may promote cell-autonomous responses directed against cytosolic bacteria, which makes future investigations into this pathway of innate defense an exciting and important research venture.

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TLR2 joins the interferon gang

Franz Bauernfeind & Veit Hornung

The induction of type I interferon is a critical checkpoint in antiviral immunity. Toll-like receptor 2 can unexpectedly induce type I interferon in the subset of inflammatory monocytes during infection with vaccinia virus.

Type I interferons (interferon-α isoforms and interferon-β) are dominant mediators of antiviral immunity in vertebrates. An intact type I interferon response is critical for the survival of the host after viral infection. Since their initial discovery as soluble factors that 'interfere' with viral proliferation half a century ago, intense research has focused on type I interferon signaling, the plethora of type I interferon–mediated effects induced and also the mechanisms of their induction. Soon after the discovery of type I interferon, it was noted that nucleic acids derived from viruses can trigger the production of type I interferon. In line with those observations, several receptor molecules have since been identified that sense nucleic acids and are able to induce type I interferons. These receptors, called 'pattern-recognition receptors', include members of the membrane-bound Toll-like receptor (TLR) family: TLR3, TLR7 and TLR9. These three TLRs can all sense viral nucleic acids and induce type I interferons[1,2]. In this issue of *Nature Immunology*, Barton and colleagues now add TLR2 to this list[3]. However, in contrast to the ligands for other TLRs, nucleic acids do not seem to be the triggering ligands[1].

TLRs can induce different transcriptional responses when activated by their cognate ligands. This can be explained largely by the recruitment of one or more adaptor proteins, which subsequently engage distinct signaling cascades. The adaptor MyD88, which is used by all TLRs with the exception of TLR3, induces a proinflammatory cytokine response, whereas the adaptor TRIF, which is used by TLR3 and TLR4, can trigger the transcription of genes encoding proinflammatory cytokines and type I interferons[2]. A critical checkpoint for the transcriptional activation of genes encoding type I interferons is phosphorylation of the interferon-regulatory factors, mainly IRF3 and/or IRF7. TRIF uses the adaptor TRAF3 to recruit the kinase TBK1, which then phosphorylates IRF3 (ref. 2). In most cell types, MyD88 signaling does not lead to the phosphorylation of IRFs and thus TLRs that signal via MyD88 fail to induce type I interferon. This MyD88-TRIF dichotomy provides an explanation for the outcomes of TLR responses in most cell types in terms of their proinflammatory versus type I interferon transcriptional profile. Nonetheless, this simple model is challenged by two notable exceptions. TLR7 and TLR9, which signal exclusively via MyD88, are potent inducers of the production of type I interferon. However, this response is operative only in a certain subset of dendritic cells (DCs), the plasmacytoid DC (pDC). Indeed, in pDCs, MyD88 forms a unique signaling complex with TRAF3, the kinases IRAK1 and IKKα, and IRF7. In this complex, IRF7 is phosphorylated in an IRAK1- and IKKα-dependent manner and is then translocated to the nucleus to regulate the expression of genes encoding type I interferons[2]. Thus, it seems that in addition to differences in adaptor use, cell type specificity is important in determining TLR-mediated type I interferon responses.

In this issue of *Nature Immunology*, Barbalat *et al.* add another level of complexity to this picture and provide one more exception to the MyD88-TRIF dogma. Studying the innate immune response to various DNA viruses, they find that murine cytomegalovirus and, most prominently, vaccinia virus can induce the production of type I interferon in a manner that does not require viral replication. Surprisingly, this type I interferon response requires TLR2. Indeed, bone marrow cells from TLR2-deficient mice fail to elicit a type I interferon response when challenged with vaccinia virus inactivated by ultraviolet irradiation. In line with numerous published studies, 'classical' TLR2 ligands such as the triacylated lipopeptide Pam_3CSK_4 do not trigger the production of type I interferon. Barbalat and colleagues go on to explain this discrepancy in a series of elegant studies. First they show that TLR2-mediated production of type I interferon requires MyD88 rather than TRIF and is independent of other nucleic acid–sensing TLRs. They find that bone marrow cells from 3d mice, which are dysfunctional in TLR3, TLR7 and TLR9 responses, have normal type I interferon production when stimulated with vaccinia virus.

