A20: linking a complex regulator of ubiquitylation to immunity and human disease

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Abstract | A20 (also known as TNFAIP3) is a potent anti-inflammatory signalling molecule that restricts multiple intracellular signalling cascades. Recent studies in three general areas have converged to highlight the clinical and biological importance of A20. First, human genetic studies have strongly linked polymorphisms and mutations in the gene encoding A20 to inflammatory, autoimmune and malignant diseases. Second, studies in gene-targeted mice have revealed that A20 regulates multiple immune cell functions and prevents experimental diseases that closely mimic human conditions. Third, biochemical studies have unveiled complex mechanisms by which A20 regulates ubiquitin-dependent nuclear factor-κB and cell-survival signals. Taken together, these studies are revealing the importance of A20-mediated regulation of ubiquitin-dependent signalling in human disease.

Ubiquitylation
The covalent attachment of single ubiquitin molecules or ubiquitin chains to proteins to regulate their interactions with proteasomal components and other proteins. Protein ubiquitylation occurs in three enzymatic steps that require a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3), respectively. The ligase catalyses the formation of an isopeptide bond between the carboxyl terminus of ubiquitin and an amino group belonging to a lysine residue of the target protein.

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Human genetic studies have implicated a wide variety of immune genes in the pathophysiology of autoimmune and inflammatory diseases. These studies have suggested a role for aberrant immune cell activation in these diseases and have guided recent investigations into the molecular mechanisms underlying disease pathogenesis. From the many polymorphisms analysed, several genes — including the gene encoding the anti-inflammatory signalling molecule A20 (*TNFAIP3*) — have been linked to susceptibility to multiple diseases. The products of these genes are likely to regulate crucial steps in immune cell homeostasis and should be attractive targets for targeted therapies.

Perhaps the best-characterized molecular pathway for triggering immune cell activation is the canonical nuclear factor-κB (NF-κB) signalling pathway, which leads to the transcription of numerous pro-inflammatory and cell-survival genes[1]. Excessive NF-κB signalling in multiple cell types has been linked to both human and experimental inflammatory diseases, and to malignant diseases. For example, dysregulated NF-κB signals underlie the pathophysiology of common subtypes of B cell lymphoma and myeloma[2], as well as colon carcinogenesis[3]. Therefore, determining how NF-κB signals are normally restricted is crucial both for understanding the pathophysiology of these diseases and for devising therapeutic strategies.

The NF-κB signalling cascade is prominently regulated by ubiquitylation, a process that can generate a series of post-translational modifications that direct proteins towards distinct biological fates[4]. For example, the attachment of K48-linked polyubiquitin chains targets proteins for proteasomal degradation, whereas the attachment of K63-linked polyubiquitin chains can result in the recruitment of downstream signalling molecules that propagate signals[4,5]. Thus, the precise synthesis, recognition and degradation of diverse types of ubiquitin chain must be specified by ubiquitin ligases, ubiquitin-binding proteins and de-ubiquitylating enzymes (DUBs), respectively, to ensure the proper regulation of intracellular signalling pathways. The functions of ubiquitylation in cell signalling have recently been described in several excellent reviews[4–6].

The ubiquitin-modifying enzyme A20 has emerged as a potent and unusually complex regulator of ubiquitin-dependent signals. A20 is a pleiotropically expressed cytoplasmic protein, the expression of which is regulated at both the transcriptional and the post-transcriptional level. Induced by NF-κB-dependent signals, A20 in turn restricts the duration and intensity of signalling by several molecules involved in the NF-κB pathway. Hence, the induction of A20 expression constitutes a negative feedback loop for NF-κB signalling[7].

