

Science. Author manuscript; available in PMC 2013 December 15.

Published in final edited form as:

Science. 2013 May 10; 340(6133): . doi:10.1126/science.1233028.

Cellular Self-Defense: How Cell-Autonomous Immunity Protects Against Pathogens

Felix Randow^{1,*}, John D. MacMicking^{2,*}, and Leo C. James^{1,*}

¹Medical Research Council Laboratory of Molecular Biology, Division of Protein and Nucleic Acid Chemistry, Francis Crick Avenue, Cambridge CB2 0QH, UK

²Department of Microbial Pathogenesis, Boyer Centre for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06510, USA

Abstract

Our prevailing view of vertebrate host defense is strongly shaped by the notion of a specialized set of immune cells as sole guardians of antimicrobial resistance. Yet this view greatly underestimates a capacity for most cell lineages—the majority of which fall outside the traditional province of the immune system—to defend themselves against infection. This ancient and ubiquitous form of host protection is termed cell-autonomous immunity and operates across all three domains of life. Here, we discuss the organizing principles that govern cellular self-defense and how intracellular compartmentalization has shaped its activities to provide effective protection against a wide variety of microbial pathogens.

The cell is an outstandingly attractive nutrient source for potential parasites and pathogens. All organisms must therefore have developed the ability to defend against such threats early during evolution (1). In metazoans, specialized immune cells stimulate innate immunity and, in vertebrates, adaptive immunity; however, most other species rely entirely on intrinsic self-defense for protection, which in some cases has reached an astonishing level of complexity. In archaea and bacteria, for example, even adaptive forms of resistance—long considered the hallmark of vertebrates—contribute to cell-autonomous immunity, as exemplified by the clustered regularly interspaced short palindromic repeats (CRISPR) system, which recognizes foreign DNA in a sequence-specific manner (2). In metazoans, cellular self-defense synergizes with the whole-body protection provided by traditional immunity to confer pathogen resistance. Here, professional immune cells patrol their environment in search of pathogens, whereas cell-autonomous immunity guards both individual immune and non-immune cells against the immediate threat of infection (3–6). Cellular self-defense thus has the potential to confer antimicrobial protection on most, if not all, cells.

Cell-autonomous effector mechanisms appear conserved across phyla. For example, nitric oxide synthases (NOSs) defend Gram-positive bacteria against other bacilli that share the same environment (7). Epithelial cells in the osmoregulatory Malpighian (renal) tubules of flies express NOS to cell-autonomously combat bacteria (8). Similar pathways exist in nonimmune cells of humans and mice (5). Note, however, that these ancient systems have not been inherited unchanged by higher eukaryotes. Viral restriction factors such as the vertebrate tripartite motif (TRIM) protein family or immunity-related guanosine triphosphatases (GTPases) (IRGs) that protect against intracellular bacteria and protozoa are encoded by some of the fastest evolving genes in all of metazoan biology (4, 5). Here, we

^{*}Corresponding author: randow@mrc-lmb.cam.ac.uk (F.R.); john.macmicking@yale.edu (J.D.M.); lcj@mrc-lmb.cam.ac.uk (L.C.J.).

outline the basic principles of cellular self-defense in animals, with an emphasis on how cells take advantage of their compartmentalized nature and how the defined composition of compartments, as well as the borders between them, are used to antagonize the invasion, replication, and spread of intracellular pathogens.

A Topological View of Cellular Self-Defense

Surveying the entire cell body for the presence of pathogens is a daunting task, particularly for eukaryotic cells whose volume greatly exceeds that of their potential foe (5). The division of eukaryotic cells into membrane-bound compartments poses an additional problem because compartments can provide excellent sanctuaries for pathogens (5, 9). Immune sensors must therefore exhibit a high degree of sensitivity; be targeted to the appropriate location; or be part of a widespread, multilayered surveillance system to detect infection.

Although compartmentalization provides potential pathogen habitats, it also facilitates unique defense strategies (Fig. 1). Pathogens entering their preferred intracellular niche must cross at least one, and often two or three, physical barriers (cellular membranes). Traversing each barrier requires specific adaptations, and at each step, pattern recognition receptors (PRRs) and other sensory machinery are positioned to alert the host cell to the presence of infection, while restriction factors are poised to inhibit replication (5, 10, 11). Compartmentalization has driven the evolution of compartment-specific sensory receptors capable of detecting pathogen-associated molecular patterns (PAMPs) (12, 13) and dangerassociated molecular patterns (DAMPs) (14). Compartmentalization also allows the cell to control the topological distribution of molecules that are either harmful or desirable to the pathogen. This principle has enabled the evolution of powerful antimicrobial effector mechanisms that, although detrimental for the cell in one compartment, are innocuous in others. Finally, compartmentalization enables steep concentration gradients across membranes. These gradients permit cells to survey the integrity of their compartments based on danger receptors that detect the entry of molecules that are normally excluded from a given compartment.