All TLRs that can induce the production of type I interferon signal from endosomal compartments. TLR3, TLR7 and TLR9 access endosomal compartments directly through the endoplasmic reticulum[4,5], where they can initiate the induction of type I interferon. TLR4 follows the classical secretory pathway and is then trafficked from the plasma membrane to endosomal compartments via endocytosis for type I interferon signaling through TRIF[6]. TLR2, however, is thought to signal mainly from the plasma membrane. Barbalat *et al.* address the role of endosomal localization by blocking endocytosis or endosomal maturation using cytochalasin D or chloroquine. In fact, these experiments reveal that TLR2 also probably signals from the endosomal membrane specifically for induction of the type I

Franz Bauernfeind and Veit Hornung are with the Institute Clinical Chemistry and Pharmacology, University of Bonn, Bonn, Germany.
e-mail: veit.hornung@uni-bonn.de

interferon response. This is in contrast to the induction of proinflammatory cytokines, which, consistent with previous work, is independent of endocytosis or endosomal maturation and is initiated directly from the plasma membrane.

Why has this TLR2–type I interferon signaling pathway been missed in previous studies? One likely explanation is that TLR2-triggered production of type I interferon is functional only in a distinct fraction of bone marrow or spleen cells: inflammatory monocytes (IMs; $CD11c^{-}CD11b^{+}Ly6C^{+}$). The authors identify this cell type in bone marrow using interferon-β reporter mice in which transactivation of the interferon-β promoter is visualized by expression of yellow fluorescent protein. Despite the fact that this unique cell type constitutes about 5% of all cells in primary bone marrow, it is lost in classical macrophage- or DC-differentiation protocols. In fact, the authors go on to show that bone marrow–derived macrophages or DCs, which are used by many researchers to study pattern-recognition receptors, do not show a TLR2-mediated type I interferon response. Interestingly, bone marrow–derived macrophages or DCs produce type I interferon independently of TLR2 when stimulated with nonreplicating vaccinia virus. In a key experiment, Barbalat *et al.* obtain a highly purified fraction of IMs from bone marrow by cell sorting and show that vaccinia virus–mediated production of type I interferon is confined to this cell type and also is TLR2 dependent (**Fig. 1**). Moreover, they show a critical role for this cell type *in vivo*. By ablating $CD11b^{+}$ cells using a CD11b-specific diphtheria toxin receptor approach, they achieve a fairly specific depletion of IMs. IM-depleted mice have much greater induction of type I interferon and higher viral titers when challenged with live vaccinia virus.

Collectively the results outlined above establish TLR2 as a true pattern-recognition receptor that can induce the transcription of genes encoding type I interferons. This is a noteworthy observation, given the fact that TLR2 has classically been considered the prototypical TLR for proinflammatory responses only. The finding that this response seems to be confined to IMs constitutes another interesting observation and might also explain why this pathway has been overlooked for so long. TLR2-mediated induction of type I interferon seems to take place from the endosomal compartment, as it is true for all other TLR-mediated type I interferon responses. Thus, it seems to be a general concept that TLR-triggered production of type I interferon is initiated from endosomal compartments.

