colon but not in extracolonic tissues such as the spleen and lymph nodes. This increase is dependent on the proliferation of both thymic T_{reg} cells and, more significantly, peripheral T_{reg} cells in the colon (distinguished by neuropilin-1 expression), and is independent of further migration into the tissue. This demonstrates true homeostatic population expansion *in situ* rather than increased induction. The authors go on to delineate the molecular mechanisms by which the host and microflora act together to drive the population expansion of colonic T_{reg} cells and to preserve tolerance to beneficial microflora. The driver of this process is the production of interleukin 2 (IL-2) by activated T cells in the colon, as the population expansion of T_{reg} cells is halted by inhibition of IL-2. IL-2 production in turn results in upregulation of expression of the epigenetic modifier Uhrf1 in local T_{reg} cells. Uhrf1 acts in concert with methylase Dnmt1 to diminish methylation of the promoter of *Cdkn1a*, which encodes p21, an important inhibitor of the cell cycle. The resulting downregulation of *Cdkn1a* enables T_{reg} cells to enter the cell cycle and undergo proliferative expansion. T cell–specific deletion of *Uhrf1* blocks T_{reg} cell proliferation in the lamina propria following microbial colonization and results in the development of a fatal colitis driven by the T_H1 and T_H17 subsets of helper T cells without causing pathology in other tissues. These results reveal a heretofore unknown molecular mechanism for the IL-2-mediated population expansion of T_{reg} cells that probably acts in synergy with previously described functions of IL-2 in diminishing apoptosis via upregulation of expression of the survival factor Mcl-1 (ref. 8) and a partially defined role for IL-2 in enhancing the metabolic fitness of T_{reg} cells[10].

The results of this study[1] add to a growing understanding of the mechanisms by which the host and commensal microflora act together to create a healthy gut environment. The colonization of the gut is a key event with important physiological consequences. Commensal microflora act to buffer the mucosa against pathogens and can aid in the digestion of complex food products into digestible forms. At the same time, colonization with commensal microorganisms drives a low-grade mucosal inflammation that, if unchecked, has the potential to develop into pathological inflammation. The symbiotic relationship between host and microflora has therefore required the evolution of processes to prevent colonization with commensal microorganisms from causing immunopathology while preserving the ability of the immune system to respond to pathogens. The discovery of a key role for Urhf1 in establishing this balance adds to a growing appreciation of the diverse mechanisms by which the host immune system harnesses the signals of beneficial colonization with commensal microorganisms—that is, the presence of commensal metabolites and the development of low-grade inflammation—to recruit conventional T cells into the peripheral T_{reg} cell lineage and to expand local thymic and peripheral T_{reg} cell populations to an abundance sufficient to prevent commensal microorganism–driven pathology (**Fig. 1**).

In their study, Hase and colleagues concentrate on the role of the IL-2–Uhrf1–p21 axis in expanding colonic T_{reg} cell populations[1]. However, it is worth noting that this may be a common molecular mechanism for the IL-2-mediated population expansion of T_{reg} cells. In the microbial reconstitution system used in this study, the effect of Uhrf1 deficiency is more profound on colonic T_{reg} cells than on systemic T_{reg} cells. However, this result is probably due to the local colonic IL-2 production that occurs during microbial colonization rather than representing an intrinsic difference in IL-2-response pathways in colonic T_{reg} cells versus systemic T_{reg} cells. Indeed, the systemic administration of complexes of IL-2 and antibody to IL-2 drives the Uhrf1-dependent proliferation of systemic T_{reg} cells, which demonstrates that Uhrf1 can function outside the colonic compartment. Systemic deficiency in T_{reg} cells results in increased IL-2 production by conventional $CD4^+$ T cells that feeds back to drive the population expansion of T_{reg} cells[8,11]. Thus, it seems likely that Uhrf1 has a similar role in T_{reg} cell homeostasis in other IL-2-dependent models of population expansion.

