Published in final edited form as:

Nat Rev Immunol. ; 12(1): . doi:10.1038/nri3111.

The role of ubiquitylation in immune defence and pathogen evasion

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Abstract

Ubiquitylation is a widely used post-translational protein modification that regulates many biological processes, including immune responses. The role of ubiquitin in immune regulation was originally uncovered through studies of antigen presentation and the nuclear factor- κ B family of transcription factors, which orchestrate host defence against microorganisms. Recent studies have revealed crucial roles of ubiquitylation in many aspects of the immune system, including innate and adaptive immunity and antimicrobial autophagy. In addition, mounting evidence indicates that microbial pathogens exploit the ubiquitin pathway to evade the host immune system. Here, we review recent advances on the role of ubiquitylation in host defence and pathogen evasion.

Ubiquitin is a highly conserved 76-amino-acid polypeptide in eukaryotes that can be covalently attached to other proteins through an enzymatic cascade involving three enzymes: E1, E2 and E3 (REF. 1). In humans, there are two E1 enzymes, around forty E2 enzymes and hundreds of E3 enzymes. Ubiquitylation can be reversed by deubiquitylation enzymes (DUBs), which also form a large family, consisting of ~100 members in humans^{2,3}. The ubiquitin 'signal' is recognized by specific ubiquitin 'receptors' that contain one or more ubiquitin-binding domains⁴. There are more than 20 different types of ubiquitin-binding domain, and these domains are embedded in a large variety of cellular proteins. Most ubiquitin-binding domains bind to ubiquitin with low affinity, which indicates that this binding is highly dynamic and specifically regulated. Thus, the ubiquitin conjugation and deconjugation systems, together with ubiquitin-binding proteins, constitute the basic molecular machineries for ubiquitin-mediated regulation of diverse cellular processes.

A protein can be modified on one lysine residue with a single ubiquitin (monoubiquitylation) or with a chain of ubiquitin (polyubiquitylation). In some cases, multiple lysine residues on a protein target can be modified by ubiquitin or ubiquitin chains. Non-linear polyubiquitin chains are linked through one of the seven lysine residues of ubiquitin. Lysine 48 (K48)-linked polyubiquitylation usually targets proteins for proteasomal degradation, whereas K63-linked polyubiquitylation is implicated in many

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Competing interests statement

The authors declare no competing financial interests.

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signal transduction cascades, such as DNA repair and protein kinase activation⁵. Polyubiquitin chains linked through the other lysines of ubiquitin (namely, K6, K11, K27, K29 and K33) have also been found in cells, but their functions are less well understood⁶. Linear ubiquitin chains — in which the carboxyl terminus of one ubiquitin is linked to the amino-terminal methionine of the next ubiquitin — are also thought to function in signal transduction and protein degradation⁷. Many ubiquitin-like proteins also function by covalently modifying other target proteins⁸. Such ubiquitin-like proteins include small ubiquitin-related modifier (SUMO), NEDD8, ISG15, ATG12 and LC3 (a homologue of yeast Atg8).

Specificity in the choice of ubiquitylation substrate and the type of ubiquitin chain is largely determined by E2 and E3 enzymes⁹. E3 enzymes typically contain either a RING (really interesting new gene) or a HECT (homologous to E6AP C-terminus) domain, which mediates E2 binding and ubiquitylation. Many E3 enzymes form multiprotein complexes. For example, cullin–RING ligases, which form the largest E3 family, are composed of multiple subunits, including one of the cullin proteins (CUL1 to CUL7) and a RING subunit such as RING-box protein 1 (RBX1; also known as ROC1)¹⁰.

As a versatile post-translational modification, ubiquitylation is extensively used as a key regulatory mechanism in many aspects of the immune system, including the recognition and clearance of pathogens by the innate immune system, antigen presentation and the activation of the adaptive immune system. However, pathogens have evolved many ways to exploit the ubiquitylation system, for example by targeting key immune proteins for degradation, by interfering with crucial ubiquitin-mediated regulatory mechanisms in antimicrobial pathways, and even by hijacking the ubiquitin system to favour their own propagation or pathogenesis.

Regulation of innate immunity by ubiquitylation

Following exposure to pathogens, the innate immune system recognizes pathogen-associated molecular patterns (PAMPs) using a set of germline-encoded pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs)¹¹. Signalling networks emanating from these receptors trigger the activation of transcription factors, including those belonging to the nuclear factor- κ B (NF- κ B) and interferon regulatory factor (IRF) families. These transcription factors then orchestrate gene expression programmes that protect cells and organisms from pathogen infection. Among the genes induced by NF- κ B and IRFs are those encoding pro-inflammatory cytokines and type I interferons (IFNs), which not only directly suppress microbial infections, but also activate the adaptive immune system to eventually eradicate invading pathogens.

TLR pathways

Each member of the TLR family recognizes a distinct type of PAMP, and together these receptors can detect a broad range of pathogens, including bacteria, viruses, fungi and parasites¹². Following engagement by their respective ligands, different TLRs trigger specific signalling outcomes by recruiting various combinations of adaptor proteins, which then relay signals to specific downstream signalling molecules. TLR pathways can be largely categorized by their use of two main adaptor proteins: myeloid differentiation primary response protein 88 (MYD88) and TIR domain-containing adaptor protein inducing IFN β (TRIF). MYD88-dependent pathways are used by all TLRs except TLR3, and generally lead to the induction of pro-inflammatory cytokines. TLR7, TLR8 and TLR9, which are localized on the endosomal membrane, also use MYD88 for the induction of type

I IFNs. TRIF-dependent pathways, which transmit signals from TLR3 and TLR4, lead to the induction of both type I IFNs and pro-inflammatory cytokines.

Ubiquitylation is involved in the activation of NF- κ B and the mitogen-activated protein kinase (MAPK) cascade downstream of both the MYD88- and TRIF-dependent pathways (FIGS 1,2). Following stimulation of MYD88-dependent pathways, MYD88 recruits kinases of the IL-1 receptor-associated kinase (IRAK) family¹³, which then recruit TNF receptor-associated factor 6 (TRAF6), a RING-domain ubiquitin E3 ligase¹⁴. TRAF6 functions together with a ubiquitin E2 complex consisting of UBC13 (also known as UBE2N) and UEV1A (also known as UBE2V1) to catalyse the synthesis of K63-linked polyubiquitin chains. These polyubiquitin chains are recognized by the novel zinc-finger ubiquitin-binding domains of TAK1-binding protein 2 (TAB2) and TAB3, which are the regulatory components of the TGF β -activated kinase 1 (TAK1) complex^{15,16}. The binding of K63-linked polyubiquitin to TAB2 and TAB3 leads to TAK1 activation, which in turn activates the MAPK cascade. K63-linked polyubiquitin also binds to NF- κ B essential modulator (NEMO; also known as IKK γ), an essential regulatory subunit of the I κ B kinase (IKK) complex, which also contains the catalytic subunits IKK α and IKK β ^{17,18}. Binding of K63-linked polyubiquitin to both the IKK and TAK1 complexes facilitates the phosphorylation of IKK β by TAK1, leading to IKK activation. IKK phosphorylates NF- κ B inhibitor (I κ B) proteins, which are then recognized by the F-box protein β TrCP — a subunit of a ubiquitin E3 ligase complex known as SCF $^{\beta$ TrCP, which also contains S-phase kinase-associated protein 1 (SKP1), CUL1 and RBX1 (FIG. 1). The SCF $^{\beta$ TrCP E3 complex polyubiquitylates phosphorylated I κ B proteins, which are subsequently degraded by the proteasome. This allows NF- κ B to enter the nucleus to turn on target gene expression¹⁹.