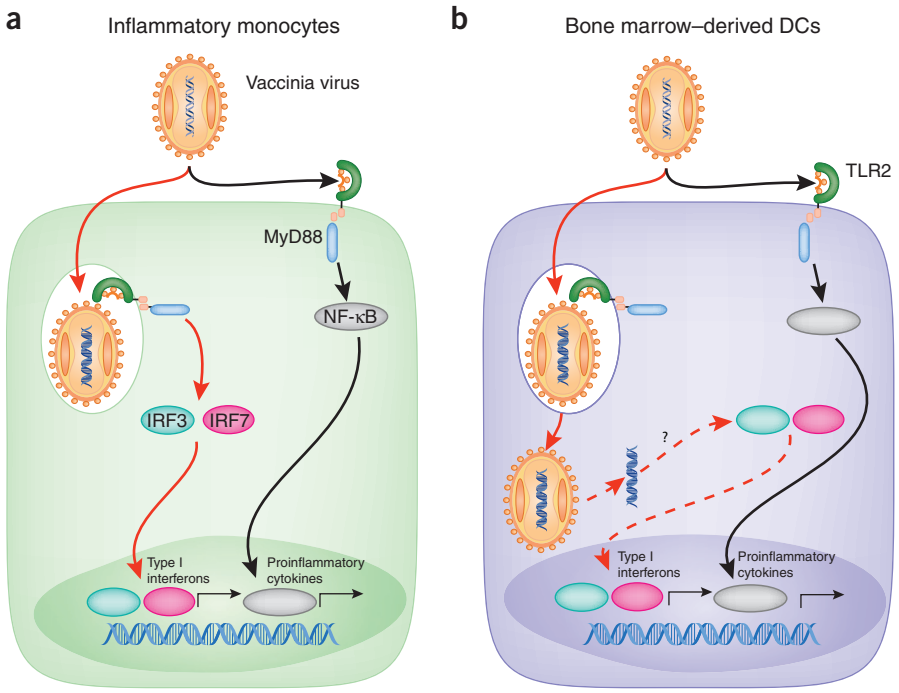

Figure 1 Vaccinia virus–dependent induction of type I interferons in IMs and bone marrow–derived DCs. (**a**) In IMs, vaccinia virus triggers TLR2-dependent activation of the transcription factor NF-κB from the plasma membrane via MyD88. In addition, after uptake of vaccinia virus into endosomal compartments, TLR2 leads to the activation of IRF3 and IRF7, which culminates in the induction of type I interferons (IFN). (**b**) In bone marrow–derived DCs, vaccinia virus also triggers TLR2-dependent activation of NF-κB and subsequent transcription of genes encoding proinflammatory cytokines, but induction of type I interferon seems to be independent of TLR2. Instead, a putative cytosolic recognition machinery senses vaccinia virus, possibly via its double-stranded DNA genome.

One common denominator that is required for TLR-meditated production of type I interferon is TRAF3 (ref. 7). It has been suggested that the spatial separation of TRAF3 from the plasma membrane explains the lack of type I interferon production by TLR2 that is activated at the plasma membrane. Indeed, a synthetic TRAF3 construct targeted to the cell membrane can convert TLR2 into a type I interferon–inducing TLR when stimulated in macrophages[6]. So it is very likely that TLR2-triggered production of type I interferon in IMs is also mediated by TRAF3 through its recruitment to endosomal membranes.

Another puzzling issue related to these results is the finding that only certain ligands of TLR2 can induce this pathway. This is reminiscent of the TLR9 field, in which a similar phenomenon has been described. Monomeric TLR9 ligands such as CpG B induce very small amounts of type I interferon in pDCs, whereas A-type CpG, which forms highly aggregated structures (20–100 nm) triggers a much greater response[8]. It has been suggested that CpG A is retained in endosomal compartments instead of being trafficked to lysosomal structures, as occurs for CpG B[9,10]. A similar scenario may account for the differences seen in TLR2-triggered signal transduction in IMs, in which vaccinia virus is probably retained in endosomal compartments for a prolonged period, rather than Pam_3CSK_4, for example.

The unique use of MyD88 for the induction of type I interferon by TLR2 in IMs highlights some striking parallels to pDCs, which also use MyD88 to induce the transcription of genes encoding type I interferons. Moreover, another interesting analogy is the fact that TLRs seem to be the dominant pattern-recognition receptor system that drives the transcription of genes encoding type I interferons in both of these cell types. Although a putative intracellular sensing pathway seems to be operational in bone marrow–derived DCs for vaccinia virus, IMs seem to use solely TLR2 to induce antiviral immunity in response to this virus. An analogous picture is seen for pDCs, which use TLR7 exclusively to sense influenza virus, whereas the same virus is sensed by the cytosolic sensor RIG-I in bone marrow–derived DCs[11]. Thus, pDCs and IMs might represent a first line of defense that function together to control viral infection by rapidly sensing viruses via the TLR system even before they

replicate. In this context, the role of pDCs as early sources of type I interferon during viral infection is well established. Depletion of pDCs severely affects the control of virus infection in various models. At the same time, indirect evidence exists suggesting that IMs have a similar role. In the absence of the chemokine receptor CCR2, IMs cannot exit the bone marrow and thus their numbers are much lower in peripheral blood and in peripheral tissues[12]. Experiments using CCR2-deficient mice have identified IMs or their progeny as important first-line producers of proinflammatory cytokines in various models of microbial infection. For example, mice infected with murine cytomegalovirus have higher viral titers and substantial mortality in the absence of CCR2 (ref. 13). It will be interesting to study whether the type I interferon response is impaired in these mice and whether IMs are the major source for this cytokine in these models.