Compartment Borders Restrict Pathogen Movement

Self-defense begins even before pathogens come into contact with the cell surface. Chemorepellents are used to deter microbes from approaching host cells, such as hydrogen peroxide (H_2O_2) generated by the apically localized DUOX2 enzyme (15). Specialized gut epithelial Paneth cells secrete the antimicrobial lectin, RegIII γ , into the lumen of the small intestine to maintain a ~50- μ m germ-free barrier (16). Lack of this sugar-binding protein in mice enables rapid microbial colonization of the intestinal mucosa.

All cells are surrounded by outer membranes, and eukaryotic cells are divided by endomembranes into multiple internal compartments. These membranes constitute physical barriers to infection. Pathogens have evolved different solutions to this challenge. Certain enveloped viruses, such as HIV, herpes simplex virus (HSV), and Rous sarcoma virus, fuse with the plasma membrane to deliver their genome into the cytosol. This fusion event is sensed by the cell using polytopic membrane proteins like STING to trigger antiviral defenses (17). Antimicrobial proteins embedded within the plasma membrane further increase its effectiveness as a barrier. The multisubunit enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2), for example, consists of a transmembrane heterodimer (gp91phox, p22phox) and cytosolic subunits that assemble into the mature holoenzyme at this site; here, it generates microbicidal reactive oxygen species (ROS) to inhibit incoming pathogens (5).

Some intracellular pathogens, including enveloped and nonenveloped viruses, bacteria, protozoa, and fungi, do not cross the plasma membrane at the cell surface but are instead taken up into vesicles. Although this strategy facilitates entry into cells, it does not provide direct access to their cytosol. Thus, pathogens entering the endocytic network must quickly subvert their vacuoles to avoid transport to the degradative lysosomal compartment or must escape into the cytosol. Bacteria, in particular, have evolved sophisticated systems to manipulate vesicle maturation by secreting effector proteins into the host cytosol in an attempt to establish a safe replicative niche for the pathogen (9). Host cells counteract this strategy by using compartmental PPRs to detect vesicular pathogens and to stimulate LC3-assisted phagocytosis (LAP) (6, 18). Viral exit from endosomes can also be blocked by the interferon-inducible transmembrane proteins (IFITM), which are effective against varied enveloped viruses including influenza, coronavirus, lentivirus, and flavivirus (5, 11).

Other compartmental borders include the nuclear envelope, which many viruses must also negotiate during their replication cycle, including adenovirus, HSV, and HIV. Except during cell division, the host genome is protected by the nuclear envelope, and traffic into and out of the nucleus is strictly controlled by the nuclear pore complex. Some viruses cannot pass through nuclear pores, while others rely on clever strategies for entry. For example, gammaretroviruses, like murine leukemia virus, cannot infect nondividing cells, but related lentiviruses, like HIV, can. Their ability to cross the nuclear envelope relies on hijacking nuclear import cofactors, including CPSF6, TNP03, and nuclear pore proteins like NUP358 and NUP153 (19). Whether antiviral factors actively prevent viral nuclear entry remains unclear, although the fact that the nuclear pore is significantly smaller (~10 to 20 nm) than most viral nucleocapsids (~20 to 100 nm) provides an effective antiviral barrier in its own right.

Compartmental borders represent a barrier both for incoming and exiting pathogens. Many enveloped viruses "bud" from the cell membrane, and several antiviral proteins inhibit this process, including tetherin and viperin (5, 11). Tetherin, a dimer comprising two long α -helices anchored at either end in the membrane, physically "tethers" the viral envelope to the plasma membrane as the virion buds, whereas viperin disrupts lipid raft microdomains used by viruses, such as influenza, to exit cells (5, 20).

How Compartmentalization Fosters Pathogen Detection by Danger Receptors

Cellular borders are not sufficient to prevent infection, and therefore, cells must have other ways of sensing and inhibiting pathogens. Cell-autonomous immunity relies on both PRRs to detect microbial signatures and danger receptors that monitor DAMPs, i.e., disturbances in cellular homeostasis caused by infection, rather than the pathogen itself. The latter process predominates in organisms that lack circulating immune cells, such as plants and nematodes, and give rise to mechanistic models known as the "guard" hypothesis and effector-triggered immunity (14, 21).

