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Viperin Is Highly Induced in Neutrophils and Macrophages during Acute and Chronic Lymphocytic Choriomeningitis Virus Infection

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Although most cells are thought to respond to IFNs, there is limited information regarding specific cells that respond in vivo. Viperin is an IFN-induced antiviral protein and, therefore, is an excellent marker for IFN-responsive cells. In this study, we analyzed viperin expression in vivo during acute lymphocytic choriomeningitis virus Armstrong infection, which induces high levels of type I IFNs, and in persistently infected lymphocytic choriomeningitis virus carrier mice, which contain low levels of type I IFNs. Viperin was induced in lymphoid cells and dendritic cells (DCs) during acute infection and highly induced in neutrophils and macrophages. The expression kinetics in neutrophils, macrophages, and T and B cells paralleled IFN-α levels, but DCs expressed viperin with delayed kinetics. In carrier mice, viperin was expressed in neutrophils and macrophages but not in T and B cells or DCs. For acutely infected and carrier mice, viperin expression was IFN dependent, because treating type I IFN receptor knockout mice with IFN-γ-neutralizing Abs inhibited viperin expression. Viperin localized to the endoplasmic reticulum and lipid droplet-like vesicles in neutrophils. These findings delineate the kinetics and cells responding to IFNs in vivo and suggest that the profile of IFN-responsive cells changes in chronic infections. Furthermore, these data suggest that viperin may contribute to the antimicrobial activity of neutrophils. The Journal of Immunology, 2010, 184: 000–000.

Type I IFNs are produced in the context of viral infections and induce a potent antiviral response that activates innate immunity and leads to a heightened antiviral state. Virally infected cells produce and secrete type I IFNs, notably IFN-α and IFN-β, which activate neighboring cells and alert them to ongoing infection. Upon IFN stimulation, cells that express the type I IFN receptor (IFNAR) undergo a complex signaling cascade that leads to the induction of hundreds of genes and limits viral infection. Although many of the functions of these gene products are still unknown, several of them have dramatic effects on cells, halting protein synthesis and inhibiting cellular proliferation (1, 2).

Although IFN production during many different viral infections has been well characterized, little is known about the ensuing cellular response. Although most tissues and cell lines express the IFNAR transcript to varying degrees, there is increasing evidence that a number of positive and negative regulatory molecules can modulate the intensity and kinetics of IFNAR signaling (3). Furthermore, although low levels of IFNs are thought to persist throughout chronic viral infections (4–6), the levels are generally below the limit of detection and are difficult to measure. The challenge of detecting IFNs in vivo and the lack of a good marker for IFN stimulation have made it difficult to evaluate the nature and extent of the IFN response during various infections.

Viperin is one of the most highly induced IFN effector proteins (7, 8). Similar to other well-characterized IFN-induced effector proteins, viperin is rapidly induced upon IFN stimulation or infection with various viruses. Viperin, also known as RSAD2, cig5 in humans, and vig1 in mice, was originally identified as a gene induced in fibroblasts upon human CMV (HCMV) infection (7). Subsequent analyses showed that viperin is induced in a variety of cell types by types I and II IFNs, polyinosinic:polycytidylic acid, dsRNA, viral DNA, and LPS (9–13). In addition, infection with numerous RNA and DNA viruses, including Japanese encephalitis virus, Sindbis virus (SIN), rhinovirus, hepatitis C virus, dengue virus, Sendai virus, vesicular stomatitis virus, pseudorabies virus, and HCMV, induces high levels of viperin (8, 9, 12, 14–17).

Although viperin is highly conserved across mammals and lower vertebrates (9), its precise mechanism of action is still largely undefined. Viperin was shown to localize to the endoplasmic reticulum (ER) and lipid droplets and to inhibit the replication of various DNA and RNA viruses (9, 18, 19). Overexpression of viperin inhibits HCMV, hepatitis C virus, SIN, and influenza A virus, whereas small interfering RNA-mediated knockdown of viperin enhances the replication of Sendai virus, SIN, and HIV-1 (9, 15, 17, 20). For HCMV, viperin overexpression was specifically shown to reduce the synthesis of late viral proteins, including pp65, glycoprotein B, and pp28, but the mechanism of reduction is not known (9). Overexpression of viperin inhibits the budding and release of influenza A virions from infected cells by altering lipid raft microdomains on the plasma membrane (18). More recent studies showed that viperin expression reduces protein secretion and alters ER membrane morphology (21).