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1. Obata, Y. *et al. Nat. Immunol.* **15**, 571–579 (2014).
2. Lathrop, S.K. *et al. Nature* **478**, 250–254 (2011).
3. Atarashi, K. *et al. Nature* **500**, 232–236 (2013).
4. Furusawa, Y. *et al. Nature* **504**, 446–450 (2013).
5. Smith, P.M. *et al. Science* **341**, 569–573 (2013).
6. Arpaia, N. *et al. Nature* **504**, 451–455 (2013).
7. Cording, S. *et al. Mucosal Immunol.* **7**, 359–368 (2014).
8. Pierson, W. *et al. Nat. Immunol.* **14**, 959–965 (2013).
9. Cording, S. *et al. Eur. J. Microbiol. Immunol.* **3**, 1–10 (2013).
10. Fontenot, J.D. *et al. Nat. Immunol.* **6**, 1142–1151 (2005).
11. Amado, I.F. *et al. J. Exp. Med.* **210**, 2707–2720 (2013).

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Rad50 and CARD9, missing links in cytosolic DNA–stimulated inflammation

Andrew G Bowie

The DNA-damage sensor Rad50 couples the sensing of cytosolic DNA to the innate immunological adaptor CARD9 to stimulate DNA-dependent activation of the transcription factor NF-κB. This facilitates DNA virus–stimulated production of the cytokine IL-1β.

Innate immunity has experienced something of a revolution in the increased appreciation of, and insights into, the mechanisms of intracellular DNA sensing. New paradigms for the discrimination of self versus non-self have emerged in the understanding how both pathogen DNA and mislocalized self DNA trigger similar immune responses, while elegant and previously unknown signal-transduction pathways that respond to cytosolic DNA have been defined[1,2]. Two cytosolic DNA–response pathways in particular have been well characterized, both of which are of immense importance for containing and ultimately clearing infections with DNA viruses and for driving pathological responses to self DNA. These pathways are activation of the DNA-stimulated inflammasome AIM2, which leads to the production of mature interleukin 1β (IL-1β), and the induction of type 1 interferon via the DNA signaling axis of cGAMP synthase–STING-TBK1-IRF3 (**Fig. 1**). However, there is very little insight into how

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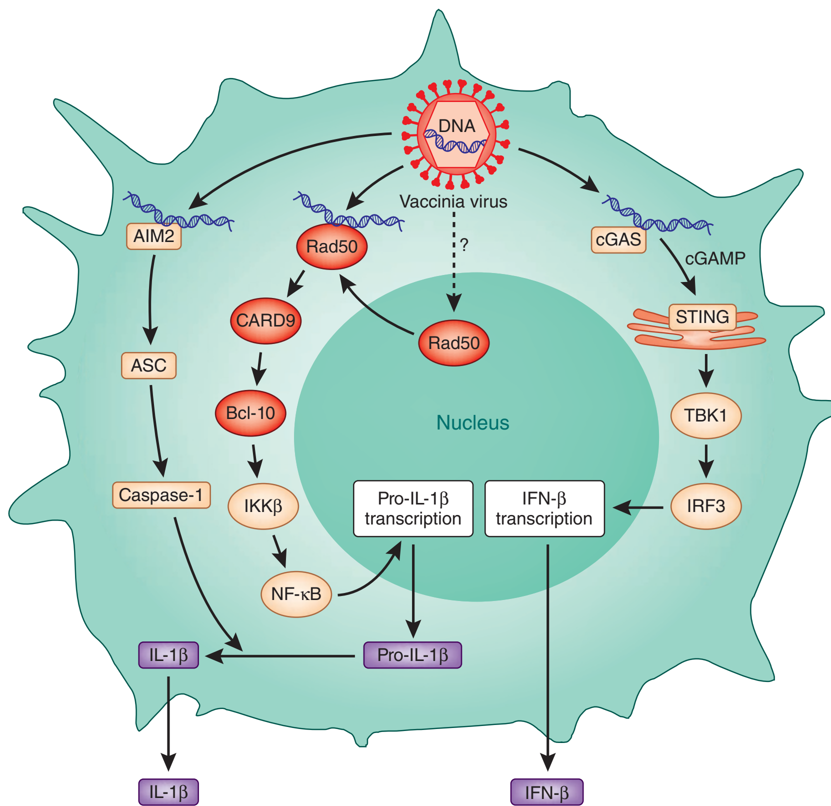