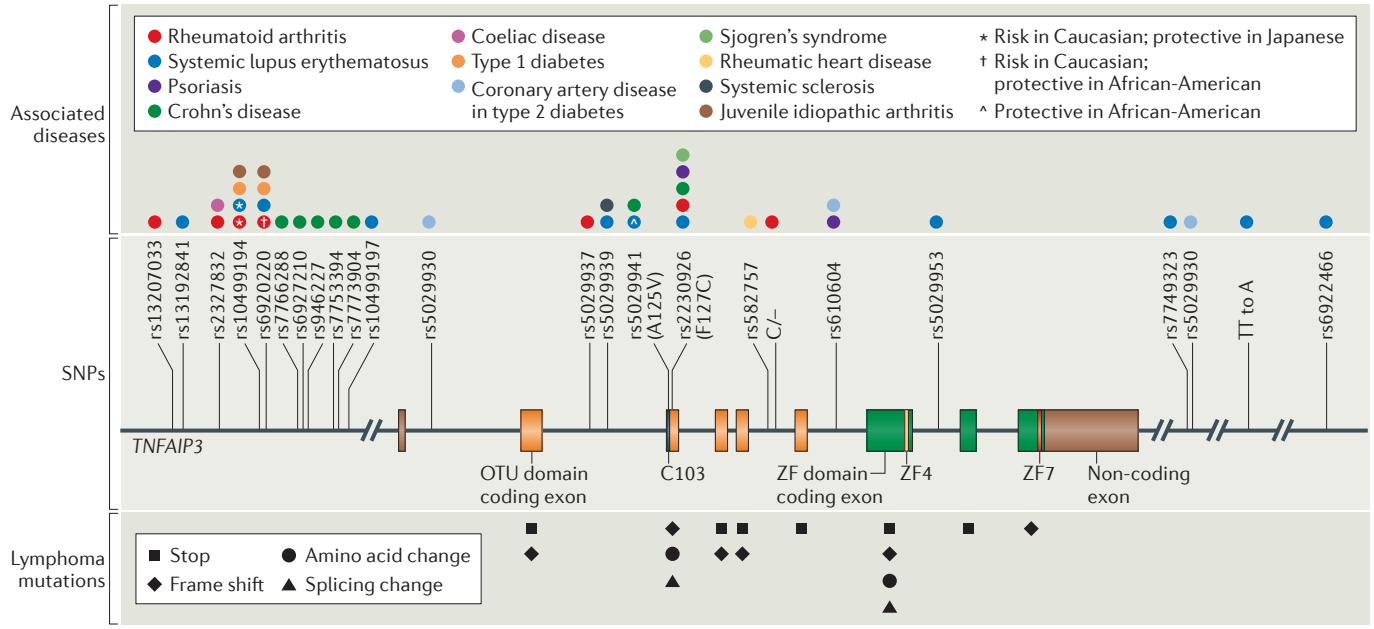

Figure 1 | **Polymorphisms or mutations in *TNFAIP3* and human diseases.** The figure shows a schematic of the *TNFAIP3* gene locus, which encodes A20. Exons encoding the amino-terminal OTU domain are shown in orange and exons encoding the carboxy-terminal zinc fingers (ZFs) of A20 are shown in green. The C103, ZF4 and ZF7 motifs are highlighted. Non-coding exons, including AT-rich sequences at the end of exon 9, are shown in brown. Human germline single-nucleotide polymorphisms (SNPs) in the *TNFAIP3* locus that are associated with autoimmune and autoinflammatory diseases are indicated (labelled with their reference SNP (rs) numbers)[26–46], as are somatic mutations that have been identified in the coding exons of *TNFAIP3* in human B cell lymphomas[50–54].

In addition, post-translational modifications of A20 — including phosphorylation, protein cleavage, glycosylation and ubiquitylation — may serve to support or restrict its activity[8,9,10].

A20 restricts NF-κB signalling downstream of tumour necrosis factor receptor 1 (TNFR1), CD40, Toll-like receptors (TLRs), NOD-like receptors (NLRs) and the interleukin-1 receptor (IL-1R)[11–19]. A20 also promotes cell-survival signals, adding another dimension to its ability to regulate dynamic immune responses[14]. Of note, the ability of A20 to inhibit cell death may be independent of its role in restricting NF-κB signalling, as decreased NF-κB signalling is typically associated with increased cell death. The molecular mechanisms by which A20 performs these diverse functions are incompletely understood, but are likely to involve the regulation of ubiquitin-dependent signalling complexes (see below).

A20 cleaves polyubiquitin chains, thereby exhibiting DUB activity[15,20–22], but also collaborates with ubiquitin-activating E1 enzymes and ubiquitin-carrier E2 proteins to build ubiquitin chains, thus displaying E3 ubiquitin ligase activity[22]. Furthermore, A20 directly binds to ubiquitin chains[23,24]. Therefore, A20 interfaces with and modifies ubiquitylated protein substrates in multiple ways and probably uses a variety of biochemical mechanisms to regulate NF-κB and cell-death signals (see below).

In this Review, we examine recent studies in three areas. First, human genetic studies have linked both germline polymorphisms and somatic mutations of *TNFAIP3* (which encodes A20) to inflammatory and malignant human diseases. Second, new strains of mice bearing lineage-specific deletions of *Tnfaip3* have revealed several cell type-specific functions for A20. Third, biochemical studies have revealed complex mechanisms by which A20 regulates a variety of ubiquitin-dependent signalling pathways in immune cells. Taken together, these studies are revealing a diverse set of genetic, cellular and molecular mechanisms by which A20 prevents disease.