In TRIF-dependent pathways downstream of TLR3, receptor-interacting protein 1 (RIP1; also known as RIPK1) is recruited to TRIF²⁰ and undergoes K63-linked polyubiquitylation by an E3 ligase of the pellino family, PELI1 (REF. 21). Ubiquitylated RIP1 then recruits TAB2 and NEMO, leading to NF- κ B activation (as described above). TRIF also recruits another ubiquitin ligase, TRAF3, which activates the kinases TBK1 and IKK ϵ , leading to IRF3 phosphorylation and subsequent type I IFN production^{22,23}. Further research is needed to understand how TRAF3 activates TBK1 and IKK ϵ .

Different types of polyubiquitylation can function cooperatively to determine specific signalling outcomes. For example, in the TLR4-induced pathway that activates MAPKs, MYD88 is rapidly recruited to the receptor on the plasma membrane following stimulation by the ligand lipopolysaccharide (FIG. 2). MYD88 then recruits several ubiquitin E3 ligases, including TRAF6, TRAF3, cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2. TRAF6 catalyses K63-linked polyubiquitylation and activates cIAP proteins, which in turn promote the K48-linked polyubiquitylation and proteasomal degradation of TRAF3 (REF. 24). Following TRAF3 degradation, the signalling complex containing MYD88, TRAF6, cIAPs, UBC13 and TAK1 is released into the cytosol, where it activates the MAPK cascade. TLR-mediated IKK β activation (and hence NF- κ B activation), by contrast, does not require TRAF3 degradation.

In the IFN-inducing branch of TLR4 signalling, endocytosed TLR4 recruits TRAF3 and TRAF6, but not cIAPs, to the endosome (FIG. 2). TRAF3 subsequently undergoes K63-linked polyubiquitylation, which leads to IFN induction. K63-linked polyubiquitylation of TRAF3 was also shown to be associated with IFN production downstream of TLR9 and retinoic acid-inducible gene I (RIG-I)^{25,26}, although the exact role of K63-linked polyubiquitylation of TRAF3 in IFN induction is not yet clear. Interestingly, the ability to induce IFNs can be conferred to TLR pathways that normally do not induce IFNs (such as the TLR2 pathway) by forced plasma membrane localization of TRAF3 (REF. 27).

RLR pathways

RIG-I is the founding member of the RLR family, which also includes melanoma differentiation-associated gene 5 (MDA5) and LGP2 (also known as DHX58)^{28,29}. All three proteins contain a DExD/H (Asp-Glu-x-Asp/His) box RNA helicase domain in their centre. In addition, RIG-I and MDA5 contain two caspase-recruitment domains (CARDs) in tandem at their N-termini, and these CARDs are important for signalling. RIG-I also contains a C-terminal domain that binds to viral RNA that has a 5' triphosphate group, which distinguishes viral RNA from mammalian RNAs, which normally have 5' modifications (for example, the 5' cap in mRNA). MDA5 is activated by long double-stranded RNA and is required for IFN induction by certain RNA viruses, such as picorna-viruses; however, the exact nature of the viral RNA recognized by MDA5 is not clear. LGP2 lacks the N-terminal CARDs and has regulatory roles in the RIG-I and MDA5 pathways³⁰. Both RIG-I and MDA5 trigger signal transduction cascades through mitochondrial antiviral signalling protein (MAVS; also known as IPS1, VISA and CARDIF)^{31–34} (FIG. 3). MAVS contains a C-terminal transmembrane domain that targets it to the mitochondrial outer membrane. The importance of this mitochondrial localization is underscored by the finding that hepatitis C virus uses the viral protease NS3–NS4A to cleave MAVS off the mitochondrial membrane, thereby assisting the virus to evade the host immune response and establish persistent infections in humans^{33,35}. From the mitochondrial surface, MAVS activates two cytosolic kinase complexes: TBK1 and IKK, which activate IRF3 and NF- κ B, respectively. IRF3 and NF- κ B then enter the nucleus to induce type I IFNs and pro-inflammatory cytokines.

Ubiquitylation has a crucial role in regulating the RIG-I signalling pathway. RIG-I activation requires tripartite motif-containing protein 25 (TRIM25), a RING-domain ubiquitin ligase that can ubiquitylate RIG-I at the second CARD³⁶. TRIM25 E3 ligase activity is specifically inhibited by the influenza virus NS1 protein, which is consistent with the important role of this E3 ligase in antiviral pathways³⁷. Another ubiquitin ligase, riplet (also known as RNF135), has been shown to polyubiquitylate RIG-I at the C-terminus, and this ubiquitylation was proposed to activate RIG-I³⁸. Recent studies have revealed another mechanism of RIG-I activation. Through *in vitro* reconstitution of the RIG-I pathway, it was found that unanchored (free) K63-linked polyubiquitin chains, which are not conjugated to any cellular protein, can directly bind to the RIG-I CARDs, and this binding was shown to be essential for RIG-I activation³⁹. Importantly, endogenous free K63-linked polyubiquitin chains can be isolated from human cells and have been shown to be highly potent in activating the RIG-I pathway.

The binding of full-length RIG-I to K63-linked polyubiquitin chains depends on the presence of 5'-triphosphorylated RNA and ATP. Recent structural studies provide the molecular basis for the regulation of RIG-I by RNA^{40–43}. In the absence of RNA binding, RIG-I adopts an auto-inhibited conformation in which the second CARD interacts with a unique helicase domain within RIG-I; this is thought to preclude the binding of polyubiquitin chains to the RIG-I CARDs. RNA binding to the C-terminal and helicase domains of RIG-I induces a conformational rearrangement that is thought to expose the N-terminal CARDs, which can then bind to K63-linked polyubiquitin chains. Following binding to K63-linked polyubiquitin chains, RIG-I promotes a massive aggregation of MAVS through a prion-like mechanism⁴⁴. These MAVS aggregates then potently activate the downstream signalling pathways to induce type I IFNs.

Interestingly, after MAVS is activated, ubiquitylation also has an important role in mediating the activation of IKK and TBK1 complexes in the cytoplasm⁴⁵. The E2 enzyme UBC5 (also known as UBE2D1) and K63-linked polyubiquitylation are required for the activation of TBK1 by MAVS, which contains binding sites for several TRAF proteins, including TRAF2, TRAF3 and TRAF6 (REF. 31). Indeed, virus infection leads to the

recruitment of TRAF2 and TRAF6 to aggregated MAVS⁴⁴. Recent studies have also suggested the involvement of other ubiquitin E3 ligases in signalling downstream of MAVS. For example, the mindbomb E3 ligases (MIB1 and MIB2) have been proposed to activate TBK1 by catalysing its K63-linked polyubiquitylation⁴⁶. Another E3 ligase, TRIM23, promotes K27-linked polyubiquitylation of NEMO, and this might facilitate the activation of IKK and TBK1 complexes⁴⁷.

