could sustain the presence of NKT_{FH} cell–dependent germinal centers.

The new insights gained from these studies could lead to the design of complex antigen formulations that combine lipid-protein antigen with T cell epitopes to prime not only B cells but also T cells. This strategy would boost not only *i*NKT cells but also conventional T cell responses through the activation of dendritic cells. This strategy would combine the antigen-specific *i*NKT cell cognate help provided to B cells plus the adjuvant effect of *i*NKT cells through dendritic cells. Considerable effort has been expended in synthesizing new *i*NKT cell agonists that stimulate various aspects of *i*NKT cell functions. At present, these ligands are assessed mainly for their ability to induce strong T helper type 1 or 2 or tolerogenic immune responses according to the therapeutic objectives. It will be important to assess the effect of *i*NKT cell agonists on inducing IL-21 production by *i*NKT cells to promote not only B cell help but also a different spectrum of cytokines such as IL-4 or interferon-γ, which are known to drive isotype switching to IgG1 and IgG2a, respectively. Modifying the lipid covalently coupled to protein antigen could affect *i*NKT cell function and therefore antigen-specific immune responses.

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$CD169^+$ macrophages take the bullet

Burkhard Ludewig & Luisa Cervantes-Barragan

Lifting the protective shield provided by the type I interferon system selectively in $CD169^+$ splenic macrophages enforces localized viral replication. Such controlled release of virus amplifies adaptive antiviral immune responses.

Type 1 interferons are pleiotropic cytokines that secure an important first line of defense against viral infection. As many viruses replicate rapidly, the interferon system must react swiftly to control viral replication in infected cells and to limit viral dissemination to as-yet-uninfected cells. Mammalian cells not only produce type I interferons under certain conditions but also respond to these cytokines, because the IFNAR receptor for type I interferon is expressed ubiquitously. Thus, the interferon system can be seen as a broadly protective shield that generates and maintains the 'antiviral state' during the early phase of viral infection. In this issue of *Nature Immunology*, Honke *et al.* describe upregulation of the ubiquitin-specific protease Usp18 as a mechanism that lifts the protective type I interferon shield selectively in $CD169^+$ macrophages in the splenic marginal zone and thus enforces locally restricted production of virus to make antigen available for the generation and maintenance of protective, virus-specific T cells and B cells[1] (**Fig. 1**).

After type I interferons bind to their receptors on the cell surface, signals are transduced via the Jak-STAT signal-transduction pathway. Two members of the Jak family of kinases, Tyk2 and Jak1, bind to the IFNAR1 and IFNAR2 subunits, respectively, and form a signal-transducing heterodimer (**Fig. 1b**). A phosphorylation cascade facilitates the binding of STAT1-STAT2 to the IFNAR proteins and subsequent translocation of a complex of STAT1, STAT2 and the transcription factor IRF9 to the nucleus. The binding of this complex to the interferon-stimulated response elements in the promoter regions of interferon-stimulated genes (ISGs) leads to the production of intracellular effectors that directly counteract viral replication[2]. Honke *et al.* now show that the cell type–specific and context-dependent regulation of type I interferon signaling in $CD169^+$ macrophages of the marginal zone via Usp18 secures survival after infection with vesicular stomatitis virus (VSV)[1]. Usp18 was initially described as an enzyme that deconjugates the interferon-induced protein ISG15; Usp18 is upregulated in cells by type I interferons or lipopolysaccharide. The conjugation of proteins encoded by ISGs of ISG15 to other has been shown to be important for antiviral defense in some infections, such as infection with Sindbis virus[3]. As a consequence, it was proposed that Usp18 would interfere with the antiviral response by deconjugating ISG15. However, only the lack of Usp18, but not the lack of ISG15, results in greater susceptibility to infection with lymphocytic choriomeningitis virus or VSV, which suggests that Usp18 might have additional functions[4]. Indeed, Usp18 interferes with the binding of Jak1 to IFNAR2 and hence acts as a negative regulator of type I interferon signaling[5] (**Fig. 1c**). Consequently, deletion of Usp18 results in improved survival of mice infected intracranially with lymphocytic choriomeningitis virus or VSV[6] because in these infection models, larger amounts of locally produced type I interferon lead to better control of viral replication in the central nervous system. Likewise, the enhanced type I interferon response in Usp18-deficient mice confers more efficient protection against infection with *Salmonella typhimurium*. However, such unleashed type I interferon production can also precipitate hypersensitivity to lipopolysaccharide-induced immunopathology[7]. Thus, the tight regulation of type I interferon signaling via Usp18 might also be critical for reaching a protective balance between the innate response to a pathogen and infection-associated immunopathology.

The retention and sampling of infectious agents in specialized zones of secondary lymphoid organs is a critical step in the initiation of immune responses[8]. In the spleen, the marginal zone efficiently filters pathogens from the blood (**Fig. 1**). The macrophages of the marginal zone that express the C-type lectin CD169 (recognized by the monoclonal antibody MOMA-1) are also known as 'metallophilic macrophages'. Similar to splenic $CD169^+$ macrophages of the marginal zone[1], $CD169^+$ subcapsular macrophages of lymph