In this study, we examined viperin expression in vivo during acute lymphocytic choriomeningitis virus (LCMV) Armstrong infection, which produces high levels of type I IFNs, and in chronically infected
LCMV carrier mice, which produce transiently detectable levels early in infection that decline to undetectable levels as the infection persists (4, 6, 22). We show that viperin is an excellent marker for IFN-responsive leukocytes because it is rapidly and highly expressed in various cell types, with an expression pattern that follows IFN-α kinetics during acute LCMV infection. Specifically, viperin is highly induced in neutrophils (NFs) and macrophages (MDs) in lymphoid organs in acutely infected mice and in carrier mice, whereas its expression in T cells, B cells, and dendritic cells (DCs) is only seen in acute infection. IFNs are necessary and sufficient to induce viperin expression in NFs and MDs, because treating mice with type I or II IFNs induced viperin expression, while inhibiting types I and II IFN stimulation in acutely infected mice, and in LCMV carrier mice it blocked viperin expression. Finally, analysis of NFs by immunoelectron microscopy showed that viperin localized to the ER and to the membrane of intracellular vesicles that are morphologically similar to lipid droplets and also contain the ER marker calreticulin.

Overall, these findings suggest that many cells respond to IFN stimulation during viral infection and that NFs and MDs are the predominant cell types that express viperin during acute and persistent infections. These findings indicate that although LCMV carrier mice produce persistently low levels of IFN, the cells capable of responding to IFN are significantly changed, which may ultimately impact their ability to clear viral infection. Furthermore, because NFs and MDs are primarily associated with phagocytic killing of extracellular microbes, the results also suggest that viperin may play a central role in bacterial or parasitic infections and may protect these cell types from infection.

Materials and Methods
Reagents

The anti-Grp94 and anti-viperin (MaP.VIP) Abs were described previously (21). Anti-calreticulin was purchased from Affinity BioReagents (Golden, CO). Fluorescently conjugated FACS Abs were purchased from eBioscience (San Diego, CA), including FITC–GR-1, PE-F4/80, PerCP-MHC II, Pacific Blue–CD11c, PeCy7–CD11b, PerCP-Cy5.5, PE, and PE Texas Red-B220. Magnetic anti-rat IgG and IgM particles were obtained from Polysciences (Warrington, PA). LPS was purchased from Sigma-Aldrich (St. Louis, MO), and the IFN-γ–neutralizing Ab (XMG1.2) was purchased from BioXCell (West Lebanon, NH). Mouse IFN-α and IFN-γ were purchased from R&D Systems (Minneapolis, MN).

Mice, viral infections, and IFN and LPS treatments

The IFNAR1 mice were obtained from Warren Shlomchik (Yale University). Tissues were homogenized using a PowerGen 700 (Fisher Scientific, Pittsburgh, PA) in 1 ml PBS containing protease inhibitors. Homogenates were serially diluted in DMEM containing 5% BCS and then added to Vero cell monolayers in six-well plates. After a 1-h incubation at 37˚C, the inoculum was removed, and the cells were overlaid with media containing 1% agarose and 0.02% Neutral red (Sigma-Aldrich, St. Louis, MO), and the IFN-α standard (HC1040; Hycult Biotechnology) were diluted in blocking buffer, added to the plate, and then incubated overnight at 4˚C. Plates were washed and incubated with the detection Ab (CP2012; Cell Sciences, Canton, MA), followed by an HRP-conjugated anti-rabbit Ab and HRP tetramethylbenzidine substrate (Pierce, Rockford, IL). The HRP reaction was stopped with 2 N H2SO4 and analyzed at a 495-nm wavelength using a Wallac Victor 1420 (PerkinElmer, Wellesley, MA).

Tissue isolation and flow cytometry

Mouse tissues were harvested on the noted days and placed into RPMI 1640 containing 1% BCS and 0.5% 2-ME. Spleens, lungs, and livers were digested in media containing 10 U/ml collagenase (Sigma-Aldrich) for 1 h at 37˚C. Tissues were manually homogenized and then filtered through a 100-μm nylon-mesh filter (BD Biosciences, San Jose, CA). Two million cells were surface stained in FACS buffer (PBS with 0.5% BCS) with the noted flow cytometry (FACS) Abs and then fixed in 4% formaldehyde. Cells were permeabilized with 0.2% saponin in FACS buffer and then stained with FITC- or Alexa 647-conjugated MaP.VIP. All FACS samples were run on an LSR-II (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR).