A20: a protein linked to multiple human diseases

Human genetic studies have linked germline single-nucleotide polymorphisms (SNPs) of *TNFAIP3* with susceptibility to multiple human diseases[25]. These diseases include systemic lupus erythematosus (SLE)[26–32], rheumatoid arthritis[30,33–37], psoriasis[38,39], type 1 diabetes[37,40], coeliac disease[37,41], Crohn's disease[42,43], coronary artery disease in type 2 diabetes[44], and systemic sclerosis[45,46] (FIG. 1). Given the potent anti-inflammatory function of A20, disease-associated *TNFAIP3* SNPs might reduce its function or expression. Indeed, one SLE-associated SNP in the coding region of *TNFAIP3* causes a phenylalanine to cysteine substitution at residue 127 and reduces A20 function[27]. Another SLE-associated SNP reduces A20 expression by altering the binding of transcription factors at a putative 3′ enhancer in *TNFAIP3* (REF. 31). Other disease-associated *TNFAIP3* SNPs are located outside of the coding regions, suggesting that they may also confer susceptibility to disease by reducing A20 expression[35,44]. Importantly, mice expressing reduced levels of A20 develop spontaneous inflammation, providing direct evidence that reduced A20 expression causes autoimmune or inflammatory disease[18,19,43].

Single-nucleotide polymorphisms (SNPs). Variations in DNA sequence in which one of the four nucleotides is substituted for another (for example, C for A). SNPs are the most frequent type of polymorphism in the genome.

Table 1 | **Mouse phenotypes resulting from cell type-specific ablation of A20 expression and the related human diseases**

Cell type	Genetic modification	Mouse phenotype	Related human disease	Refs
B cells	Cre-mediated deletion of *Tnfaip3* in cells that express CD19	Germinal centre and plasma cell dysplasia; production of autoantibodies; renal immunoglobulin deposition; B cell resistance to FAS-mediated cell death	Systemic lupus erythematosus	18,19,60
DCs	Cre-mediated deletion of *Tnfaip3* in cells that express CD11c	DC activation; expansion and activation of T cell and myeloid cell populations; colitis; spondyloarthritis	Inflammatory bowel disease	43
DCs	Cre-mediated deletion of *Tnfaip3* in cells that express CD11c	DC activation; expansion of T cell and plasma cell populations; increased uptake of apoptotic cells by DCs; autoantibody production; nephritis	Systemic lupus erythematosus	62
Macrophages and granulocytes	Cre-mediated deletion of *Tnfaip3* in cells that express lysozyme M	Increased IL-6 production; production of collagen-specific autoantibodies; protection against influenza A virus infection	Rheumatoid arthritis	67,68
Intestinal epithelial cells	Cre-mediated deletion of *Tnfaip3* in cells that express villin	Hypersensitivity to experimental colitis	Inflammatory bowel disease	69
Intestinal epithelial cells	Villin-driven expression of a *Tnfaip3* transgene	Protection against DSS-induced colitis	Inflammatory bowel disease	72
Keratinocytes	Cre-mediated deletion of *Tnfaip3* in cells that express keratin 14	Epidermal hyperproliferation; hair and skin defects; sebaceous gland hyperplasia	?	73

DC, dendritic cell; DSS, dextran sulphate sodium; IL-6, interleukin-6.

Potential benefits of correlating SNPs in specific genes with genetically complex human diseases include the ability to associate genotypes and/or molecular phenotypes with prognostic or therapeutic responses. *TNFAIP3* polymorphisms and altered A20 expression have been correlated with therapeutic responses to TNF blockade in the treatment of rheumatoid arthritis, inflammatory bowel disease and psoriasis[47–49]. Moreover, the presence of certain *TNFAIP3* SNPs strongly correlates with the risk of severe renal or haematological complications in patients with SLE[31]. Therefore, in addition to providing insights into the pathogenesis of these inflammatory disorders, further genetic analyses might link *TNFAIP3* polymorphisms with distinct subtypes of these diseases.

In addition to the effects of germline polymorphisms of *TNFAIP3* in conferring susceptibility to inflammatory diseases, biallelic somatic mutations of this gene are pathogenic in human lymphomas, suggesting that A20 acts as a tumour suppressor[50–54]. Biallelic mutations in the coding sequence of *TNFAIP3* have been identified in ~18% of B cell lymphomas, including diffuse large B cell lymphoma, mucosa-associated lymphoid tissue-type lymphoma and Hodgkin's lymphoma[50,51]. Such mutations occur throughout the *TNFAIP3* gene and result in the introduction of stop codons, frame shifts, amino acid changes or splicing alterations[55,56] (FIG. 1). The expression of *TNFAIP3* may also be inhibited by epigenetic methylation of its promoter[53,57]. The degree to which mutations affect A20 protein stability and/or function has yet to be determined for most lymphomas.