Figure 1 Signaling to elicit the production of IL-1β and the induction of type 1 interferon after cytosolic DNA is sensed. Cytosolic DNA from viruses such as vaccinia virus is recognized both by cGAMP synthase (cGAS), which leads to the activation of IRF3 via STING and TBK1, and by AIM2, which leads to the processing of pro-IL-1β via ASC and caspase-1. However, how DNA activates NF-κB to upregulate pro-IL-1β mRNA has remained unclear. Roth *et al.* show that the presence of DNA or a DNA virus in the cytosol somehow signals (dashed arrow) a Rad50-containing complex to exit the nucleus and engage with free DNA in the cytosol[3]. That interaction then results in the recruitment of CARD9 to couple the CARD9–Bcl-10–NF-κB pathway to the sensing of cytosolic DNA by Rad50, which stimulates the induction of pro-IL-1β mRNA.

cytosolic DNA causes activation of the transcription factor NF-κB, which is essential for the DNA-stimulated induction of genes encoding cytokines such as pro-IL-1β, tumor-necrosis factor (TNF) and IL-6. In this issue of *Nature Immunology*, Roth *et al.* shed light on this puzzle by defining an additional DNA-sensing pathway in dendritic cells (DCs) that is responsible for DNA-stimulated activation of NF-κB and induction of pro-IL-1β mRNA[3].

Apart from its general role in virus-induced inflammation, IL-1β has a key function in mobilizing the $CD8^+$ T cell antiviral response, and its importance is emphasized by the multiple mechanisms that cytosolic DNA poxviruses in particular have evolved to counter IL-1β function. AIM2 is known to directly sense DNA in the cytosol and to trigger the formation of an inflammasome that facilitates, via activation of caspase-1 by the adaptor ASC, the processing of pro-IL-1β into mature IL-1β[4]. However, the pathways that lead to the DNA-dependent induction of pro-IL-1β mRNA in myeloid cells, which would require activation of NF-κB, have remained less clear. Coupled to this is the observation that DNA-stimulated activation of NF-κB is weak at best in many cell types, particularly relative to activation of the transcription factor IRF3. Because the adaptor STING is essential for DNA-stimulated activation of IRF3 and has been linked more broadly to DNA-mediated transcriptional responses, a STING–NF-κB pathway has been assumed to be involved in the induction of the gene encoding pro-IL-1β and of other NF-κB-dependent genes. However, although STING clearly engages the IRF3 kinase TBK1, STING has not been shown to activate IKKβ, a kinase critical for the activation of NF-κB. Here Roth *et al.* reveal a pathway to the activation of NF-κB by cytosolic DNA in which STING is not central but in which CARD9, another adaptor for the detection of pathogens, is involved[3] (**Fig. 1**). CARD9 is required for signaling via C-type lectin receptors and the cytosolic receptor Nod2 and has also been shown to couple sensing by the cytosolic RNA sensor RIG-I to inflammasome activation and IL-1β production[5].