Other PRR pathways

Ubiquitin E3 ligases are essential signalling components in other PRR pathways (FIG. 3). For example, in the cytosolic DNA-induced IFN pathway that is dependent on stimulator of interferon genes (STING), TRIM56 stimulates K63-linked polyubiquitylation of STING, and this promotes the recruitment of the IRF3 kinase TBK1 (REFS 48,49).

Nucleotide-binding oligomerization domain protein 2 (NOD2) — a protein that is associated with inflammatory disorders of the gastrointestinal tract — detects bacterial peptidoglycans. NOD2-mediated activation of MAPKs and NF- κ B requires K63-linked polyubiquitylation of the protein kinase RIP2 by cIAP proteins and/or TRAF proteins^{50,51}.

A crucial role of ubiquitylation in restricting retroviral infection was demonstrated by recent studies of the RING-domain ubiquitin ligase TRIM5 (REF. 52). TRIM5 is known to restrict retroviruses (including HIV) by ubiquitylating viral capsid proteins and targeting them for proteasomal degradation. Interestingly, TRIM5 also functions as an innate immune sensor by detecting incoming retroviral capsids. It was shown that viral capsid proteins strongly stimulate TRIM5 to synthesize free K63-linked polyubiquitin chains⁵², which then activate the TAK1 kinase complex to trigger an innate immune response⁵³.

As illustrated in the above examples, polyubiquitylation is a recurring theme in diverse PRR signalling pathways, in which polyubiquitin functions as a signal to recruit ubiquitin-binding proteins and to coordinate the formation of signalling complexes. Thus, the assembly and disassembly of these ubiquitin chains must be tightly regulated (see below).

Deubiquitylation in innate immunity

Consistent with a key role of ubiquitylation in activating immune signalling cascades, several DUBs have been shown to negatively regulate immune responses. A20 (also known as TNFAIP3) and CYLD are two DUBs that are best known for their inhibition of NF- κ B activation in diverse signalling pathways, including PRR pathways³. A20 contains an ovarian tumour (OTU) domain that is known to catalyse deubiquitylation. In addition, A20 contains multiple zinc-finger domains that mediate ubiquitin binding and ubiquitin ligase functions^{54,55}, both of which have been proposed to have a role in downregulating NF- κ B signalling. In several PRR signalling pathways, A20 interacts with specific adaptor proteins and inhibits NF- κ B activation by removing or inhibiting the K63-linked polyubiquitylation of TRAF6 (REFS 56,57), RIP1 (REF. 54) and RIP2 (REFS 51,58), thereby restricting the pro-inflammatory outcomes of PRR pathways. A20 can also prevent polyubiquitin chain synthesis by blocking the interaction between ubiquitin E3 ligases (such as TRAF2, TRAF6 and cIAPs) and their E2 enzymes (UBC13 and UBC5)⁵⁹. As a significant regulator of NF- κ B signalling, A20 also broadly controls the function of many immune cell types, including B cells, T cells and dendritic cells (DCs)³. The function of A20 in restricting inflammatory responses is not only crucial for preventing exaggerated deleterious inflammation when combating microbial infections, but is also important for maintaining homeostasis of the immune system under physiological conditions.

CYLD is a tumour suppressor, and loss of CYLD function has been linked to several types of skin tumour, including familial cylindromatosis and Brooke–Spiegler syndrome. CYLD contains a ubiquitin protease domain, and it specifically cleaves K63-linked polyubiquitin chains. This catalytic activity negatively regulates IKK activation in several NF- κ B signalling pathways, including those downstream of the TNF receptor, RLRs, the B cell receptor and the T cell receptor (TCR)². Interestingly, a recent genetic study uncovered an unexpected role of CYLD in antiviral immunity downstream of IFN receptor signalling, the exact mechanism of which is unclear⁶⁰. Genetic studies have shown that A20 and CYLD have non-redundant physiological functions in the immune system, although they seem to target several common substrates. The difference in their physiological functions might be a result of differential regulation of their temporal and spatial expression patterns or their enzymatic activities and specificities, or might be due to the use of different adaptor proteins.

Role of ubiquitylation in antimicrobial autophagy

Autophagy is a process by which eukaryotic cells dispose of intracellular organelles and large protein aggregates to maintain cellular homeostasis. Such activity can also target non-self entities such as microbial pathogens. Autophagy involves the formation of a double-membrane structure, known as the autophagosome, which engulfs a portion of cytosolic constituents. The ubiquitin-like protein LC3 is conjugated to the membrane of autophagosomes. Autophagosomes then fuse with lysosomes to degrade the contents. It is not entirely clear what defines the substrate specificity for an autophagosome, but ubiquitin has emerged as a specificity factor for selective autophagy. In the ubiquitin–proteasome pathway, ubiquitylated cargos are recognized by ubiquitin receptors that deliver them to the 26S proteasome. In a similar manner, ubiquitin-mediated recognition of autophagy targets is mediated by adaptor proteins — such as p62 (also known as sequestosome 1) and NBR1 — that recognize both the ubiquitylated cargo (through their ubiquitin-binding domains) and LC3 on autophagosomes (through their LC3-interacting regions)^{61,62}.

Ubiquitin adaptors in autophagy

The involvement of ubiquitin in antimicrobial autophagy is so far best characterized in studies of autophagy induced by intracellular bacteria⁶³ (FIG. 4). Polyubiquitin has been shown to accumulate on the surface of cytosolic bacteria such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium⁶⁴. This observation led to the hypothesis that antimicrobial autophagy uses ubiquitin-dependent mechanisms for cargo recognition that are similar to those used for cellular aggregates. Indeed, three adaptor proteins — p62, NDP52 and optineurin — have been shown to target intracellular bacteria for autophagy^{65–67}. All three proteins contain a ubiquitin-binding domain and an LC3-interacting region⁶⁸. In cells infected with *S. Typhimurium*, these adaptor proteins colocalize with ubiquitin-coated bacteria in the cytosol. Although these adaptor proteins are independently recruited to the same bacterium, they seem to be mutually dependent in promoting bacterial autophagy^{67,69}.

In addition to recognizing pathogen-associated ubiquitin targets, p62 also mediates the recruitment of certain ubiquitylated cellular proteins into autophagosomes, where they are processed into antibacterial peptides⁷⁰.

Interestingly, not all autophagy adaptor proteins that can bind to both ubiquitin and LC3 are involved in antibacterial autophagy^{61,66}. This indicates that additional determinants make an adaptor protein specific for pathogens. It is known that PRR signalling can induce autophagy^{71,72}, so there might be mechanisms that integrate PRR signalling with the autophagic machinery, thereby allowing coordination between the two processes. For example, phosphorylation of optineurin by TBK1 enhances the binding of optineurin to LC3

(REF. 67). As TBK1 can be activated by the TLR4 signalling pathway, this regulation of optineurin-mediated autophagy by TBK1 indicates coordinated regulation of pathogen sensing and autophagy to ensure the rapid elimination of invading pathogens.