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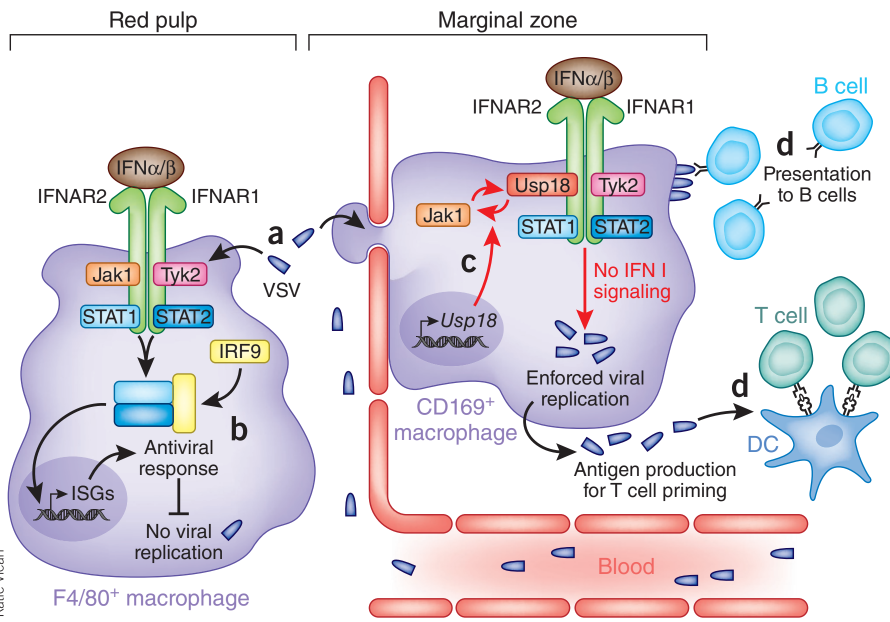

Figure 1 Inhibition of type I interferon signaling in CD169+ macrophages of the marginal zone through Usp18 enforces local virus production and improves activation of antiviral T cell and B cell responses. (**a**) After systemic infection with VSV, viral particles enter the spleen via the blood stream and 'preferentially' infect F4/80+ macrophages in the red pulp and CD169+ macrophages in the marginal zone. (**b**) Type I interferons trigger a potent antiviral intracellular response via IFNAR in F4/80+ macrophages of the red pulp to control the viral infection. (**c**) In contrast, CD169+ macrophages 'take the bullet', as Usp18-mediated inhibition of type I interferon signaling enforces viral replication in these cells. Usp18 functions as a negative regulator of type I interferon signaling by interfering with the binding of Jak1 to IFNAR2. (**d**) The production of live virus by CD169+ macrophages facilitates direct antigen presentation of virus to B cells and generates sufficient antigen for processing and presentation via classical dendritic cells (DCs) for T cell priming.

nodes capture and retain VSV[9,10]. The depletion of CD169+ macrophages specifically from lymph nodes by chlodronate-loaded liposomes can lead to the spread of VSV to other lymph nodes and blood[9] or can facilitate viral neuroinvasion in the infected lymph node[10]. Such studies note not only that CD169+ macrophages are critical for containment of VSV but also that these cells are necessary to achieve high viral loads in lymph nodes. Honke *et al.* provide a molecular explanation for such findings and show that CD169+ macrophages do not exert a passive 'flypaper' function but instead function as a virus-replicating 'sink' for VSV[1].

In their experimental approach, the authors show first that intravenous infection of wild-type mice restricts VSV replication to the spleen, whereas either IFNAR deficiency or treatment with chlodronate liposomes results in the systemic spread of the virus to almost all organs. Second, all IFNAR-deficient macrophages (such as Kupffer cells in the liver, F4/80+ red pulp macrophages and CD169+ macrophages) support VSV replication, whereas in IFNAR-competent mice, only the CD169+ macrophages have substantial VSV production. When assessing whether Usp18 is involved in the cell type–specific support of VSV replication, the authors find that CD169+ macrophages upregulate Usp18 considerably during VSV infection. Consequently, Usp18-deficient mice have much lower viral titers in spleens, which emphasizes the potent negative regulatory effect of Usp18 on type I interferon responses. However, the survival of Usp18-deficient mice is compromised because the virus gains rapid access to the central nervous system. Detailed analysis of the VSV-specific adaptive immune response shows that the Usp18-enforced replication of the virus in CD169+ macrophages of the marginal zone is necessary for the generation of protective adaptive immune responses, including the generation of neutralizing antibodies and antiviral T cells (**Fig. 1d**).

In summary, the study by Honke *et al.* underscores the central function of CD169+ macrophages in controlling antiviral immunity[1]. Furthermore, this study supports the proposal that the early events in the antigen-sampling zones of secondary lymphoid organs, such as the subcapsular sinus in lymph nodes or the splenic marginal zone, are decisive for the outcome of antimicrobial immune responses[9–11]. Nevertheless, important issues still remain to be addressed, such as how viral particles are transferred to classical dendritic cells in the T cell zone, and how viruses are contained by CD169+ macrophages in the splenic marginal zone or the lymph node subcapsular sinus. We predict that the various myeloid cells of antigen-sampling zones of the secondary lymphoid organs (that is, CD169+ macrophages and plasmacytoid and classical dendritic cells) provide the appropriate 'tuning devices' to facilitate controlled release of live virus, either alone or in concert with other cells. However, viruses may have developed counter mechanisms to interfere with the selectively enforced viral replication to remain 'under the radar' of the adaptive immune system[12]. Future studies should characterize these intracellular regulatory and counter-regulatory circuits. Such studies will deliver important information for vaccine development, because targeting of antigen to and 'tuning' of innate effectors in the antigen-sampling zone are critical for the induction of protective adaptive immune responses. Finally, the study of Honke *et al.* provides, at least in part, an explanation for the finding that attenuated live vaccines generally elicit better protection than do inactivated or subunit vaccines[1]. The 'enforced replication' of rationally designed live vaccines in CD169+ macrophages may provide the critical advantage for the successful kick-off of the generation of long-lasting protective immunity.

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