Compartmentalization is a prerequisite for DAMP recognition, because only strict internal organization allows the detection of molecules outside their proper spatial context. Galectins are a family of cytosolic lectins with specificity for β-galactosides that detect damage to endosomes or lysosomes when luminal glycans, otherwise hidden inside vesicles, become accessible to the cytosol (Fig. 2). Galectins perceive even sterile damage to membranes and, thus, serve as versatile proxies of membrane damage caused by a broad spectrum of intracellular pathogens, including Gram-negative *Salmonella*, *Shigella*, and *Legionella*; Gram-positive *Listeria*; and non-enveloped viruses, such as adenovirus (22–25). Although the precise function of most galectins remains to be established, recruitment of galectin-8 to damaged *Salmonella* Typhimurium–containing vacuoles brings in the autophagy cargo

receptor NDP52, which induces antibacterial autophagy by means of LC3C to restrict bacterial proliferation (23, 26, 27).

Membrane damage also serves as a danger signal for different forms of programmed cell death (PCD) to limit pathogen spread. Plant cells have long been known to enlist this method against phytopathogens (21), but animals could potentially use it as well. Stimulation of sensory inflammasome complexes by membrane-disrupting bacteria induces pyroptosis, a form of PCD reliant on caspase-1 and/or -11 signaling, to inhibit bacterial growth in a cell-autonomous fashion (22, 28).

DAMP-mediated defense is not confined to detecting the altered redistribution of intracellular self-components. Translocation of extracellular antibodies into the host cell during infection can also trigger a protective response. Serum antibodies bound to bacteria or nonenveloped viruses are carried into the cytosol where they are detected by TRIM21, a cytosolic mammalian Fc receptor and E3 ubiquitin ligase (Fig. 2) (29). This detection triggers the synthesis of lysine 48 (K48) and K63 ubiquitin chains, which target virions for degradation by the proteasome and AAA adenosine triphosphatase (ATPase) valosin-containing protein (VCP), and activates nuclear factor κB (NF κB), activating protein–1 (AP-1), and interferon regulatory factors IRF3, 5, and 7, respectively, which induce a potent antiviral state (30, 31). TRIM21 exhibits broad-spectrum defense as a result of antibody diversity and links cell-intrinsic defense with adaptive immunity.

The evolutionary advantage of DAMP-mediated cellular defense lies in its independence of pathogen structures. PAMPs may become invisible to a given PRR after genetic or chemical modification; however, avoiding detection by danger receptors is difficult for pathogens that rely on pore- or toxin-mediated membrane damage to enter the cell or escape from the phagosome. In addition, because DAMPs are not pathogen-specific, they offer protection against "new" pathogens, like emerging zoonotic viruses for which a given host may lack PRRs (14).

How Compartmentalization Fosters Pathogen Detection by PRRs

In bacteria, foreign DNA is sensed and destroyed by the CRISPR system and restriction endonucleases (2). Because recognition motifs for most restriction endonucleases occur frequently in the host's own genome, these enzymes are paired with matching methyltransferases, which modify host DNA to demarcate it as "self." In eukaryotic cells, rather than being modified, DNA is largely sequestered inside the nucleus, which fosters the detection of foreign DNA in other compartments and allows the deployment of enzymes that mutate and/or degrade DNA without risk to the host genome. The abundance of cytosolic DNA receptors and sensors—such as AIM2, DAI, DNA-PK, IFI16, LRRFIP1, and cyclic guanosine monophosphate—adenosine monophosphate (cyclic GMP-AMP or cGAMP) synthase—underscores the idea that packaging DNA into the nucleus provides an important advantage to host cells against invading pathogens by physically separating self from nonself (32–34).

Cytosolic RNA polymerase III also contributes to DNA sensing by transcribing AT-rich double-stranded DNA into uncapped RNA that is subsequently recognized by RIG-I (11). The addition of caps to the 5' end of mRNAs and other RNA modifications help distinguish host from foreign nucleic acids. Here, the lack of 5' N-7- or 2'-O-methyl-guanosine on unprocessed viral RNAs betrays their presence in the cytosol, just as a lack of 3' polyadenyl groups may reveal the existence of bacterial mRNA species (35, 36). Thus, host cells have devised a number of strategies to detect microbial DNA and RNA in the cytosol.