TNFAIP3 mutations are likely to be pathogenic in cancerous transformation, as re-expressing A20 in tumour cells induces apoptosis or cell-cycle arrest[50,51,53,54]. As A20 deficiency can lead to exaggerated NF-κB signalling and/or increased cell death, and as enhanced NF-κB signalling is linked to several types of human B cell lymphoma, A20-mediated restriction of this crucial signalling cascade may explain the tumour-suppressive function of A20 in B cells. In addition, whether A20 inactivation contributes to other cancer cell types or predicts responses to therapies warrants further investigation. In summary, polymorphisms and mutations of *TNFAIP3* have an impact on a wide range of human diseases. These links to human disease provide a compelling rationale for further investigations into the mechanisms by which A20 regulates immune cell functions.

Cellular mechanisms of A20 and disease

A20 is expressed by virtually all cell types, which complicates our understanding of the physiological functions of A20 and its roles in preventing disease. In addition, A20-regulated signals, such as canonical NF-κB signals and cell-survival signals, are shared by multiple ligands and cell types. Given the pleiotropic functions of NF-κB and cell-death signalling in various cell types that contribute to autoimmune and inflammatory diseases, the regulation of these signalling cascades by A20 may contribute to disease pathogenesis (TABLE 1). However, the multiorgan inflammation and perinatal lethality of A20-deficient mice largely prevents detailed studies of the functions of A20 in adult mice[14]. The recent development of mice with conditionally targeted *Tnfaip3* alleles (*Tnfaip3*$^{flox/flox}$ mice) has enabled lineage-specific and temporally controlled deletions of *Tnfaip3*, thereby facilitating studies of the functions of A20 in specific immune cell types.

B cell-mediated autoimmunity and lymphomas. The genetic association of *TNFAIP3* SNPs with SLE and of somatic *TNFAIP3* mutations with B cell lymphomas suggests that A20 might have important functions

in B cells. The expression of A20 is transcriptionally induced in B cells and may also be regulated at the post-translational level via cleavage by the paracaspase MALT1 (REFS 9,58,59). Insights into how A20 regulates B cell functions have emerged from studies of mice lacking A20 specifically in B cells (*Tnfaip3*$^{flox/flox}$ *Cd19*-Cre mice). Three independent groups have generated such mice[18,19,60], and the phenotypes of these mice are largely similar.

A20-deficient B cells display exaggerated NF-κB-mediated responses and are hyperresponsive to multiple stimuli, including lipopolysaccharide (LPS) and B cell receptor (BCR) and CD40 ligation. They produce higher levels of IL-6 following stimulation than wild-type B cells, which may account for the moderate increase in the number of A20-sufficient T cells in *Tnfaip3*$^{flox/flox}$ *Cd19*-Cre mice. As these mice age, a progressive increase in spontaneous B cell activation, the expansion of myeloid cell populations and plasma cell hyperplasia are observed[18,19]. In addition, the expression of A20 in B cells may be important for the development of marginal zone B cells[19,60].

Tnfaip3$^{flox/flox}$ *Cd19*-Cre mice spontaneously develop an autoimmune condition similar to SLE that is characterized by elevated numbers of plasma cells and germinal centre B cells, IgM and IgG autoantibodies, and renal immunoglobulin deposits[18,19,60]. These phenotypes are observed by 3–4 months of age, suggesting that A20 expression in B cells might prevent an early step in SLE pathogenesis. Although *Tnfaip3*$^{flox/flox}$ *Cd19*-Cre mice were not shown to develop renal failure, the severity of the clinical disease in these mice may be more apparent when they are bred onto lupus-prone backgrounds.

The increase in the number of germinal centre B cells in *Tnfaip3*$^{flox/flox}$ *Cd19*-Cre mice may be due to the resistance of A20-deficient B cells to FAS-mediated apoptosis[18]. This resistance may be mediated by the markedly elevated levels of NF-κB-dependent anti-apoptotic proteins, including B cell lymphoma X (BCL-X), in stimulated A20-deficient B cells. This finding was somewhat surprising given the increased sensitivity of A20-deficient fibroblasts and thymocytes to TNF-mediated programmed cell death[14]. Thus, the regulation of cell survival or death by A20 is likely to be dependent on both the cell type and the context[61].

Focused genetic studies — including deep sequencing of the human *TNFAIP3* gene — have revealed that a SNP in the 3′ non-coding region confers susceptibility to SLE by reducing A20 expression[31]. The spontaneous development of autoantibodies in heterozygous *Tnfaip3*$^{+/-}$ mice may thus genetically mimic the human condition (G. E. Hammer and A.M., unpublished observations). Furthermore, heterozygous *Tnfaip3*$^{flox/+}$ *Cd19*-Cre mice, which express reduced levels of A20 in B cells, also contain increased numbers of germinal centre B cells and develop autoantibodies[18]. In contrast to many mouse models of autoimmune disease in which homozygous null alleles are used to approximate human heterozygous SNPs, mice with spontaneous SLE-like disease owing to reduced A20 expression may provide a closer genetic approximation to the human condition.