The story begins with a search for proteins that interact with CARD9 through the use of yeast two-hybrid screening, which reveals Rad50 as a partner of CARD9. Rad50, together with Mre11 and Nbs1, is known to form the MRN complex that senses and responds to double-strand breaks in DNA in the nucleus. Interestingly, Rad50, together with Mre11, has already been linked to the innate sensing of DNA[6]. Here, through the use of biophysical techniques and immunoprecipitation of endogenous proteins, the Rad50-CARD9 interaction is confirmed to exist in mammalian cells[3]. Because Rad50 is known to bind DNA, the authors go on to show a Rad50-dependent association between CARD9 and immunostimulatory viral double-stranded DNA. Furthermore, transfection of DCs with DNA causes the relocalization of Rad50 (and Mre11 and Nbs1) from the nucleus to the cytoplasm, where a complex with the DNA and CARD9 is formed. Since the cytosolic localization of Rad50 and DNA does not require CARD9, this suggests that Rad50 senses cytosolic DNA and subsequently recruits CARD9 as a signaling adaptor. Crucially, IL-1β production in response to pathogen DNA is impaired in CARD9-deficient DCs relative to that of wild-type cells. Interestingly, the production of DNA-stimulated interferon-β (IFN-β) is normal in CARD9-deficient DCs, while the opposite result is obtained for STING-deficient DCs, in which IL-1β production is normal but, as expected, IFN-β is absent. Also, after depletion of Rad50 from DCs (through deletion of the gene encoding Rad50 via tamoxifen-stimulated Cre recombinase), DNA-stimulated production of IL-1β is significantly impaired. Hence, a novel DNA-sensing pathway required for IL-1β production, involving the detection of cytosolic DNA and recruitment of CARD9 by Rad50, is revealed.

Roth *et al.* next address how Rad50 and CARD9 contribute to the DNA-stimulated production of IL-1β and show that, consistent with the known ability of CARD9 signaling to activate NF-κB for other pathogen-sensing pathways, here CARD9 controls DNA-stimulated translocation of NF-κB to the nucleus and subsequent induction of pro-IL-1β mRNA[3]. Thus, Rad50 couples the sensing of cytosolic DNA to the CARD9–NF-κB signaling axis to facilitate the induction of pro-IL-1β. The fact that this is the point at which IL-1β production is regulated by Rad50 and CARD9, rather than an effect on the inflammasome, is further clarified by the finding that DNA-stimulated activation of caspase-1 is normal in CARD9-deficient cells. Furthermore, in those cells, IL-1β production

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stimulated by lipopolysaccharide and ATP (which activates the NLRP3 inflammasome) is not affected, which indicates that there is no general defect in the IL-1β response.

CARD9 is also shown to be required for the DNA-stimulated induction of other NF-κB-dependent genes (those encoding IL-6 and TNF) in DCs[3]. For signaling via C-type lectin receptors and RIG-I, CARD9 engages the adaptor Bcl-10 for the activation of NF-κB, and here Bcl-10 is also required for DNA-stimulated IL-1β (but not IFN-β), and is recruited to the DNA-Rad50 complex in a CARD9-dependent manner.

Finally, Roth *et al.* show that the sensing of DNA by Rad50-CARD9 is also important for the detection of a live DNA virus, because vaccinia virus (known to activate the AIM2 inflammasome[4]) stimulates the production of IL-1β, IL-6 and TNF in DCs in a CARD9- and Bcl-10-dependent manner, and, in the context of the viral infection, DNA associates with Rad50 in the cytosol[3]. After infection with vaccinia virus, CARD9-deficient mice show defective IL-1β production and an impaired $CD8^+$ T cell response relative to that of wild-type mice.