DNA or RNA occurring in the endolysosomal system is also conspicuously out of place. In mammals, compartmentalized Toll-like receptors (TLRs) detect pathogens that reside within this network (10). TLRs 3, 7, 8, and 9 are delivered by endoplasmic reticulum (ER) chaperones plus sorting adaptors to endosomes and lysosomes for sensing foreign nucleic acids (10). These luminal TLRs remain inactive until their ectodomain is cleaved by proteases in the endolysosomal system, a possible safeguard against activation by self RNA and DNA (10). Their strategic location also enables sampling of genetic material exposed after endolysosomal degradation. Targeting PRRs to compartments that harbor their respective nucleic acid ligands thus enables immune surveillance of its enclosed cargo.

Besides DNA and RNA, structural PAMPs also elicit strong cellular self-defenses. Translocation of flagellin or rod components of bacterial type III secretion systems into the cytosol elicits robust NAIP-NLRC4 inflammasome activity, interleukin-1β production, and pyroptosis (37, 38). Viral capsids are detected by a different set of cell-intrinsic proteins. TRIM5a and TRIMCyp target the capsids of primate lentiviruses, including HIV (11). TRIMCyp binds directly to the capsid by means of a C-terminal CypA-like domain, which it obtained by gene duplication from CypA, an HIV host cofactor. The need for HIV to preserve binding to CypA poses a substantial hurdle for the virus to avoid detection by TRIMCyp, a problem compounded by the ability of certain TRIM-Cyps to isomerize between multiple conformations that are complementary to different lentiviruses (39). TRIM5a targets viral capsids by means of a C-terminal PRYSPRY domain containing six flexible loops reminiscent of antibody CDRs. Once bound, TRIM5a uses the repetitive nature of the capsid structure as a template to form higher-order multimers and eventually a complete lattice, which prematurely uncoats the virus (11). Because capsid binding by TRIM5a also activates innate immune signaling, it represents both a sensor and terminator of viral invasion (11).

How Compartmental Detection Promotes Pathogen Elimination

Host cells have evolved methods to link pathogen detection with pathogen disposal. One of the best examples is the recognition of cytosolic pathogens and their subsequent delivery to lysosomes by the process of macroautophagy (6, 18). Macroautophagy sequesters cytosol destined for degradation into a de novo generated, membrane-bound compartment, the autophagosome. Autophagosome biogenesis proceeds by means of crescent-shaped membrane structures (phagophores) that grow around and, ultimately, enclose portions of the cytosol (18). During infection, eukaryotic cells co-opt autophagy to defend the cytosol against bacterial invaders, to attack microbe-containing vacuoles, and to remove pathogen-derived inclusion bodies (5, 6, 18). Selective autophagy relies on cargo receptors that crosslink "eat-me" signals on prospective cargo to ubiquitin-like proteins of the ATG8 family displayed on the phagophore membrane. Antimicrobial autophagy ("xenophagy") uses many of the same eat-me signals that alert the autophagic machinery to engulf host protein aggregates and damaged organelles (6, 18).

Autophagy efficiently restricts the subpopulation of vesicle-inhabiting bacteria that, by damaging the limiting membrane of their vacuole, become exposed to the cytosol, for example, *Mycobacterium tuberculosis* and *Salmonella* Typhimurium (18). Professional cytosol-dwelling pathogens like *Listeria monocytogenes* avoid such attacks [see review in this issue (40)]. Phagophore recruitment to bacteria depends on three nonredundant cargo receptors, i.e., NDP52 (41), p62 (42), and optineurin (43). Their bacteria-associated eat-me signals arise from (i) active ubiquitin deposition by host cells around susceptible bacteria, for example, by the E3 ligase LRSAM1 (6, 18, 44); (ii) cytosolic exposure of intravesicular glycans upon bacterial damage of the vacuolar membrane, bound by galectin-8 and recruiting NDP52 (23); or (iii) release of mycobacterial DNA after phagosome

permeabilization by the type VII secretion system ESX-1, detected by STING and causing ubiquitin-deposition (45). In the case of viruses, capsids may be directly bound by p62 to activate the autophagic cascade (46), which facilitates the removal of toxic capsomere aggregates from infected cells and tolerizes them against the pathogenic consequences of infection, consistent with the idea of disease tolerance as a defense strategy (47).