Immunofluorescence with tissue sections

For immunofluorescence of spleen sections, the tissues were snap-frozen in Cryo-OCt compound and then sectioned. Thin tissue sections were placed on glass slides and then fixed in 4% paraformaldehyde. Sections were permeabilized with 0.1% Triton X-100 in PBS and stained with the indicated Abs. All immunofluorescence samples were mounted with Prolong Gold AntiFade (Invitrogen, Carlsbad, CA) and analyzed using a Leica TCS SP2 confocal microscope (Leica Microsystems, Wetzlar, Germany).

Western blot analysis of tissue homogenates

Homogenized tissue samples were brought to 1% Triton X-100 and then boiled in reducing sample buffer. Lysates were separated on 12% SDS-PAGE gels, transferred to polyvinylidene difluoride membranes, and then probed with the indicated Abs.

Cell sorting and immunoelectron microscopy

Splenocytes from six LPS-treated mice were stained with rat anti-B220 (ATCC TIB-146), anti-CD4 (GK1.5), and anti-CD8 (ATCC TIB-210) Abs, washed extensively, and then incubated with magnetic anti-rat IgG and anti-rat IgM, as previously described (23). After pulling down these cells with a magnet, the B cell- and T cell-depleted splenocytes were stained with anti-GR-1 and anti-CD11b and then FACS sorted on a FACSaria (BD Biosciences). The sorted cells were collected into 2% paraformaldehyde in 0.25 M HEPES (pH 7.4) and then fixed in 4% paraformaldehyde containing 0.1% glutaraldehyde in 0.25 M HEPES (pH 7.4) for 30 min on ice. Finally, the cells were washed and incubated in 4% paraformaldehyde in 0.25M HEPES (pH 7.4) at 4˚C overnight. The cells were prepared for immunocytochemistry, as previously described (21), and stained with MaP.VIP and anti-calreticulin, followed by 10- and 5-nm gold (Cell Microscopy Center, Utrech University, The Netherlands), respectively. A Tecnai 12 Biotwin electron microscope and charge-coupled device camera (Morada, Olympus, Munich, Germany) were used to examine and capture images.

Results

Viperin is expressed in most lymphoid cells during LCMV Armstrong infection

To establish that viperin is a good marker for IFN-responsive cells during viral infections, we used LCMV Armstrong as a model of an acute, systemic viral infection that produces high levels of IFN-α. C57BL/6 mice were infected with 200,000 PFU LCMV i.p. On days 1–5, spleens, lungs, and livers were harvested from infected and naive mice, and tissue homogenates were analyzed for viperin expression by Western blot. Viperin was detectable in these tissues with fairly rapid kinetics, and expression declined around day 4 or 5 (Fig. 1A). To determine the specific cell types within these tissues that expressed viperin, hematopoietic cells within the spleens, lungs, livers, and lymph nodes (LNs) were analyzed for viperin expression by FACS analysis, whereas viral titers and IFN-α levels in tissue homogenates and the serum were assayed by plaque assay
and ELISA, respectively. The specificity of the anti-viperin mAb, MaP.VIP, was confirmed using wild-type, heterozygous, and viperin knockout mice, which showed a dose-dependent decrease in viperin expression by FACS analysis (Supplemental Fig. 2A). During infection, viperin was expressed in most cell types, including T cells, B cells, DCs, NΦs, MΦs, and NK cells (Fig. 1B–G, Supplemental Fig. 1). CD4 and CD8 T cells and B cells in the spleen expressed moderate levels of viperin with rapid kinetics (Fig. 1B), whereas...
DCs also expressed moderate levels but with delayed kinetics. Viperin expression in DCs in the liver, lung, and LNs was not readily detectable until day 3, and it rapidly declined by days 4 and 5 (Fig. 1C). Viperin expression was highest in NØs and MΦs in the liver, spleen, lung, and LNs (Fig. 1D–G). These cell types were distinguished by their levels of GR-1 and CD11b; FACS sorting and electron microscopic analysis of the polynuclear morphology of GR-1 high, CD11b high cells confirmed that they were NØs (24). Furthermore, these cell types rapidly expressed viperin by day 1 postinfection and maintained this level of expression until day 4 or 5.

**Viperin expression corresponds to IFN-α levels in LCMV Armstrong-infected mice**

Next, we examined IFN-α levels in LCMV Armstrong-infected mice to determine whether viperin expression correlated with IFN-α levels. IFN-α levels were detectable in the serum, liver, and spleen on day 1 and gradually declined to undetectable or lower levels at days 4 and 5 (Fig. 2A). This decline in IFN-α levels in the serum and target organs on day 4 or 5 in LCMV Armstrong-infected mice correlated with a reduction in viperin expression. IFN-α was detected in the spleen and liver, which are the two organs predominantly associated with LCMV Armstrong infection, but it was not detected in the lung or LNs (25).