Role of ubiquitylation in antigen presentation

MHC class II cell-surface expression

MHC class II molecules on antigen-presenting cells (APCs) present antigenic peptides to helper T cells. Such peptides include those derived from extracellular pathogens that enter APCs by endocytosis. MHC class II molecules use intracellular protein trafficking pathways to communicate between different endosomal compartments and the plasma membrane⁷³. Their expression at the cell surface is subject to regulation through endocytosis and subsequent delivery to lysosomes for degradation. The endocytosis and sorting of membrane proteins is often regulated by ubiquitylation of the cytoplasmic domains of such proteins (FIG. 5). In mouse immature DCs, the cytoplasmic tail of the MHC class II β -chain undergoes polyubiquitylation, resulting in low levels of MHC class II surface expression^{74,75}. Following DC maturation, MHC class II molecules are no longer ubiquitylated and, as a result, mature DCs accumulate MHC class II molecules at the cell surface, leading to an enhanced capacity for antigen presentation.

The MARCH (membrane-associated RING-CH) family of E3 ligases, which contain variant RING domains, have been linked to MHC class II ubiquitylation. The first mammalian MARCH E3 ligase was identified as a homologue of a family of viral E3 enzymes known as modulator of immune recognition (MIR) proteins, which were known to downregulate MHC class I surface expression⁷⁶. These viral proteins include the murine gammaherpesvirus 68 (MHV68) protein mK3 and the Kaposi's sarcoma-associated herpesvirus (KSHV) proteins K3 and K5, as discussed below. Although multiple MARCH enzymes have E3 ligase activity towards MHC class II molecules, only MARCH1 has been identified as a physiological E3 ligase for MHC class II molecules in mouse knockout studies^{77,78}. In the absence of MARCH1, the cell-surface expression of MHC class II molecules is increased on B cells and DCs.

As MARCH1 is an important regulator of MHC class II cell-surface expression, its expression is regulated at different DC maturation stages. *MARCH1* gene expression is downregulated by factors that promote DC maturation^{79,80}, so that mature DCs display on their surface long-lived MHC class II molecules loaded with peptides derived from antigens that were captured at the time of activation. Conversely, plasmacytoid DCs (pDCs) continue synthesizing MARCH1 after activation, resulting in sustained MHC class II turnover. This property renders pDCs inefficient at presenting exogenous antigens, but instead pDCs are able to continuously process and present endogenous or viral antigens, which might be important for an effective antiviral response⁸¹. MARCH1 is also regulated at a post-transcriptional level. CD83 — a surface immunoglobulin protein expressed on mature DCs — blocks the association of MHC class II molecules with MARCH1, thereby enhancing MHC class II cell-surface expression on mature DCs⁸².

MHC class I degradation and cell-surface expression

Antigenic peptides generated from cytosolic proteins are translocated to the endoplasmic reticulum (ER) and presented by MHC class I molecules to cytotoxic T cells. Pioneering studies carried out nearly two decades ago uncovered an important role of the ubiquitin–proteasome pathway in the generation of antigenic peptides in the cytosol^{83,84}. How endogenous or foreign proteins in the cytosol are selected for ubiquitylation and proteasomal degradation is still not understood. It is known, however, that antigen presentation can be

enhanced by IFN γ , which increases the expression of several proteasome subunits. These subunits are encoded by genes located in the MHC clusters, and they include LMP2, LMP7 and MECL1 (also known as LMP10). These subunits substitute the conventional proteasome subunits to form the immunoproteasome, which is specialized to generate peptide fragments that are optimal for MHC class I antigen presentation⁸⁵.

Mature MHC class I complexes consist of a heavy chain, β_2 -microglobulin (β_2m) and a peptide ligand. The folding and assembly of MHC class I complexes is under stringent quality control in the ER. Misfolded MHC class I molecules are degraded by the ER-associated degradation (ERAD) pathway, which involves ubiquitylation and dislocation from the ER to the cytosol for proteasomal degradation. The human cytomegalovirus (HCMV) protein US11 uses the cellular protein derlin 1 to induce the removal of MHC class I molecules by the ERAD machinery^{86,87}. Recently, the E3 ligase TRC8 (also known as RNF139) was shown to be required for ERAD-mediated MHC class I degradation induced by the HCMV protein US2; however, *TRC8* knockdown had little effect on steady-state MHC class I degradation in the absence of US2 (REF. 88). The degradation of MHC class I molecules in the absence of viral infection requires another E2–E3 pair — UBE2J1 and HMG-CoA reductase degradation 1 (HRD1; also known as synoviolin)⁸⁹. It will be interesting for future studies to investigate how the specific mechanisms used by viral proteins to induce ERAD-mediated MHC class I degradation compare with those of physiological quality control.

Cell-surface expression of MHC class I molecules is controlled by endocytosis and endolysosomal degradation. Monoubiquitylation of MHC class I molecules is not sufficient for efficient endocytosis from the cell surface; two E2 enzymes, UBC5 and UBC13, work sequentially to catalyse the initial monoubiquitylation and subsequent polyubiquitylation of MHC class I molecules. These E2 enzymes function with certain viral E3 enzymes, such as K3 and K5 from KSHV⁹⁰, to induce efficient endocytosis of surface MHC class I molecules, thereby helping the virus to escape killing by cytotoxic T cells.

Thus, ubiquitylation is involved in important regulatory mechanisms for MHC molecule function and antigen presentation, which have an essential role in bridging the innate and adaptive immune systems.

Role of ubiquitylation in adaptive immunity

Adaptive immunity is mediated by B and T cells. As discussed earlier, MHC molecules present antigenic peptides to T cells; the activation of T cells requires both TCR–peptide–MHC interactions and co-stimulatory signals. Adequately activated T cells then clonally expand and carry out effector functions such as cytotoxicity and cytokine secretion. B cells are activated through antigen interaction with B cell receptors. Ubiquitylation is important for the proper activation of adaptive immunity, as well as for the prevention of autoimmunity.

T cell activation and anergy induction

T cell activation triggers a complex network of signalling pathways, among which are the NF- κ B and MAPK pathways. TCR signalling activates protein kinase C θ , which recruits a protein complex consisting of CARMA1, BCL-10 and MALT1 (FIG. 6). The recruitment of TRAF6 to this complex activates its E3 ligase activity⁹¹, leading to NF- κ B and MAPK activation and subsequent T cell proliferation, survival and cytokine production. However, T cell-specific knockout of *Traf6* did not impair NF- κ B activation following TCR activation⁹². This may be due to a compensatory function of TRAF2, which could also bind to MALT1 (REF. 91). Recently, another E3 ligase, MIB2, was identified as a BCL-10-interacting

protein that has a role in TCR signalling-induced NF- κ B activation⁹³. Thus, multiple E3 ligases might function downstream of the TCR to mediate IKK activation.

TCR stimulation in the absence of co-stimulation leads to the induction of a tolerant state known as anergy. Several E3 ligases — such as GRAIL (also known as RNF128), ITCH and CBL-B — have been implicated in the induction of T cell anergy in the absence of co-stimulation⁹⁴. Recent studies indicate that these E3 enzymes can directly target components of the TCR complex. For example, GRAIL-deficient T cells are hypersensitive to TCR stimulation. This transmembrane RING-domain E3 ligase can down-regulate TCR cell-surface expression after TCR activation⁹⁵, probably through endocytosis-mediated degradation, a process that involves the recognition of ubiquitin modification by the endosomal sorting machinery⁹⁶. ITCH and CBL-B cooperate to mediate K33-linked polyubiquitylation of TCR ζ , and this suppresses the recruitment of downstream signalling proteins in a proteasome- and endocytosis-independent manner⁹⁷. Downstream signalling components in T cells are also regulated by ubiquitylation. For example, deficiency of the E3 ligase PELI1 results in T cell hyperactivation, possibly owing to defects in the degradation of c-REL, an NF- κ B subunit that is important for T cell activation and the prevention of T cell anergy⁹⁸.