Besides sequestering cytosolic pathogens, cargo receptors also select cytosolic proteins for autophagy that yield antimicrobial peptides upon digestion, which contribute to the bactericidal properties of autophagosomes (48). Interferon (IFN)-inducible 65-kD guanylate-binding proteins (GBPs) help traffic p62-bound cytosolic proteins to autophagic vacuoles for generating bacteriolytic peptides (49). GBPs also directly target bacteria and protozoa in damaged vacuoles or after escape into the cytosol for subsequent elimination; how they recognize these pathogens is unknown (4, 5, 49, 50). Members of the related IRG family of immune GTPases target vacuolar pathogens by recognizing self-components on these structures. Irgm1 binds phosphatidylinositol 3,4,5-trisphosphates generated by host class I phosphatidylinositol 3-kinases on mycobacterial phagosomes to assemble soluble NSF attachment protein receptor (SNARE) and autophagy-related proteins for fusion with lysosomes (4, 5, 51). Other IRGs require Atg5 and GBPs to target *Toxoplasma* vacuoles, which then undergo disruption (50, 52). Thus, it appears both self and nonself signals solicit autophagy receptors and IFN-inducible GTPases to eliminate intracellular pathogens as part of the cell-autonomous defense program.

How Cells Control Compartmental Composition to Limit Microbial Growth

Controlling compartment composition allows cells to create conditions unfavorable for microbial growth. For example, the concentration of deoxynucleotide triphosphates (dNTPs) in the cytosol is significantly higher in virally permissive than in certain nonpermissive cell types (~40 to 70 nM versus ~1 to 15 μ M) (53). In order to remove excess dNTPs, human dendritic cells and macrophages express an IFN-inducible deoxyguanosine triphosphate triphosphohydrolase, SAMHD1, which hydrolyzes dNTPs to block reverse transcription and cDNA synthesis in HIV-1 (53). Another compositional strategy to restrict microbial growth is depletion of amino acids, exemplified by the IFN-induced indoleamine-2,3-deoxygenase (IDO) which degrades L-tryptophan. IDO potently inhibits human viruses (HSV and hepatitis B virus), bacteria (*Chlamydia, Francisella*, and *Rickettsia*) and protozoan parasites (*Leishmania, Trypanosoma*, and *T. gondii*) (5).

Perhaps nowhere else in the cell is control of compartmental composition more effective than in phagolysosomes and autophagolysosomes, acidified organelles designed to kill and degrade internalized pathogens. Their sterilizing power comes from the concerted action of several factors. Mammalian cells express proton-dependent efflux pumps, such as natural resistance–associated macrophage protein–1 (NRAMP1), that export Mn²⁺ and Fe²⁺ from vacuoles to prevent access of captured microbes to these essential metals (5). Antimicrobial peptides disrupt the outer envelope of pathogens, and luminal proteases, lipases, and glycosidases imported through the Golgi-late endosome pathway further degrade this material. Reactive oxygen and nitrogen species oxidize and nitrosylate, respectively, pathogen lipids, DNA, and proteins (5). Together, they create a hostile environment for most incoming pathogens. The low pH (~ 4.5 to 5.0) generated inside these compartments by a proton-pumping vacuolar ATPase and maintained by antiporters, such as the sodiumhydrogen exchanger-1 (NHE1), is optimal for lysosomal hydrolase activity and the conversion of superoxide (O2⁻) to H2O2 and of nitrogenous end products back to the toxic radical, nitric oxide (NO). Import of copper ions by the P-type ATPase Cu²⁺ pump ATP7A promotes the formation of toxic hydroxyl (·OH) radicals (5). Concentrating diverse antimicrobial activities in a specialized organelle thus promotes microbial killing, with the

pH difference between lysosomes and cytosol safeguarding the cell against the consequences of potential lysosomal rupture.

How Multilayered Self-Defense Effectively Restricts Invading Pathogens

The multiple barriers and compartments that define cellular architecture provide a series of obstacles, all of which pathogens must overcome in order to replicate. Therefore, although the protection provided at each stage is not complete, their combination is extremely effective, as illustrated for HIV in Fig. 3. Although a cell might be challenged by many infectious particles, the fraction that navigates each step is small, cumulatively reducing the probability of a productive infection. Some virions do not correctly engage with their receptor and co-receptor and thus fail to deposit their capsid into the cytosol. Virions that begin reverse transcription are substrates for APOBEC3G, a host restriction factor that mutates the viral genome by deaminating deoxycitidine (11). Reverse transcription is also targeted by SAMHD1, which depletes dNTPs (53). Meanwhile, as the capsid traverses the cytosol, TRIM5a and TRIMCyp interfere with its uncoating (11). Virions that make it past these defenses have to negotiate nuclear entry by using cofactors to pass through the pore. Once inside the nucleus, the viral genome becomes a substrate for host DNA repair enzymes, which circularize it into long terminal repeat 1-LTR or 2-LTR circles (54). Viral genomes can also be degraded by DNA repair enzymes XPB and XPD (55). Even integration does not yet constitute successful completion of the retroviral "life cycle." Transcription of integrated virus is repressed by TRIM22 and TRIM28 (56, 57), while particle assembly and viral budding are inhibited by 2',3'-cyclic-nucleotide 3'phosphodiesterase (CNP) and tetherin, respectively (20, 58).