Consistent with the IFN-α results, LCMV Armstrong viral titers were only detected in the spleen and liver, with higher levels in the spleen (Fig. 2B). Furthermore, the decline in LCMV viral titers in the spleen correlated with IFN-α levels and viperin expression in this organ. Consistent with previous findings, LCMV titers in the spleen decreased by 1 log on day 5, whereas in the liver, viral titers increased by ~1 log on days 4 and 5 (Fig. 2B) (25).

**Viperin is expressed in NØs and MΦs in LCMV carrier mice**

To further determine whether viperin can be used as a marker of IFN-responsive cells in a persistent viral infection that produces low levels of IFNs, we examined viperin expression in LCMV carrier mice (6). One day after birth, C57BL/6 mice were infected with 200,000 PFU of LCMV Clone 13 intracranially (black histograms), and 6-wk-old C57BL/6 mice infected with 2,000,000 PFU of LCMV Clone 13 i.v. (gray histograms) were analyzed 6 mo postinfection. The spleens were harvested and examined by FACS staining for viperin expression in NØs (gray boxes), MΦs (black boxes) (A), B cells (B), and CD4+ T cells (black boxes) and CD8+ T cells (gray boxes) (C), as described in Fig. 1. The data are representative of three independent experiments, for a total of three mice per group.

**IFN-α– and IFN-γ–treated mice express moderate levels of viperin in NØs and MΦs**

Because viperin expression closely paralleled the IFN kinetics in LCMV Armstrong-infected mice, and viperin has been consistently shown to be upregulated upon IFN stimulation in vitro, we examined viperin expression in IFN-α– or IFN-γ–treated mice to confirm that IFNs are sufficient to induce viperin expression in vivo. C57BL/6 mice were treated with 1.5 μg IFN-α and IFN-γ. Sixteen hours posttreatment, the lymphoid organs were harvested from naive and IFN-treated mice and examined for viperin expression. Viperin was moderately expressed in GR-1+ CD11b⁺
cells, which could not be further delineated into $N\Phi$s and $M\Phi$s (Fig. 4A). Viperin was also moderately expressed in DCs but was not detectable in CD4 or CD8 T or B cells (Fig. 4B, 4C).

To further examine where viperin-expressing cells are located within the spleen after IFN-α or -γ treatment, we also analyzed spleen sections by immunofluorescence. First, viperin-expressing cells were examined in the context of splenic architecture. B220 and F4/80 staining was used to delineate the white and red pulp, respectively. Staining with an anti-viperin Ab showed that viperin-expressing cells were predominantly located in the red pulp, which is consistent with the high level of viperin expression in MΦs (Fig. 4D). These spleen sections were also stained with anti-GR-1, anti-CD11b, and anti-viperin Abs. Consistent with the FACS analysis, viperin was highly expressed in GR-1+ and CD11b+ cells (Fig. 4E). As expected, viperin was not expressed in the spleens of type I or II IFNAR knockout mice (data not shown).

**FIGURE 4.** Viperin is expressed in NΦs/ MΦs and DCs after IFN-α and -γ stimulation. A–C, C57BL/6 mice were left untreated (gray-filled histograms) or treated with 1.5 μg IFN/mouse (black histograms). Sixteen hours posttreatment, the spleens, LNs, liver, and lung were harvested and analyzed by FACS staining for viperin expression in GR1+ CD11b+ cells (A). Splenocytes in A were analyzed for viperin expression in DCs (MHC II+ CD11c+ cells) (B) or CD4+ T cells (black histograms) and CD8+ T cells (gray histograms) (C). D and E, Spleens sections from IFN-treated mice were fixed, permeabilized, and stained for viperin and B220 or F4/80 as markers of the white and red pulp, respectively (D), or with GR-1 and CD11b (E). Original magnification ×20 and ×63. The data are representative of two independent experiments, for a total of four to six mice per group.