B cell signalling

In B cells, ubiquitylation-mediated mechanisms have been proposed for the activation of MAPK and non-canonical NF- κ B signalling pathways downstream of CD40 and the BAFF receptor, two proteins of the TNF receptor superfamily^{99,100}. In these pathways, cIAP proteins and TRAF2 are recruited to the plasma membrane, where cIAPs are activated by TRAF2 through K63-linked polyubiquitylation. cIAPs then induce K48-linked polyubiquitylation and degradation of TRAF3, resulting in the release of a signalling complex containing MEKK1 (a MAPK kinase kinase) into the cytosol. MEKK1 then activates JUN N-terminal kinase (JNK) and other MAPK cascades. TRAF3 degradation also enables NF- κ B-inducing kinase (NIK) to dissociate from its E3 ligases (cIAPs), thereby stabilizing NIK. Stabilized NIK phosphorylates and activates IKK α , which in turn phosphorylates the NF- κ B precursor p100. p100 is subsequently polyubiquitylated by the SCF ^{β TrCP} ubiquitin E3 ligase complex and processed to the mature p52 subunit by the proteasome. p52 then functions together with another NF- κ B subunit, REL-B, to turn on the expression of genes that are important for B cell survival, maturation and activation¹⁰¹.

As discussed so far, ubiquitylation regulates many aspects of the host immune system. Not surprisingly, targeting the ubiquitylation machinery is also a common mechanism used by pathogens to evade and manipulate host immune responses.

Ubiquitylation in immune evasion by pathogens

Many pathogens have evolved mechanisms to subvert the host immune response to favour their survival. Viral and bacterial pathogens lack a classical ubiquitylation system; to manipulate the host immune response through ubiquitylation, they have acquired ubiquitylation-related components through gene flow and/or convergent evolution, and this underscores the significance of the ubiquitylation system in immune defence. Many viral and bacterial E3 ligases have been identified (TABLE 1). Some of these have sequence similarities with host proteins, which indicates that they have been acquired by gene transfer. Others have little sequence similarity with known E3 enzymes^{102,103}. Pathogens can also use DUBs and adaptor proteins to recruit host ubiquitylation machineries. Some pathogen components are themselves targeted by the host ubiquitylation machinery, so these pathogens also have evasion strategies to avoid being ubiquitylated.

Targeting host defence proteins for degradation

A common survival strategy used by many pathogens is the degradation of key antimicrobial proteins that are produced by the host. These include proteins involved in NF- κ B activation and IFN production and signalling, as well as MHC molecules and proteins encoded by interferon-stimulated genes. A few recent findings are discussed here.

Several pathogens target the I κ B ubiquitin ligase, SCF $^{\beta$ TrCP, to block NF- κ B activation. For example, the rotavirus protein NSP1 contributes to infection-induced degradation of β TrCP and the subsequent inhibition of NF- κ B activation¹⁰⁴. The *S. enterica* protein SseL has DUB activity that inhibits I κ B α ubiquitylation and degradation¹⁰⁵. By contrast, the HIV protein Vpu exploits β TrCP to induce the degradation of CD4 and bone marrow stromal antigen 2 (BST2)¹⁰⁶, two host factors with a key role in controlling HIV infection. Thus, the same ubiquitylation machinery can be controlled by viruses and bacteria in different ways to manipulate the host immune response.

The gammaherpesvirus MuHV-4 encodes a protein, known as ORF73, that can trigger polyubiquitylation and proteasomal degradation of the NF- κ B family member p65 (also known as REL-A), thereby inhibiting NF- κ B activation and allowing for virus proliferation. ORF73 contains an unconventional SOCS (suppressor of cytokine signalling) box, which mediates its assembly into a cullin-RING E3 ligase complex (containing ORF73, elongin C and CUL2 or CUL5) that is responsible for p65 degradation. MuHV-4 that has a mutation in the SOCS box of ORF73 fails to cause persistent infection in mice, demonstrating the physiological importance of this motif¹⁰⁷.

Several bacterial E3 enzymes inhibit the NF- κ B pathway by causing NEMO destruction. *In vitro*, the *Shigella flexneri* protein IpaH9.8 can induce K27-linked polyubiquitylation of NEMO at K309 and K321. This might account for NEMO degradation *in vivo* and thus control inflammatory responses¹⁰⁸. Intriguingly, although IpaH9.8 is sufficient as an E3 ligase on its own, the cellular protein A20-binding inhibitor of NF- κ B activation 1 (ABIN1; also known as TNIP1) is also involved in IpaH9.8-induced NEMO degradation. It is possible that IpaH9.8 uses cellular factors to regulate its activity; for example, such factors might promote specific interactions with substrates.

Several viral proteins trigger the degradation of IRF3 and IRF7 (REF. 109). The rotavirus protein NSP1 and the pestivirus protein Npro can both bind to IRF3 and induce its ubiquitylation-mediated proteasomal degradation in cell culture^{110,111}. The KSHV protein replication and transcription activator (RTA) has no sequence similarity with known E3 ligase domains, but nonetheless has E3 ligase activity towards IRF7 and induces IRF7 proteasomal degradation¹¹².

The binding of IFNs to IFN receptors triggers down-stream signalling pathways that induce a broad spectrum of interferon-stimulated genes, which function together to establish an antiviral state for host cells. IFN receptor signalling is mediated by the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways. The paramyxoviruses are well-studied examples of viruses that block IFN receptor signalling by causing the degradation of STAT proteins¹¹³. This effect is mediated by the viral V protein, which is assembled into a cullin-RING ubiquitin E3 ligase complex that also contains the host proteins CUL4A and damage-specific DNA binding protein 1 (DDB1). The V protein recruits STATs to the E3 complex for ubiquitylation and subsequent degradation¹¹⁴.

The APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like) family of proteins are virus restriction factors that have intrinsic antiviral properties¹¹⁵. APOBEC3G was the first host inhibitor of HIV-1 infection to be identified¹¹⁶. It is

incorporated into virus particles, where it causes extensive cytidine to uridine editing during reverse transcription of the viral genome to restrict viral propagation. Cytidine deaminase-independent antiviral mechanisms mediated by APOBEC proteins have also been described¹¹⁷. HIV-1 can overcome these host antiviral effects through the viral protein virion infectivity factor (Vif), which targets APOBEC3G and APOBEC3F for degradation^{118,119}. In a similar manner to the V protein of paramyxoviruses, HIV Vif functions as an adaptor protein for a cullin-RING E3 complex (containing Vif, CUL5 and elongin B or elongin C), to which it recruits APOBEC proteins for ubiquitylation and subsequent proteasomal degradation. Another example of a host intrinsic antiviral factor is SAMHD1 (SAM-domain and HD-domain-containing protein 1). SAMHD1 interferes with HIV-1 infection of macrophages by preventing efficient viral cDNA synthesis. The HIV-2 Vpx protein was recently shown to induce the degradation of SAMHD1 through the assembly of a cullin-RING E3 complex containing CUL4A, RBX1, DDB1 and DCAF1 (DDB1- and CUL4-associated factor 1; also known as VPRBP)^{120,121}.