In conclusion, the organization of eukaryotic cells into compartments has driven the evolution of highly effective cellular self-defense, which has forced pathogens to adapt to each environment they encounter. Not only must pathogens survive these environments, but they must also have means of transportation between them. Controlling the movement of pathogens is therefore another defense strategy, realized, for example, by the enclosure of professional cytosol-dwelling bacteria into septin cages that prevent bacteria from usurping the host actin cytoskeleton for mobility and cell-to-cell spread (59).

Outlook

This Review has focused on how cells use their spatial organization to defend themselves against infection, although the temporal sequence of events is equally important. A cell invaded in the first few hours of pathogen exposure relies on preformed defense factors (11). However, after these first few hours, innate immune signaling activates additional cellautonomous responses, as exemplified by IFN-induced protection that elicits transcription of hundreds of new genes (5). Remarkably, we know almost nothing about the protein functions encoded by the vast majority of these genes. After this innate response, tailormade adaptive immunity helps clear pathogens and prevent persistence. Again, we now realize that innate and adaptive immunity may not be so easily demarcated. This point is well illustrated by the Fc receptor TRIM21, which recognizes antibody-opsonized pathogens in the host cell cytosol (31). It is also becoming clear that traditional immunity must cooperate with cells outside the immune system for optimal protection of the host. Such cells are often endowed with potent antimicrobial capacity. Neurons, for example, exhibit powerful antiviral programs against a wide spectrum of RNA viruses (46, 60). Our attempts to understand the functional anatomy of this "nonclassical" immune system will thus represent a major scientific frontier in the years ahead.

Acknowledgments

We apologize to colleagues whose work could not be cited due to space constraints. Work in the authors' laboratories was supported by the Medical Research Council (U105170648 and U105181010) (F.R. and L.C.J.), the National Association for Colitis and Crohn's Disease (M/11/3) (F.R.), European Research Council (281627-IAI) (L.C.J.), NIH grant AI068041-06 (J.D.M.), Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Disease Award (1007845) (J.D.M.), Searle Foundation Scholars Program (05-F-114) (J.D.M.), Cancer Research Institute Investigator Award Program (CRI06-10) (J.D.M.), Crohn's and Colitis Foundation of America Senior Investigator Award (R09928) (J.D.M.), and Yale's W. W. Winchester Fund (J.D.M.).