**Types I and II IFN signaling is necessary to induce viperin expression during acute and chronic LCMV infections**

The above data suggested that viperin was a marker of IFN-stimulated cells during infection in vivo and that IFN stimulation was sufficient to induce viperin expression in NΦs and MΦs. In addition, the kinetics of viperin expression paralleled IFN-α levels in LCMV-infected mice, suggesting that IFN-α may be the major contributor to viperin expression during LCMV infection and potentially other infections. To determine whether viperin expression during LCMV infection was solely IFN dependent, C57BL/6 wild-type and IFNAR knockout mice were infected i.p. with LCMV Armstrong (200,000 PFU) and treated with a neutralizing IFN-γ Ab on days 0 and 2 postinfection. When viperin expression was examined on day 3 postinfection by FACS, IFNAR mice had dramatically reduced viperin expression in splenic NΦs and MΦs (Fig. 5A). Treating LCMV-infected IFNAR mice with the neutralizing
IFN-γ Ab completely abolished viperin expression. Analyses in LCMV carrier mice showed similar results. Viperin expression in NΦs and MΦs was reduced to baseline levels in IFNAR knockout mice and in IFNAR mice that were treated with neutralizing IFN-γ Abs (Fig. 5B). Therefore, viperin expression in LCMV Armstrong-infected and LCMV carrier mice is IFN dependent.

Viperin localizes to the ER and lipid droplets in NΦs

Next, we examined the intracellular localization of viperin within NΦs. Because viperin was reported to localize to lipid droplets, and inflammatory reactions have been associated with lipid body formation in NΦs, we examined NΦs in LPS-treated mice (26). Mice were treated with 500 μg LPS i.p., which also induces IFN. Consistent with our previous findings, viperin was highly expressed in NΦs, MΦs, and DCs in the liver, LN, and spleen but not in T or B cells (Fig. 6A, data not shown). To further examine viperin expression in NΦs, GR-1 high, CD11b high cells were FACs sorted (Supplemental Fig. 3) and then examined by immunoelectron microscopy. Electron microscopy confirmed the purity of the NΦ population, because 100% of the cells were polyendocytosed and contained electron-dense granules (Fig. 6B1). Furthermore, immunoelectron microscopy with an anti-viperin Ab showed that viperin localized to the ER, along with the ER marker calreticulin, and was also present at the limiting membranes of cytoplasmic vesicles that morphologically resembled lipid droplets (Fig. 6B). These vesicles, which also contained calreticulin, contained low electron-dense material, consistent with their definition as lipid droplets.

Discussion

This analysis of viperin expression revealed several novel findings that elucidated the kinetics of IFN stimulation, identified IFN-responsive cells during acute and persistent viral infections, and indicated how viperin may function during infection. Because viperin is one of the most highly induced genes in response to IFNs, we used viperin as a marker for cells that responded to IFN stimulation. We showed that many hematopoietic cell types rapidly respond to IFN stimulation during an acute LCMV Armstrong infection but that NΦs and MΦs are the only examined cell types that express viperin in LCMV carrier mice. During acute LCMV infection, the induction of viperin closely paralleled the kinetics of IFN-α production, and preventing stimulation by types I and II IFN in acutely infected and carrier mice returned viperin expression to baseline. Because viperin expression in an acute and chronic viral infection model was strictly IFN dependent, these results indicate that viperin is an excellent marker for IFN-stimulated leukocytes. Although not examined in this study, viperin is likely to be a general marker of IFN-responsive cells, including nonhematopoietic cells. Previous studies showed that viperin is induced in many cell types by types I, II, and III IFNs (27). Although elimination of the types I and II response eliminated viperin expression in leukocytes (Fig. 5), type III IFNs could be important for induction in nonhematopoietic cell types.

Our findings also indicate that chronic LCMV carrier infection, and likely other infections, persistently induce IFN that activates downstream gene expression. Although previous studies showed that low levels of IFN are produced during chronic infections (6, 22, 28), there is little information on how these low levels affect and alter downstream gene expression and the innate immune response. In this study, we showed that these IFN levels are sufficient to upregulate viperin expression in NΦs and MΦs, albeit to lower levels than in acutely infected mice. Interestingly, other cell types, notably T cells, B cells, and DCs, did not express viperin during chronic LCMV infection. The absence of viperin expression in lymphoid cells of carrier mice could be a direct consequence of low IFN levels. A threshold of IFN stimulation may be required to upregulate viperin expression in certain cell types, and this level may not be reached during chronic LCMV infection. Low IFN levels are thought to be present in LCMV carrier mice (6).

Previous studies showed that LCMV encodes a nucleoprotein that inhibits IFN-β production by blocking the nuclear translocation of IFN regulatory factor-3. This protein is expressed at high levels in mice chronically infected with LCMV Clone 13 (29), which may reduce IFN production and limit cell types that respond to low IFN
levels. Alternatively, chronic viral infection may suppress the IFN responsiveness of certain cells by an unknown mechanism. Studies with chronic HIV patients showed that certain cell types, notably plasmacytoid DCs, have a dampened response to IFN-α stimulation in vitro, despite high levels of circulating IFN-α (30, 31). These findings suggest that constant IFN stimulation leads to unresponsiveness or even cellular exhaustion.