The increased NF-κB signalling and enhanced survival of A20-deficient germinal centre B cells also provide a potential molecular underpinning for the role of A20 in preventing human B cell lymphomas. Several human B cell lymphoma subtypes are thought to arise from germinal centre B cells, and dysregulated NF-κB signalling is a known feature of the oncogenic mutations in these cells. A failure to eliminate germinal centre B cells could also lead to prolonged activation-induced cytidine deaminase (AID) activity and increased numbers of somatic mutations, including oncogenic mutations. Thus, the effects of exaggerated NF-κB signalling in causing increased expression of anti-apoptotic proteins and resistance to cell death suggest several ways in which A20 deficiency may contribute to the transformation of germinal centre B cells. Although mice bearing A20-deficient B cells have not yet been shown to spontaneously develop B cell lymphomas, future studies of potentially collaborative oncogenes may better define the tumour-suppressor functions of A20.

In summary, A20 regulates the homeostasis of germinal centre B cells and marginal zone B cells. In addition, important roles for A20 in other B cell subtypes may become apparent with the generation of mice that lack A20 expression in B cells at other stages of their development. Nevertheless, the studies to date indicate that precisely regulated A20 expression levels in B cells are essential for preventing B cell-mediated autoimmunity and B cell malignancies in mice and humans.

Dendritic cells and immune homeostasis. Early physiological evidence for the importance of A20 in regulating innate immune responses emerged from the observation that mice deficient in both A20 and recombination-activating gene 1 (RAG1; which is required for the development of B and T cells) develop myeloid cell-dependent inflammation, cachexia and perinatal lethality in a similar manner to A20-deficient mice[14]. In addition to restricting TNF-induced NF-κB signals, A20 has been shown to restrict NF-κB signals triggered by both TLRs and NLRs[14–16]. Indeed, A20-dependent restriction of TLR signals may be particularly important for immune homeostasis, as mice lacking both A20 and the TLR and IL-1R adaptor MYD88 (myeloid differentiation primary-response protein 88) exhibit markedly less inflammation and prolonged survival compared with mice lacking A20 only[17].

Given the central role for dendritic cells (DCs) in initiating immune responses, the functions of A20 in DCs have been investigated via the generation of mice with a DC-specific deletion of *Tnfaip3* (*Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice)[43,62] and via short hairpin RNA-mediated knockdown of *Tnfaip3* expression in DCs[63–65]. *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice rapidly develop profound disturbances in immune homeostasis, suggesting that A20 expression in DCs has major roles in regulating immune homeostasis, although these mice do not develop the cachexia and perinatal lethality observed in globally A20-deficient mice[14,43,62]. A20-deficient DCs are spontaneously activated in *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice and produce high levels of pro-inflammatory cytokines. Spontaneous activation of B cells, T cells and myeloid cells, as well as the

Apoptosis
A common form of cell death. Many physiological and developmental stimuli cause apoptosis, and this mechanism is frequently used to delete unwanted, superfluous or potentially harmful cells, such as those undergoing transformation.

Activation-induced cytidine deaminase
(AID). An enzyme that is required for two crucial events in the germinal centre: somatic hypermutation and class-switch recombination.

development of lymphadenopathy and splenomegaly, was observed in young (~4–6-week-old) *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice. DCs from these mice drive the rapid activation and proliferation of normal adoptively transferred naive T cells and prevent the induction of anergy and the deletion of antigen-specific T cells[43].

Although the ligands that trigger the activation of A20-deficient DCs under basal conditions are unknown, A20 is known to be a crucial regulator of TLR and IL-1R signals[15,17]. Breeding of *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice with *Myd88*$^{flox/flox}$ mice revealed that A20 restricts MYD88-dependent signals in DCs that lead to enhanced IL-6 expression and T cell clonal expansion under basal conditions[43]. Persistent DC and T cell activation in these *Tnfaip3*$^{flox/flox}$*Myd88*$^{flox/flox}$ *Cd11c*-Cre mice indicated that A20 also restricts MYD88-independent signals that drive these phenotypes[43]. Thus, even under basal conditions, DCs probably receive a variety of potentially stimulatory signals. A20 restricts these intracellular signals and prevents aberrant immune activation.

Surprising and potentially informative differences were observed in the phenotypes of two independently generated strains of *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice. In one strain, increased levels of B cell-activating factor (BAFF) and increased numbers of plasma cells were associated with elevated levels of serum immunoglobulin and double-stranded DNA-specific autoantibodies[62]. These mice developed nephritis, antiphospholipid syndrome and autoimmune arthritis, which are phenotypes that resemble human SLE. By contrast, the other strain of mice developed lymphocyte-dependent colitis, seronegative arthritis, enthesitis and ankylosing spondylitis. This phenotype mimics a stereotypical syndrome of human inflammatory bowel disease with associated arthritides[43]. One intriguing potential explanation for these phenotypic differences could reside in divergent luminal microbiota in the two colonies[66].