References and Notes

- 1. Ronald PC, Beutler B. Science. 2010; 330:1061. [PubMed: 21097929]
- 2. Horvath P, Barrangou R. Science. 2010; 327:167. [PubMed: 20056882]
- 3. Beutler B, et al. Annu Rev Immunol. 2006; 24:353. [PubMed: 16551253]
- 4. Kim BH, Shenoy AR, Kumar P, Bradfield CJ, MacMicking JD. Cell Host Microbe. 2012; 12:432. [PubMed: 23084913]
- 5. MacMicking JD. Nat Rev Immunol. 2012; 12:367. [PubMed: 22531325]
- 6. Randow F, Münz C. Trends Immunol. 2012; 33:475. [PubMed: 22796170]
- 7. Gusarov I, Shatalin K, Starodubtseva M, Nudler E. Science. 2009; 325:1380. [PubMed: 19745150]
- 8. McGettigan J, et al. Insect Biochem Mol Biol. 2005; 35:741. [PubMed: 15894191]
- 9. Alix E, Mukherjee S, Roy CR. J Cell Biol. 2011; 195:943. [PubMed: 22123831]
- 10. Kagan JC. Cell. 2012; 151:1168. [PubMed: 23217704]
- 11. Yan N, Chen ZJ. Nat Immunol. 2012; 13:214. [PubMed: 22344284]
- 12. Medzhitov R, Janeway CA Jr. Science. 2002; 296:298. [PubMed: 11951031]
- 13. Akira S, Uematsu S, Takeuchi O. Cell. 2006; 124:783. [PubMed: 16497588]
- 14. Stuart LM, Paquette N, Boyer L. Nat Rev Immunol. 2013; 13:199. [PubMed: 23411798]
- 15. Botteaux A, Hoste C, Dumont JE, Van Sande J, Allaoui A. Microbes Infect. 2009; 11:537. [PubMed: 19298864]
- 16. Vaishnava S, et al. Science. 2011; 334:255. [PubMed: 21998396]
- 17. Holm CK, et al. Nat Immunol. 2012; 13:737. [PubMed: 22706339]
- 18. Levine B, Mizushima N, Virgin HW. Nature. 2011; 469:323. [PubMed: 21248839]
- 19. Lee K, et al. Cell Host Microbe. 2010; 7:221. [PubMed: 20227665]
- 20. Neil SJD, Zang T, Bieniasz PD. Nature. 2008; 451:425. [PubMed: 18200009]
- 21. Jones JDG, Dangl JL. Nature. 2006; 444:323. [PubMed: 17108957]
- 22. Creasey EA, Isberg RR. Proc Natl Acad Sci USA. 2012; 109:3481. [PubMed: 22308473]
- 23. Thurston TLM, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Nature. 2012; 482:414. [PubMed: 22246324]
- 24. Dupont N, et al. Cell Host Microbe. 2009; 6:137. [PubMed: 19683680]
- 25. Maier O, Marvin SA, Wodrich H, Campbell EM, Wiethoff CM. J Virol. 2012; 86:10821. [PubMed: 22855481]
- 26. von Muhlinen N, et al. Mol Cell. 2012; 48:329. [PubMed: 23022382]
- 27. Li S, et al. Sci Signal. 2013; 6:ra9. [PubMed: 23386746]
- 28. Aachoui Y, et al. Science. 2013; 339:975. [PubMed: 23348507]
- James LC, Keeble AH, Khan Z, Rhodes DA, Trowsdale J. Proc Natl Acad Sci USA. 2007;
 104:6200. [PubMed: 17400754]
- 30. McEwan WA, et al. Nat Immunol. 2013; 14:327. [PubMed: 23455675]
- 31. Mallery DL, et al. Proc Natl Acad Sci USA. 2010; 107:19985. [PubMed: 21045130]
- 32. Hornung V, Latz E. Nat Rev Immunol. 2010; 10:123. [PubMed: 20098460]
- 33. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. Elife. 2012; 1:e00047. [PubMed: 23251783]
- 34. Sun L, Wu J, Du F, Chen X, Chen ZJ. Science. 2013; 339:786. [PubMed: 23258413]

- 35. Daffis S, et al. Nature. 2010; 468:452. [PubMed: 21085181]
- 36. Sander LE, et al. Nature. 2011; 474:385. [PubMed: 21602824]
- 37. Kofoed EM, Vance RE. Nature. 2011; 477:592. [PubMed: 21874021]
- 38. Zhao Y, et al. Nature. 2011; 477:596. [PubMed: 21918512]
- 39. Caines MEC, et al. Nat Struct Mol Biol. 2012; 19:411. [PubMed: 22407016]
- 40. Baxt LA, Garza-Mayers AC, Goldberg MB. Science. 2013; 340:697. [PubMed: 23661751]
- 41. Thurston TLM, Ryzhakov G, Bloor S, von Muhlinen N, Randow F. Nat Immunol. 2009; 10:1215. [PubMed: 19820708]
- 42. Zheng YT, et al. J Immunol. 2009; 183:5909. [PubMed: 19812211]
- 43. Wild P, et al. Science. 2011; 333:228. [PubMed: 21617041]
- 44. Huett A, et al. Cell Host Microbe. 2012; 12:778. [PubMed: 23245322]
- 45. Watson RO, Manzanillo PS, Cox JS. Cell. 2012; 150:803. [PubMed: 22901810]
- 46. Orvedahl A, et al. Cell Host Microbe. 2010; 7:115. [PubMed: 20159618]
- 47. Medzhitov R, Schneider DS, Soares MP. Science. 2012; 335:936. [PubMed: 22363001]
- 48. Ponpuak M, et al. Immunity. 2010; 32:329. [PubMed: 20206555]
- 49. Kim BH, et al. Science. 2011; 332:717. [PubMed: 21551061]
- 50. Yamamoto M, et al. Immunity. 2012; 37:302. [PubMed: 22795875]
- 51. Tiwari S, Choi HP, Matsuzawa T, Pypaert M, MacMicking JD. Nat Immunol. 2009; 10:907. [PubMed: 19620982]
- 52. Zhao Z, et al. Cell Host Microbe. 2008; 4:458. [PubMed: 18996346]
- 53. Goldstone DC, et al. Nature. 2011; 480:379. [PubMed: 22056990]
- 54. Sloan RD, Wainberg MA. Retrovirology. 2011; 8:52. [PubMed: 21722380]
- 55. Yoder K, et al. Proc Natl Acad Sci USA. 2006; 103:4622. [PubMed: 16537383]
- 56. Kajaste-Rudnitski A, et al. J Virol. 2011; 85:5183. [PubMed: 21345949]
- 57. Wolf D, Goff SP. Cell. 2007; 131:46. [PubMed: 17923087]
- 58. Wilson SJ, et al. Cell Host Microbe. 2012; 12:585. [PubMed: 23084924]
- 59. Mostowy S, et al. Cell Host Microbe. 2010; 8:433. [PubMed: 21075354]
- 60. Cho H, et al. Nat Med. 2013; 19:458. [PubMed: 23455712]