Interfering with protein modification by ubiquitin and ubiquitin-like proteins

A dramatic example of how pathogens use innovative strategies to manipulate host cells is provided by the effector protein CHBP from *Burkholderia pseudomallei* and its homologue, Cif, from enteropathogenic *Escherichia coli*. CHBP directly modifies ubiquitin and the ubiquitin-like protein NEDD8 by deamidating a specific glutamine residue to glutamic acid¹²². Deamidated ubiquitin is defective in supporting ubiquitin-chain synthesis, and deamidated NEDD8, when conjugated to cullin-RING ligases, suppresses their ubiquitin ligase activity. Thus, CHBP family proteins can potentially affect many cellular functions, including the antibacterial immune response.

Sumoylation is another fundamental post-translational modification involved in many biological processes. Although the role of sumoylation in antimicrobial immune responses has not been extensively studied, some new evidence points to interesting connections. The adenovirus protein Gam1 causes overall inhibition of protein sumoylation by forming a cullin-RING E3 complex and targeting the SUMO E1 enzyme for degradation¹²³. Infection with *Listeria monocytogenes* (and possibly other bacterial infections) leads to a decrease in the overall level of sumoylated proteins and to the specific degradation of UBC9 (REF. 124), the sole SUMO E2 enzyme identified so far. Interestingly, overexpression of SUMO in host cells suppresses bacterial infection, indicating that sumoylation has a role in antimicrobial defence. Also, a SUMO protease of the plant bacterial pathogen *Xanthomonas campestris* can mediate changes in many aspects of host cell biology and promote bacterial infection¹²⁵.

Reversing ubiquitin modification

Many viral and bacterial pathogens encode their own DUBs that are involved in pathogenesis and immune evasion. A few virus-encoded DUBs that have no sequence similarity to eukaryotic DUBs have been identified in the *Herpesviridae* family¹²⁶. For example, a protein with DUB activity known as ORF64 from KSHV (a member of the *Herpesviridae* family) was recently shown to inhibit RIG-I-mediated signalling¹²⁷. Some other viral proteins are predicted to have OTU protease domains (which are known to mediate deubiquitylation); although these domains have limited sequence similarity to cellular OTU domains, they contain a few highly conserved residues, including predicted catalytic active sites¹²⁸. For example, the L protein from Crimean-Congo haemorrhagic fever virus is a large protein that has an OTU domain at its N terminus. The OTU domain of the L protein has deconjugating activity *in vitro* for proteins modified by ubiquitin and the ubiquitin-like protein ISG15 (REF. 129). Expression of the OTU domain, but not of OTU domain mutants that lack catalytic activity, decreases the overall level of ubiquitylated and

ISGylated cellular proteins. Both DUB and de-ISGylation activities are associated with the immune-evasion function of the L protein: expression of the OTU domain inhibits TNF-induced NF- κ B activation and counteracts the antiviral activity of ISG15 *in vivo*, supporting the notion that ubiquitylation and ISGylation have active roles in antiviral immunity.

Instead of encoding DUBs directly, some pathogens hijack host DUBs. Herpes simplex virus type 1 (HSV-1)-encoded ICP0 is a multifunctional protein that has a wide range of cellular targets. Some of its proposed functions — such as inducing the degradation of cellular proteins involved in cell cycle control and cell death — are associated with its ubiquitin ligase activity¹³⁰. Recently, ICP0 was found to mediate the recruitment of the cellular ubiquitin protease USP7 to deubiquitylate TRAF6 and NEMO, leading to the inhibition of the TLR–NF- κ B pathway¹³¹. USP7 knock-down enhances TLR4-induced NF- κ B activation, which indicates that USP7 might be a physiological regulator of NF- κ B activation in a manner similar to that of the DUB A20. Another example of a pathogen that uses host DUBs to inhibit the immune response comes from a study of persistent measles virus infection in monocytes. Measles virus infection leads to impaired NF- κ B activation by TLR4 signalling, which can be explained in part by increased transcription of the gene encoding A20 mediated by the measles virus P protein. Knockdown of A20 expression restored lipopolysaccharide-induced cytokine production in measles virus-infected monocytes¹³². In addition, the human T lymphotropic virus 1 (HTLV-1)-encoded protein Tax associates with TAX1BP1, a host ubiquitin-binding adaptor protein that regulates A20 function. Through this association, Tax disrupts the A20 complex, thereby contributing to the persistent activation of NF- κ B, which is important for T cell transformation by HTLV-1 (REF. 3).

Evading autophagy

To prevent detection by the host autophagic machinery, cytosol-dwelling bacteria, such as *L. monocytogenes* and *S. flexneri*, use various strategies to efficiently avoid polyubiquitin tagging. Certain mutant bacteria, such as *L. monocytogenes* deficient in ActA, undergo ubiquitylation and recruit the autophagy adaptor protein p62 (REF. 133). The *S. flexneri* protein IcsB prevents autophagy by blocking the interaction of another bacterial protein, VirG, with the host autophagy component ATG5 (REF. 134); whether ubiquitin is involved in this process is unknown. Another cytosolic bacterium, *Mycobacterium marinum* sheds ubiquitylated cell walls to prevent sequestration into lysosome-like organelles¹³⁵.

Antagonizing antigen presentation

Pathogens can target MHC molecules to modulate the host immune response by decreasing the presentation of pathogen antigens. *S. Typhimurium* infection induces the polyubiquitylation of peptide-loaded MHC class II molecules in monocyte-derived DCs, and this decreases their cell-surface expression¹³⁶. *Francisella tularensis* infection seems to induce a secreted factor that functions in an autocrine or paracrine manner to induce ubiquitin-dependent MHC class II degradation in bone marrow-derived macrophages¹³⁷.

Several viral MIR proteins function as E3 ligases that target MHC class I molecules into the ERAD pathway or for lysosomal degradation to decrease MHC class I cell-surface expression⁷⁶. Interestingly, two viral E3 ligases — mK3 from MHV68 and K3 from KSHV — conjugate ubiquitin to non-conventional amino acids such as serine, threonine or cysteine^{138,139}, a property that might allow these viral E3 ligases to have an expanded repertoire of potential targets.

The degradation of MHC class I molecules could sensitize virus-infected cells to natural killer (NK) cell-mediated killing. KSHV resolves this conundrum by using another viral E3

ligase, K5, to downregulate the expression of surface ligands (such as NKG2D) that bind to NK cell activating receptors, thus preventing NK cell-mediated killing induced by MHC class I downregulation¹⁴⁰.