These findings reveal an important new signal-transduction pathway that explains how DNA induces pro-IL-1β in DCs, but they also raise several pertinent issues. For example, although the data clearly show that CARD9 regulates the DNA-stimulated activation of NF-κB in DCs, a minor role for STING is also demonstrated, although this does not 'translate' into any effect on pro-IL-1β mRNA when STING is absent. Although evidence of the involvement of STING in the DNA-stimulated activation of NF-κB in myeloid cells is sparse, there are reports of STING-dependent phosphorylation of the NF-κB subunit p65 in macrophages and DCs. This phosphorylation event can be mediated by TBK1 (which STING strongly activates) and is required for full activation of NF-κB. Furthermore, a very clear role for STING in regulating NF-κB activation after transfection of mouse embryonic fibroblasts with DNA has been reported[7]. CARD9 would not be expected to have a role in the activation of NF-κB in fibroblasts, since it is selectively expressed in myeloid cells[8]. Thus, STING can be expected to contribute to the DNA-mediated activation of NF-κB, but its relative contribution is very context dependent, which does not seem to be the case for the STING-mediated activation of IRF3. In any case, it would be interesting to measure IL-1β in STING-deficient mice after infection with poxvirus to assess any overall requirement for STING in the expression of this important product of an NF-κB-dependent gene *in vivo*.

Another issue highlighted by this work is how communication between nuclear proteins and cytosolic DNA occurs to facilitate the sensing of cytosolic DNA. Specifically in this context, what signals Rad50 to move out of the nucleus to engage with transfected DNA or DNA from vaccinia virus? The sensing of DNA from herpesviruses by the DNA sensor IFI16 in the nucleus can somehow signal to the STING-TBK-IRF3 signaling axis in the cytosol, while IFI16 itself (which is normally more abundant in the nucleus than the cytosol), like Rad50, can be mobilized to the cytosol to sense DNA in that compartment[1]. More work is needed to understand this issue of nuclear-cytosolic communication during the sensing of DNA, especially given that an intimate relationship between nuclear sensing of DNA damage and cytosolic sensing of DNA is emerging. Apart from the discovery of the role of Rad50 in this study[3], Mre11, which is part of the MRN complex with Rad50, has been shown in some cell types to be required for the activation of IRF3 stimulated by cytosolic DNA (but not DNA from viruses) via STING[6]. Furthermore, in fibroblasts, DNA-PK (a kinase that participates in repairing double-strand DNA breaks in the nucleus) is involved in the activation of IRF3 (but not NF-κB) mediated by DNA and poxvirus and contributes to antiviral immunity[9].

Exploration of the role of CARD9 in the sensing of DNA in myeloid cells also warrants further attention. For example, it would be very interesting to determine whether other DNA sensors such as p204 (the mouse ortholog of IFI16) and DDX41, both of which have been shown to mediate NF-κB activation in myeloid cells[10,11], can couple to the CARD9–Bcl-10–NF-κB pathway. Also, the role of CARD9 in signaling to the induction of other DNA-stimulated NF-κB–dependent genes should be comprehensively addressed. Collectively, the work by Roth *et al.*[3] further improves the understanding of how cells sense cytosolic DNA and also opens up new avenues of investigation of the roles of Rad50 and CARD9 in innate immunity.

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1. Paludan, S.R. & Bowie, A.G. *Immunity* **38**, 870–880 (2013).
2. Bhat, N. & Fitzgerald, K.A. *Eur. J. Immunol.* **44**, 634–640 (2014).
3. Roth, S. *et.al. Nat. Immunol.* **15**, 538–545 (2014).
4. Rathinam, V.A. *et al. Nat. Immunol.* **11**, 395–402 (2010).
5. Poeck, H. *et al. Nat. Immunol.* **11**, 63–69 (2010).
6. Kondo, T. *et al. Proc. Natl. Acad. Sci. USA* **110**, 2969–2974 (2013).
7. Abe, T. & Barber, G.N. *J. Virol.* **88**, 5328–5341 (2014).
8. Roth, S. & Ruland, J. *Trends Immunol.* **34**, 243–250 (2013).
9. Ferguson, B.J., Mansur, D.S., Peters, N.E., Ren, H. & Smith, G.L. *eLife* **1**, e00047 (2012).
10. Unterholzner, L. *et al. Nat. Immunol.* **11**, 997–1004 (2010).
11. Zhang, Z. *et al. Nat. Immunol.* **12**, 959–965 (2011).

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