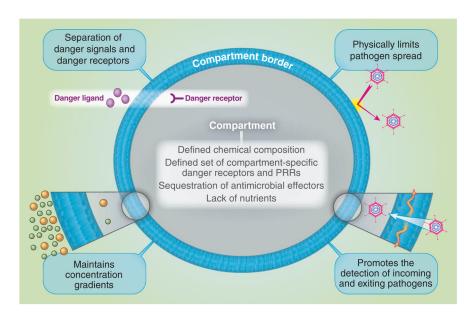
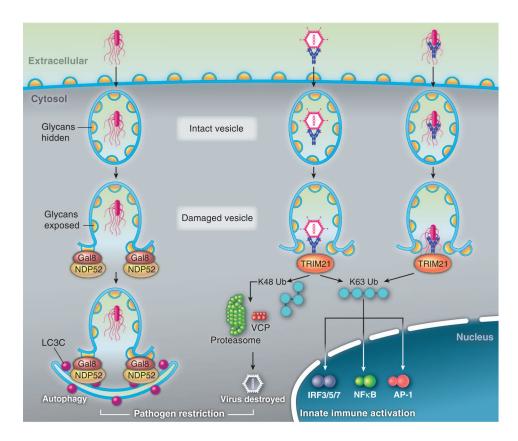


Fig. 1. Compartmentalization promotes cellular self-defense

Eukaryotic cells are composed of compartments separated by selectively permeable borders that control their composition. Cell and compartment borders can physically prevent pathogen invasion, and they house sensors that are "tripped" as pathogens try to cross them. Pathogen-induced damage to borders alters compartment composition; the resulting mislocalization of host molecules can be perceived as a danger signal. Control over compartment composition allows potent antimicrobial effectors that otherwise might damage the cell to be safely sequestered. Finally, each cellular compartment represents its own microenvironment that can be made hostile to pathogens.



 $\label{eq:continuous} \textbf{Fig. 2. Breakdown of compartment borders generates DAMPs, which trigger potent antimicrobial defenses }$

Membrane damage causes the translocation of extracellular molecules into the cytosol, which cells sense and interpret as a danger signal. Host glycans on burst phagosomes are detected by the cytosolic lectin galectin-8, whose accumulation provides an eat-me signal for the autophagy cargo receptor NDP52, causing LC3C-dependent autophagy and restriction of bacterial proliferation. Antibodies bound to bacteria and nonenveloped viruses are translocated into the cytosol upon release of pathogens from their internalized compartment. Cytosolic antibodies are detected by the E3 ligase and Fc receptor TRIM21, which targets virions for degradation by the proteasome and activates innate immune signaling.

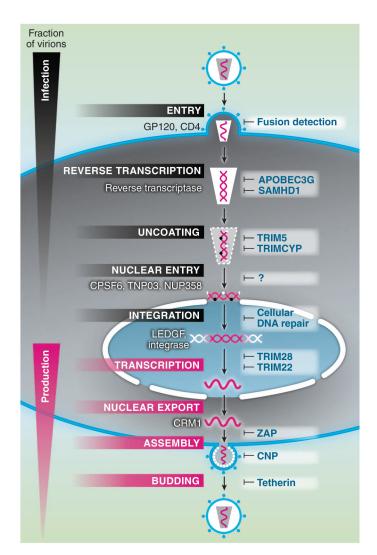


Fig. 3. Multilayered defenses synergistically inhibit infection and pathogen replication

To infect cells and complete their life cycle, retro-viruses, such as HIV-1, must pass through multiple compartment borders and access distinct cellular compartments. Virions must recruit and retask cellular cofactors to negotiate their way through the cell and to replicate successfully. Meanwhile, antiviral factors adapted to specific cellular microenvironments target and inhibit specific steps of the viral life cycle. At each stage, only a fraction of virions are successful, which provides a highly synergistic defense system capable of inhibiting even quickly evolving pathogens. LEDGF, PC4 and SFRS1 interacting protein–1; ZAP, zinc finger CCCH-type antiviral protein–1.