Exploiting the host ubiquitylation machinery

SOCS proteins were initially discovered as important physiological feedback inhibitors of cytokine-induced JAK–STAT pathways, and were later found to be also involved in many other aspects of both innate and adaptive immunity¹⁴¹. One of the mechanisms through which they function is using their SOCS box to interact with other subunits of cullin–RING E3 ligase complexes that control the degradation of many key immune mediators. Some SOCS proteins, such as SOCS1 and SOCS3, can be transcriptionally induced or stabilized at the protein level by several viruses, such as influenza A virus¹⁴², HSV-1 (REF. 143) and HTLV-1 (REFS 144,145), thereby inhibiting the host immune response.

With their compact genomes, pathogens usually have multifunctional proteins that carry out a multitude of different functions in a highly regulated manner. For example, *S. enterica* uses the host ubiquitylation machinery to regulate its multifunctional effector protein SopB¹⁴⁶. Following entry of SopB into host cells, it promotes actin-mediated bacterial internalization and AKT activation at the plasma membrane. Ubiquitylation of SopB is required for its translocation from the plasma membrane to vacuolar compartments, where it regulates vesicular trafficking and *S. enterica* intracellular replication.

Concluding remarks

Our understanding of ubiquitylation as an essential regulatory mechanism in immune responses is still in its infancy. As more components in the ubiquitin pathway are linked to the immune system, we face the challenge of understanding the underlying biochemical mechanisms. What are the physiological ubiquitylation targets? How do different E3 enzymes, DUBs and ubiquitin-binding proteins integrate signals to achieve stringent regulation with precise signalling specificity? Even after thorough biochemical or *ex vivo* characterization of particular proteins, genetic studies in knockout animals more often than not yield surprises as to the physiological functions of certain proteins. Thus, it is equally important to connect the biochemical functions of ubiquitylation-related components to their functions *in vivo*. Therapeutically, as ubiquitylation is a crucial part of immune regulation, understanding the molecular bases of ubiquitylation in the immune system might hold the key to the development of effective approaches for enhancing host defence, as well as for preventing the deleterious breakdown of immune homeostasis.

Studies of pathogen immune-evasion strategies not only provide an understanding of the biology of pathogens, but also pinpoint those immunological events that are most detrimental to a given pathogen species. It is worth noting that *in vitro* studies have shown that many pathogen proteins are highly multifunctional. However, in physiological situations, it is possible that a few particular functional aspects of a pathogen protein are more important than others, or that different aspects function together. Thus, it is important to evaluate the physiological relevance of *ex vivo* findings, and their relative contributions *in vivo*. Such knowledge will provide clues to effective therapeutic strategies for infectious diseases, and help the rational design of attenuated pathogens to create vaccine strains or gene therapy vectors.

Acknowledgments

Research in the Chen laboratory is supported by grants from the US National Institutes of Health, the Cancer Prevention and Research Institute of Texas and the Welch Foundation. Z.J.C. is an Investigator of Howard Hughes Medical Institute.

Glossary

Non-canonical NF-κB signalling	An NF- κ B activation pathway that processes the NF- κ B precursor p100 into p52, uses an NF- κ B dimer containing p52 and REL-B subunits, and occurs during B cell maturation and activation. By contrast, canonical NF- κ B signalling involves the degradation of I κ B and the activation of NF- κ B dimers comprising p65 (also known as REL-A), c-REL and p50
Gene flow	The transfer of genes from one population to another, or across species
Convergent evolution	The process by which lineages that are not closely related in evolutionary terms independently acquire similar traits
Sumoylation	A type of protein modification in which the ubiquitin-like protein SUMO is covalently attached to target proteins by an enzymatic cascade that is analogous to that involved in ubiquitylation

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Figure 1. Ubiquitin-mediated signalling in TLR and IL-1R pathways

Stimulation of Toll-like receptors (TLRs) and the interleukin-1 receptor (IL-1R) leads to the recruitment of the adaptor protein myeloid differentiation primary response protein 88 (MYD88), which in turn recruits the kinases IL-1R-associated kinase 4 (IRAK4) and IRAK1, forming the ‘myddosome’. The E3 ligase TNF receptor-associated factor 6 (TRAF6) is then recruited and activated, and synthesizes K63-linked polyubiquitin chains. These polyubiquitin chains recruit kinase complexes containing TGFβ-activated kinase 1 (TAK1) or IκB kinase (IKK) through their ubiquitin-binding subunits — TAK1-associated binding protein 2 (TAB2)–TAB3 and NF-κB essential modulator (NEMO), respectively. Binding of K63-linked polyubiquitin to TAB2 and TAB3 leads to TAK1 activation, which in turn activates the mitogen-activated protein kinase (MAPK) cascade. Binding of K63-linked polyubiquitin to both the IKK and TAK1 complexes facilitates the phosphorylation of IKKβ by TAK1, leading to IKK activation. IKK phosphorylates NF-κB inhibitor (IκB) proteins and targets them for polyubiquitylation by the SCFβTrCP ubiquitin E3 ligase complex. The polyubiquitylated IκB proteins are degraded by the proteasome, allowing nuclear factor-κB (NF-κB) to enter the nucleus to turn on target genes involved in immune and inflammatory responses.

Figure 2. Different types of polyubiquitylation in the TLR4 signalling pathways

Toll-like receptor 4 (TLR4) that is activated at the cell membrane recruits myeloid differentiation primary response protein 88 (MYD88) through TIR domain-containing adaptor protein (TIRAP). Signalling complexes containing MYD88, IL-1 receptor-associated kinases (IRAKs) and TNF receptor-associated factor 6 (TRAF6) are formed at the plasma membrane. TRAF6 activates the E3 ligases cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2, which induce the ubiquitylation and subsequent degradation of TRAF3. The degradation of TRAF3 releases TRAF6 signalling complexes from TLR4 into the cytosol, where additional components are recruited and activated. Another branch of signalling pathways is stimulated when TLR4 is endocytosed. The adaptor protein TRAM recruits TIR domain-containing adaptor protein inducing IFN β (TRIF) to endocytosed TLR4. Through the action of several E3 ligases — such as pellino 1 (PELI1), TRAF3 and TRAF6 — which mediate the ubiquitylation of signalling proteins such as receptor-interacting protein 1 (RIP1) and TRAF3, downstream pathways leading to nuclear factor- κ B (NF- κ B) activation and type I interferon (IFN) induction are activated. Deubiquitylation enzymes (DUBs), such as A20, provide mechanisms for the downregulation of various pro-inflammatory signalling pathways, by disassembling polyubiquitin chains. I κ B, NF- κ B inhibitor; IKK, I κ B kinase; IRF3, interferon regulatory factor 3; MAPK, mitogen-activated protein kinase; NEMO, NF- κ B essential modulator; TAB, TAK1-binding protein; TAK1, TGF β -activated kinase 1; TBK1, TANK-binding kinase 1.