DC-based immunization strategies have been used for vaccinating humans, and approaches for optimizing DC-based immunization have focused on increasing the immunogenicity of transferred DCs. In this context, it is notable that A20-deficient DCs induce enhanced antigen-specific immune responses in *Tnfaip3*$^{flox/flox}$ *Cd11c*-Cre mice[43]. In addition, knockdown of *Tnfaip3* expression in bone marrow-derived DCs leads to enhanced antigen-specific immune responses that overcome regulatory T cell-mediated suppression[63] and result in enhanced immune responses to tumours[63,64] and HIV[65]. Hence, the attenuation of A20 expression in DCs could significantly improve the efficacy of DC-based vaccines.

A20, myeloid cells and autoimmunity. Macrophages share a number of innate immunostimulatory functions with DCs, although their localization and subspecialization distinguishes their physiological functions from those of DCs. Studies with mice in which expression of A20 was ablated in macrophages and granulocytes (*Tnfaip3*$^{flox/flox}$ *Lysm*-Cre mice) revealed that A20 expression in these cells is necessary for maintaining innate immune homeostasis[67]. Indeed, these mice developed spontaneous polyarthritis that was associated with collagen-specific autoantibodies and increased systemic and local cytokine production, reminiscent of human rheumatoid arthritis. This phenotype involved IL-6- and TLR4–MYD88-dependent signals, but was not ameliorated by broad-spectrum antibiotics or by the genetic elimination of adaptive immune cells or of TNF signals[67]. Thus, A20-deficient myeloid cells appear to drive erosive polyarthritis via IL-6- and TLR4-dependent signals. These mice also exhibited increased osteoclast function, which probably contributed to the osteoporotic changes. Hence, A20 expression in myeloid cells restricts signals that drive arthritis and osteoporosis. These findings provide pathophysiological insights into how A20 deficiency may lead to arthritis in mice and possibly humans.

In addition to rendering mice susceptible to inflammatory disease, selective loss of A20 in myeloid cells protected mice against influenza A virus infection[68]. This finding shows that enhanced A20-dependent inflammatory responses may be protective, rather than detrimental, during influenza A virus infection. It also provides a tantalizing hint that the evolutionary preservation of hypomorphic A20 expression and the associated increased susceptibility to inflammatory disease may have resulted from an evolutionary pressure to resist certain types of infection.

A20 and intestinal immune homeostasis. In addition to being expressed by immune cells, A20 is expressed by intestinal epithelial cells (IECs), suggesting that it might have a role in the response to microorganism-derived molecules in the intestinal lumen. A20 ablation in IECs (in *Tnfaip3*$^{flox/flox}$ villin-Cre mice) renders mice hypersensitive to dextran sulphate sodium (DSS)-induced colitis and TNF-induced inflammation[69]. The sensitivity of these mice to DSS-induced colitis is rescued by TNFR1 deficiency, suggesting that A20 may protect IECs from TNF-induced apoptosis during acute damage. *Tnfaip3*$^{flox/flox}$ villin-Cre mice exhibited more severe intestinal damage when rendered deficient of MYD88, consistent with previous studies showing that TLR-mediated sensing of commensal bacteria contributes to intestinal health[70]. The effect of A20 loss in IECs was most dramatic during intestinal recovery immediately after the removal of DSS treatment, suggesting a role for A20 in tissue repair. Complementary studies using enforced expression of A20 in IEC cell lines suggested that A20 mediates IEC tolerance to LPS[71]. Moreover, transgenic expression of A20 in IECs protects mice against DSS-induced colitis by supporting tight junctions between epithelial cells and preserving intestinal barrier function[72]. Hence, A20 expression in IECs may help to maintain mucosal immune homeostasis during episodes of gross inflammation.

A20 and skin pathology. The differentiation of keratinocytes is dependent on NF-κB signalling, and increased expression of NF-κB-dependent gene products is associated with skin inflammation and psoriasis. Although the pathophysiology of human psoriasis remains somewhat enigmatic, recent genome-wide association studies have strongly linked polymorphisms in *TNFAIP3* with susceptibility to psoriasis[38,39,49].

Dextran sulphate sodium (DSS)-induced colitis
A commonly used experimental model of colitis induced in mice by ingestion of the sulphated polysaccharide DSS. This model causes acute colonic epithelial damage and inflammation via unknown mechanisms.

Tight junctions
A belt-like region of adhesion between adjacent epithelial or endothelial cells that regulates paracellular flux. Tight-junction proteins include the integral membrane proteins occludin and claudin, in association with cytoplasmic zonula occludens proteins.