Regardless of the mechanism that suppresses IFN production and/or subsequent unresponsiveness, alterations in the types of cells that respond to IFN stimulation may ultimately affect the ability to clear the virus. During viral infections, IFNs play a critical role in inducing antiviral innate immune responses, as well as in activating various cells that are involved in the adaptive immune response and viral clearance. For example, IFN was shown to play a central role in activating T, NK, and B cells during HIV infection (30). During LCMV infection, IFN-α is necessary to induce the maximal expansion of Ag-specific CD8 T cells (32). Because T cells did not express viperin during chronic LCMV infection, their ability to sense and/or respond to IFN may be partially or fully compromised, and this may extend to their ability to expand and clear virus.

The profile of viperin expression indicates which cells respond to the low IFN level in LCMV carrier mice, and it illustrates the kinetics of IFN stimulation during acute LCMV infection. NΦs, MΦs, T cells, and B cells upregulated viperin expression within 1 d postinfection. Viperin expression persisted for 24–48 h and then began to decline around day 4 or 5. Furthermore, the expression of viperin in these cell types was consistent with the peak of IFN levels in the serum, spleen, and liver. These findings indicate that these cell types rapidly respond to IFN stimulation during infection. However, DCs expressed viperin with delayed kinetics, peaking on day 3. DCs become rapidly phenotypically activated by type I IFNs during various infections, including LCMV (33, 34), indicating that they are IFN responsive during the initial phase of infection. The fact that viperin expression was delayed in DCs suggests that these cells may have different mechanisms that regulate downstream IFNAR signaling and delay upregulation of certain IFN-induced genes. This delay may correlate with the function of DCs during LCMV infection and potentially other infections. During LCMV infection, DCs are thought to be able to prime T cells only during a short window of infection, with optimal T cell priming occurring within the first 24 h of infection (33). Because viperin and other IFN-induced genes were shown to significantly impact cellular functions (1, 35), DCs may delay the expression of these genes to successfully initiate the adaptive immune response. Especially, viperin was shown to alter plasma membrane fluidity and inhibit protein secretion. Because the T cell–DC interaction largely depends on the rigidity of plasma membrane microdomains, viperin expression could affect the ability of DCs to efficiently prime T cells, and viperin expression may be delayed in DCs until after T cell priming.

Although the expression of viperin was reported in T cells, DCs, and MΦs, it has never been reported in NΦs (14, 18, 36). Furthermore, given that NΦs and MΦs are mostly associated with antibacterial and antiparasitic immunity, and viperin has been primarily associated with antiviral immunity, the high levels of viperin in these populations are informative and surprising (37, 38). NΦs are primarily associated with phagocytic killing of extracellular microbes. During microbial infections, they are the first immune cells that are recruited from the circulation to the site of infection. At the site of infection, NΦs engulf bacteria, parasites, and fungi into phagosomes that then fuse with specialized lysosomes containing antimicrobial peptides, enzymes, and reactive oxygen species (38, 39). Although NΦs have a very short half-life of several hours, these cells are essential for bacterial and parasitic immunity, and defects in NΦ function, such as in chronic granulomatous disease, are associated with a severe susceptibility to bacterial and fungal infections (38, 40).

NΦs have also been associated with viral infections. Several research groups detected HCMV transcripts in polymorphonuclear leukocytes in which HCMV was proposed to undergo abortive replication (41, 42). Because viperin was shown to inhibit HCMV, viperin may be responsible for limiting its replication in NΦs. It is also conceivable that viperin is highly induced in NΦs during viral infection to protect them from subsequent bacterial coinfections.
Regardless of the evolutionary rationale for robust viperin expression in Nöbs, the established functions of viperin, including alterations in ER morphology, protein secretion, and plasma membrane fluidity, could impact a number of these microbes at various stages in their life cycle. For example, bacteria and parasites were shown to alter lipid rafts to generate platforms that facilitate the internalization of these pathogens into cells (43, 44). In addition, several bacteria use the host secretory pathway to secrete cilitate the internalization of these pathogens into cells (43, 44). In addition, several bacteria use the host secretory pathway to secrete

...fritin and role in antiviral response. J. Virol. 81: 1665–1678.


