reports of NIK-mutant T cells from mice with alymphoplasia (*aly/aly*) suggested a global defect in IL-2 secretion, later reports found that the primary requirement for NIK was in memory T cells, not naive T cells¹⁰. Assuming that Shaw *et al.*³ analyzed naive TCR-transgenic Nod2-deficient T cells, a requirement for a NIK-c-Rel-Nod2 complex may not explain the defect. Another potential issue is with the availability of NIK to participate in such a complex. The current view, from studies of the TNFR family, is that NIK is continuously degraded by TRAF3 until TRAF3 is recruited and itself degraded by TNFR engagement¹¹. Thus, for NIK to complex with Nod2 and c-Rel, this might require prior binding of a TNFR ligand to a T cell, and it is not clear whether any TNFR-ligand interactions were occurring in the experimental systems examined by Shaw *et al.*³. Lastly, whether this complex is physiologically induced in T cells was not determined and needs to be investigated; whether complex formation is the result of signaling from the TCR, CD28 and/or cytokine receptors also remains to be determined. Shaw *et al.*³ hypothesized that a Nod2-c-Rel-NIK complex might be downstream of CD28, but CD28 is not required for protection against *T. gondii*¹².

The findings of Shaw *et al.*³ in many ways raise more questions than they provide answers. It is an intriguing study that will attract the attention of those focused on the requirement of Nod2 in host defense, as well as those interested in understanding the molecular regulation of T cell activation. The idea of a T cell-intrinsic role for Nod2 will undoubtedly generate much debate. A recent report assessed the role of Nod2 in models of graft-versus-host disease and found no apparent role in T cells. In particular, a mixed lymphocyte reaction with Nod2-deficient CD4⁺ and CD8⁺ T cells did not mimic the defective responses of Nod2-deficient TCR-transgenic T cells when responding to

antigen¹³. Obvious differences between this study and Shaw *et al.*³ will spur further research into the influence of Nod2 in other T cell-driven immune responses. The subcellular localization, trafficking and expression of Nod2 in T cells remains poorly defined. Therefore, when and where Nod2 will and will not be active in an intrinsic signaling role in T cells needs to be determined. It is also not clear whether exogenous Nod2 ligands can stimulate T cells, and this issue is of obvious interest. Furthermore, it will be important to define

the targets up- and downstream of Nod2 in T cells and to compare their cross-talk and roles with those involved in signaling from TLRs and TNFRs, which have already been proven key for NF- κ B-dependent pathways.

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B cell memory: how to start and when to end

Nadege Pelletier & Michael G McHeyzer-Williams

Antigen-driven selection in germinal centers lays the foundation of effective B cell memory. Two reports in this issue reveal novel mechanisms that control effective formation of germinal centers and their long-term persistence *in vivo*.

B cell memory is a carefully orchestrated developmental program that requires antigen-specific CD4⁺ T cell help after the initial priming of naive B cells. Entry into germinal centers (GCs) signifies the beginning of diversification and selection that ends with the production of long-lived memory B cell compartments¹. In this issue of *Nature Immunology*, studies led by Cornall and Goodnow² identify loss-of-function mutations in the gene encoding DOCK8, a member of the Rho-Rac family of GTP-exchange factors, that grossly disrupt affinity maturation and memory B cell development in the GC reaction. A second study by Weill and colleagues³ documents extraordinary longevity for GCs initiated with particulate antigens. Both groups not only provide new insights into the regulation of antigen-specific B cell memory but also introduce valuable new animal models that will help guide future studies in this important research arena.

It is becoming increasingly clear that naive B cells encounter antigen in a cell-associated form⁴. After contact with antigen, B cells can focus their antigen receptors into central areas of a cellular interface and polarize adhesion molecules such as LFA-1 into the periphery of the contact area that defines the B cell immune synapse⁵. To progress

in development, antigen-primed B cells require antigen-specific contact with follicular helper T (T_{FH}) cells for proliferation, antibody isotype switching, the production of short-lived plasma cells and establishment of the GC reaction (Fig. 1). GCs appear within the first week of priming and contain dense networks of follicular dendritic cells (FDCs) coated with antigen-antibody complexes. Importantly, GC B cells seem to be in continuous movement along FDC processes, making occasional stable contacts with small numbers of antigen-specific T_{FH} cells^{6–8}. Most models of affinity maturation in the GC propose that GC B cells test their randomly variant B cell antigen receptors (BCRs) for increased antigen binding in the context of FDC networks. Those GC B cells that receive sufficient signal then compete for the GC T_{FH} cell contact required for exit from the GC and consolidation of the memory B cell and long-lived plasma cell programs.

To search for defects in the machinery that drives affinity maturation, Randall *et al.*² use a protein immunization strategy to screen *N*-ethyl-*N*-nitrosourea-derived mutants for intact early antibody titers but lower later antibody responses. The DOCK8 mutants that emerge clearly satisfy these selection criteria and have intact T cell-independent responses. Mixed-bone marrow chimeras convincingly demonstrate the intrinsic effect of the *Dock8* mutations on the normal initiation of the GC reaction but abnormally rapid decay of mutant GC B cells with substantially less affinity maturation. Interestingly, naive B

cells with the DOCK8 defect are able to focus antigen into the B cell immune synapse but are unable to polarize the intercellular adhesion molecule ICAM-1 into the peripheral region of the synapse. This defect in synapse formation is unusually selective, with no resultant deficits in signaling events, intracellular calcium concentrations, or the capacity to proliferate *in vitro* or to upregulate CD25 or CD69. Nevertheless, once exposed to antigen *in vitro*, mutant B cells are able to form normal numbers of antigen-specific conjugates with T cells. Hence, the role of DOCK8 seems to be highly selective for ICAM-1 polarization in the BCR-associated B cell immune synapse.

The *Dock8* mutation also affects the normal development of marginal zone B cells and B-1 cells. We are tempted to speculate that the selective defect in ICAM-1 polarization is also responsible for this developmental deficit through the selective decrease in the duration of B cell synapse formation *in vivo*. If such a deficit emerges only at specific stages in B cell development, it remains plausible that loss of DOCK8 function similarly influences effective BCR engagement only at the GC phase of the immune response and not before. In this scenario, the initial B cell immune synapse remains intact, allowing subsequent T_{FH} cell contact and normal initiation of the GC reaction. Hence, testing variant BCRs on FDC processes may be the nonproductive weak link that leads to cell death and the rapid decay of mutant B cells from the GC. In this way, the *Dock8* mutation would affect maintenance of the GC and

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