A published study has also linked TLR2 to the sensing of vaccinia virus[14]. In that report, vaccinia virus induced proinflammatory cytokine production via TLR2, yet it induced the production type I interferon independently of TLR2. Those observations are not at odds with the results of Barbalat and colleagues, as the former study used bone marrow–derived DCs and not primary bone marrow cells. Thus, the role of IMs was not addressed. However, although Barbalat *et al.* have observed a critical requirement for IMs *in vivo* in terms of the production of type I interferon, the other study did not observe a TLR2-dependent type I interferon response *in vivo*. As IMs confer the TLR2-dependent interferon production in response to vaccinia virus, depletion of this cell type should produce a phenotype similar to that of a TLR2-deficient mouse for the sensing of vaccinia virus. Given the fact that the modalities of vaccinia virus infection were not identical, further studies will be required to clarify this issue.

In conclusion, the study by Barbalat *et al.* contains two key findings that challenge some aspects of the present understanding of innate immunology: TLR2 joins the 'club' of pattern-recognition receptors that can initiate antiviral immunity by inducing type I interferons. IMs, in which this pathway is at work, seem to constitute a distinct subset of innate immune cells that are able to produce large amounts of type I interferon in a TLR-dependent manner. Taking a closer look at this cell type might provide important clues about antiviral immunity and identify new avenues for therapeutic intervention in virus-induced infectious diseases.

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The gut feeling of T_{reg} cells: IL-10 is the silver lining during colitis

Derya Unutmaz & Bali Pulendran

Regulatory T cells that express the transcription factor Foxp3 are pivotal in suppressing autoimmune responses. A report in this issue describes a key role for interleukin 10 produced by lamina propria macrophages in maintaining Foxp3 expression during inflammatory responses in the intestine.

Regulatory T cells (T_{reg} cells) are a subset of $CD4^+$ T cells specialized in suppressing unwanted immune responses. T_{reg} cells require expression of the transcription factor Foxp3 to maintain their suppressive function[1]. The role of T_{reg} cells in preventing autoimmune inflammatory diseases is demonstrated by a mouse colitis model that mimics an inflammatory bowel disease (IBD)-like syndrome. The immunosuppressive cytokine interleukin 10 (IL-10) has also been critically linked to the prevention of colitis, as mice deficient in IL-10 or the IL-10 receptor β-chain (IL-10Rβ) develop spontaneous inflammation of the large intestine. In this issue of *Nature Immunology*, Murai *et al.* link IL-10 to T_{reg} cell–mediated suppression by demonstrating that T_{reg} cells require IL-10 to sustain expression of Foxp3 and their suppressive function in inflamed intestine[2].

A cardinal feature of IBD is extensive inflammation characterized by infiltrates of $CD4^+$ T cells and macrophages and higher concentrations of interferon-γ; these characteristics are evidence of T helper type 1–like responses. Although IL-10 can in some situations control T helper type 1 cell–mediated immune pathologies, the precise mechanism by which IL-10 prevents colitis remains unclear.

It is well established in both human and murine systems that maintaining Foxp3 expression is necessary for T_{reg} cell contact–dependent suppressive functions[3]. Published findings suggest that instability of Foxp3 expression in T_{reg} cells can result in the generation of pathogenic T_{reg} cells[4] that may express IL-17 and/or interferon-γ[5]. However, the mechanisms that regulate Foxp3 stability in T_{reg} cells remain poorly defined.

In an elegant set of experiments, Murai *et al.* demonstrate that IL-10 secreted by a unique subset of macrophages in the lamina propria is responsible for maintaining Foxp3 expression in T_{reg} cells in inflammatory conditions. They arrive at this conclusion with a widely used experimental model for colitis, in which transfer of $CD4^+CD45RB^{hi}$ T cells into immunodeficient mice

Derya Unutmaz is in the Department of Microbiology, Department of Pathology, New York University School of Medicine, New York, New York, USA. Bali Pulendran is with the Emory Vaccine Center, Emory University School of Medicine, Atlanta, Georgia, USA.
e-mail: derya@mac.com or bpulend@emory.edu