Ectodysplasin A receptor (EDAR). A member of the TNFR family that can activate NF-κB-, JNK- and caspase-independent cell signalling pathways. EDAR pathways are important for the development of ectodermal structures, such as hair follicles, sweat glands and teeth.

Mice in which A20 expression is specifically ablated in keratinocytes (*Tnfaip3*$^{flox/flox}$ *Krt14*-Cre mice) exhibited keratinocyte hyperproliferation, dishevelled hair and sebocyte hyperplasia[73]. These phenotypes were consistent with dysregulated ectodysplasin A receptor (EDAR)-mediated signalling, and *in vitro* studies suggested that A20 can inhibit EDAR-triggered NF-κB signalling in a manner independent of its DUB activity[73]. No spontaneous inflammation was observed in these mice, suggesting that A20 does not restrict basal pro-inflammatory signals in keratinocytes. Alternatively, cylindromatosis protein (CYLD), another DUB, might partly compensate for the loss of A20 expression and prevent exaggerated NF-κB signalling activity in these cells under steady-state conditions. *CYLD* is highly expressed in the skin, and mutations in this gene cause the skin disease cylindromatosis[74]. Compensation for the loss of A20 by CYLD would also be consistent with the ability of CYLD to remove ubiquitin chains from proteins similar to those de-ubiquitylated by A20 (REF. 75). Given the crucial roles of A20 in restricting NF-κB signals, challenging *Tnfaip3*$^{flox/flox}$ *Krt14*-Cre mice with pro-inflammatory ligands might reveal whether A20 expression by keratinocytes restricts the duration or intensity of skin inflammation.

A20 and endothelial cells. A20 was originally cloned from endothelial cells, and heterologous expression of A20 in endothelial cells suppresses inflammation in neighbouring tissues (including transplanted tissues)[76–78]. The role of A20 in endothelial cells might also contribute to links between human *TNFAIP3* polymorphisms and vascular disease; for example, SNPs in *TNFAIP3* are associated with increased atherosclerotic disease in patients with type 2 diabetes[44] (FIG. 1). In addition, a genome-wide screen for genes regulating susceptibility to atherosclerosis in apolipoprotein E-deficient mice identified a mutation in the coding sequence of *Tnfaip3*, and transgenic expression of A20 reversed this susceptibility[79,80]. One pathophysiological suggestion from these studies was that A20 expression in endothelial cells might suppress the expression of adhesion proteins for monocytes at sites of atherosclerotic plaque formation. Given the emerging evidence that type 2 diabetes and atherosclerosis are associated with chronic low levels of inflammation in humans, the anti-inflammatory functions of A20 may help to prevent these diseases. Endothelial cell-specific deletion of *Tnfaip3* may provide further insight into these issues.

The studies above indicate that the level of A20 expression in specific cell types influences the type of inflammation that occurs *in vivo* and the susceptibility to inflammatory diseases. Reduced A20 expression in several cell types may thus collaborate with additional genes or environmental triggers to define disease phenotypes (TABLE 1). Moving forward, analyses of mice with reduced or absent expression of A20 in other specific cell types (such as T cells) or combinations of cell types (such as both B and T cells) should provide new insights into disease pathologies and potential therapeutic strategies.

A20: biochemical mechanisms of action

A20 and the regulation of ubiquitin-dependent signalling. The A20 protein exhibits DUB, E3 ubiquitin ligase and ubiquitin-binding activities in cell-free and cellular systems (FIG. 2). Its DUB activity is mediated by an amino-terminal motif containing a catalytic cysteine residue