Figure 3. Roles of ubiquitylation in diverse PRR signalling pathways

a | Nucleotide-binding oligomerization domain protein 2 (NOD2) detects bacterial peptidoglycans and triggers the ubiquitylation of receptor-interacting protein 2 (RIP2), which leads to the activation of the mitogen-activated protein kinase (MAPK) cascade and nuclear factor- κ B (NF- κ B). **b** | In the retinoic acid-inducible gene I (RIG-I)-mediated pathway that senses viral RNA, K63-linked polyubiquitylation and the E3 ligases tripartite motif-containing protein 25 (TRIM25) and riplet are important for RIG-I activation. RIG-I transduces the signal to mitochondrial antiviral signalling protein (MAVS) on mitochondria, and this induces the activation of the I κ B kinase (IKK) and TANK-binding kinase 1 (TBK1) complexes through NF- κ B essential modulator (NEMO). Ubiquitylation of NEMO and TBK1 by TRIM23 and mindbomb (MIB) proteins, respectively, has been suggested to mediate TBK1 activation. IKK and TBK1 activate NF- κ B and interferon regulatory factors (IRFs) to induce the transcription of pro-inflammatory cytokines and type I interferons (IFNs), respectively. **c** | Activation of the stimulator of interferon genes (STING)-mediated pathways that sense intracellular DNA also involves ubiquitylation (for example, by TRIM56). cIAP, cellular inhibitor of apoptosis protein; ER, endoplasmic reticulum; TANK, TRAF family member-associated NF- κ B activator.

Figure 4. Ubiquitin adaptor proteins mediate selective autophagy of pathogens

Ubiquitylation tags invading bacterial pathogens by targeting bacterial proteins or associated host components. Adaptor proteins, such as p62, NDP52 and optineurin (OPTN), that bind to both ubiquitin and the ubiquitin-like protein LC3, link ubiquitin-tagged bacteria with the autophagic machinery. Antibacterial peptide precursors are targeted in a similar manner by p62 so that the antibacterial peptides are produced in the intracellular compartments that contain the bacteria.

Figure 5. Ubiquitylation controls the expression of MHC molecules

MARCH1 (membrane-associated RING-CH 1)-mediated ubiquitylation of MHC class II molecules promotes their endolysosomal degradation, thus controlling the level of cell-surface expression of MHC class II molecules. MHC class I expression is regulated by the endoplasmic reticulum (ER)-associated degradation pathway and by endolysosomal degradation, both of which are controlled by ubiquitylation. MHC class I ubiquitylation can be mediated by viral E3 ligases, such as modulator of immune response (MIR) proteins, or by cellular E3 enzymes, such as HMG-CoA reductase degradation 1 (HRD1). Other cellular E3 ligases can be exploited by viral proteins; for example, the TRC8-mediated ubiquitylation of MHC class I molecules is induced by the human cytomegalovirus (HCMV) protein US2.

Figure 6. Ubiquitin E3 ligases regulate TCR signalling

Stimulation of the T cell receptor (TCR) leads to the recruitment and activation of protein kinase C θ (PKC θ), which in turn recruits a protein complex comprising CARMA1, BCL-10 and MALT1. MALT1 binds to and activates TNF receptor-associated factor 6 (TRAF6), which then activates nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPKs) through K63-linked polyubiquitylation. Proper T cell activation requires both TCR stimulation and co-stimulatory signals. In the absence of co-stimulation, several E3 ligases (such as GRAIL, CBL-B and ITCH) keep T cell activation in check by targeting multiple stages of the TCR signalling pathway, including the TCR complex and downstream signalling molecules, such as PKC θ . Co-stimulatory signals release the inhibition of TCR signalling mediated by E3 ligases.

Table 1

Microbial proteins that harness ubiquitin pathways to evade host immune defence

Pathogen	Factor	Function	Host target
<i>Pathogens that target key antimicrobial proteins for degradation</i>			
MuHV-4	ORF73	E3 subunit	NF- κ B
Rotavirus	NSP1	ND	β TrCP, IRF3
KSHV	RTA	E3 ligase	IRF7
HIV	Vpu	ND	CD4, BST2
<i>Shigella flexneri</i>	IpaH9.8	E3 ligase	NEMO
Paramyxovirus	V protein	E3 subunit	STAT proteins
HIV-1	Vif	E3 subunit	APOBEC proteins
HIV-2	Vpx	E3 subunit	SAMHD1
Adenovirus	Gam1	E3 subunit	Sumoylation
<i>Listeria monocytogenes</i>	ND	ND	UBC9, sumoylation
<i>Xanthomonas campestris</i>	XopD	SUMO protease	ND
<i>Salmonella</i> Typhimurium	ND	ND	MHC class II
<i>Francisella tularensis</i>	ND	ND	MHC class II
MHV68	mK3	E3 ligase	MHC class I
KSHV	K3, K5	E3 ligase	MHC class I, NKG2D
<i>Pathogens that interfere with antimicrobial pathways by preventing or reversing ubiquitin modification</i>			
EPEC	Cif	Deamidase	NEDD8
<i>Burkholderia pseudomallei</i>	CHBP	Deamidase	Ubiquitin, NEDD8
<i>Salmonella enterica</i>	SseL	DUB	I κ B α
KSHV	ORF64	DUB	RIG-I
CCHFV	L protein	DUB	Ubiquitylation, ISGylation
HSV-1	ICP0	E3 ligase, recruits cellular DUBs	Recruits USP7 to deubiquitylate TRAF6 and NEMO
Measles virus	P protein	Upregulates DUBs	A20
HTLV-1	Tax	Induces persistent NF- κ B activation	TAX1BP1, A20
<i>Pathogens that evade autophagy</i>			
<i>Listeria monocytogenes</i>	ActA	Prevents ubiquitylation	Host autophagy machinery
<i>Shigella flexneri</i>	IcsB	Prevents ubiquitylation	Host autophagy machinery
<i>Mycobacterium marinum</i>	ND	Prevents ubiquitylation	ND
<i>Pathogens that exploit the host ubiquitylation machinery</i>			
Influenza A virus	ND	Induces or stabilizes SOCS proteins	ND
HSV-1	ND	Induces or stabilizes SOCS proteins	ND
HTLV-1	Tax	Induces or stabilizes SOCS proteins	ND
<i>Salmonella enterica</i>	SopB	Ubiquitin-dependent localization	ND

ActA, actin assembly-inducing protein A; APOBEC, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like; BST2, bone marrow stromal antigen 2; CCHFV, Crimean-Congo haemorrhagic fever virus; CHBP, Cif homologue from *Burkholderia pseudomallei*; Cif, cell inhibiting factor; DUB, deubiquitylation enzyme; EPEC, enteropathogenic *Escherichia coli*; HSV, herpes simplex virus; HTLV, human T lymphotropic virus; ICP0, infected cell protein 0; IRF, interferon regulatory factor; KSHV, Kaposi's sarcoma-associated herpesvirus; MHV68, murine

gammaherpesvirus 68; MuHV-4, murid herpesvirus 4; ND, not determined; NEDD8, neural precursor cell expressed, developmentally downregulated 8; NEMO, NF- κ B essential modulator; NF- κ B, nuclear factor- κ B; NKG2D, natural killer group 2D; NSP1, non-structural protein 1; RIG-I, retinoic acid-inducible gene I; RTA, replication and transcription activator; SAMHD1, SAM-domain- and HD-domain-containing protein 1; SOCS, suppressor of cytokine signalling; SseL, *Salmonella* secreted effector L; STAT, signal transducer and activator of transcription; SUMO, small ubiquitin-related modifier; TRAF6, TNF receptor-associated factor 6; β TrCP, β -transducin repeat-containing protein; UBC, ubiquitin-conjugating enzyme E2; USP7, ubiquitin-specific protease 7; Vif, virion infectivity factor; XopD, *Xanthomonas* outer protein D.