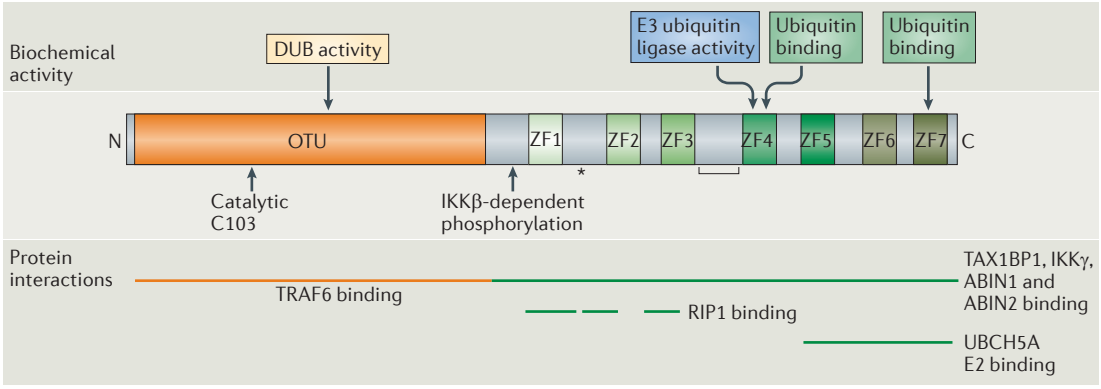

Figure 2 | **Biochemical characteristics of A20 protein function.** The de-ubiquitylating (DUB) activity of A20 is mediated by the catalytic cysteine at position 103 (C103) within the OTU domain. A20 also contains seven zinc fingers (ZFs), which mediate its E3 ubiquitin ligase activity (via ZF4) and its ubiquitin-binding activity. Indeed, A20 binds to ubiquitylated E2 enzymes such as UBCH5A via ZF4–ZF7, to K63-linked polyubiquitin chains via ZF4 (REF. 23) and to linear polyubiquitin chains via ZF7 (REFS 24,90,91). A20 also interacts with substrates such as receptor-interacting protein 1 (RIP1) via ZF1–ZF3 (with ZF1 being crucial for binding)[22,23], with E3 enzymes such as TNFR-associated factor 6 (TRAF6) via the OTU domain, and with ubiquitin-binding proteins such as TAX1-binding protein 1 (TAX1BP1), IκB kinase-γ (IKKγ), A20-binding inhibitor of NF-κB activation 1 (ABIN1) and ABIN2 via the ZF domain. Some of these latter interactions may occur through the mutual binding of A20 and the interacting protein to ubiquitin chains. The regions that mediate the interaction of A20 with the E3 enzymes RING-finger protein 11 (RNF11) and ITCH, as well as with itself, have not been clearly defined. In addition to their other functions, the C103 and ZF4 motifs have been shown to support the degradation of E2 enzymes[81]. A20 also undergoes post-translational modifications; for example, a site of A20 phosphorylation by IKKβ is indicated. Human A20 is cleaved by the paracaspase MALT1 at the site indicated by an asterisk. The site at which mouse A20 is cleaved has not been precisely determined, but the region where it is cleaved is indicated by a bracket.

(C103) in its OTU domain, whereas zinc finger 4 (ZF4) in the carboxy-terminal domain of A20 binds ubiquitin and supports E3 ubiquitin ligase activity[15,22,23,81,82]. These biochemical roles — considered together with emerging evidence of the crucial roles of ubiquitylation in regulating protein–protein interactions, protein stability and intracellular signals — suggest that A20 regulates intracellular signals by regulating ubiquitylated signalling complexes.

The attachment of polyubiquitin chains to signalling proteins stimulates the recruitment of ubiquitin-binding proteins. The attachment of K48-linked polyubiquitin chains has long been known to target proteins for proteasomal degradation, whereas polyubiquitin chains linked via other lysine residues, such as K63-linked chains, can recruit downstream signalling proteins, thereby propagating signals. The DUB activity of A20 cleaves anchored K63-linked polyubiquitin chains, so this biochemical function may help to restrict ubiquitin-dependent signals. During TNF signalling, the conjugation of receptor-interacting protein 1 (RIP1; also known as RIPK1) with K63-linked polyubiquitin chains leads to the recruitment of the signalling molecules TAK1-binding protein 2 (TAB2), TGFβ-activated kinase 1 (TAK1) and IκB kinase-γ (IKKγ; also known as NEMO) to form a complex with RIP1. This in turn leads to the phosphorylation of NF-κB inhibitor-α (IκBα) and the activation of NF-κB[4]. The increased ubiquitylation of RIP1 in stimulated A20-deficient cells indicates that A20 is crucial for restricting RIP1 ubiquitylation[22] (FIG. 3). In this context, the DUB activity of A20 probably restricts TNF-induced NF-κB signalling by removing K63-linked polyubiquitin chains from RIP1.

The assembly of K63-linked polyubiquitin chains also supports TNFR-associated factor 6 (TRAF6)-mediated activation of NF-κB during IL-1R and TLR signalling, and the removal of these chains by A20 may be a mechanism by which A20 restricts TLR signals[4,15,17,20,83,84] (FIG. 3). Furthermore, A20-restricts the ubiquitylation of RIP2 (also known as RIPK2) during NOD signalling[16] and the ubiquitylation of TBK1 and IKKε (also known as inducible IKK) during double-stranded RNA-induced signalling[85], which suggests other potential targets for the DUB activity of A20 (FIG. 3). A20 also restricts NF-κB signalling triggered by the T cell receptor (TCR), BCR and CD40, so A20 may also de-ubiquitylate targets in these pathways[9,18,19,86]. These studies are consistent with the notion that the DUB activity of A20 restricts cellular activation signals by cleaving activating K63-linked polyubiquitin chains from target signalling proteins.

In addition to limiting ubiquitylation by cleaving polyubiquitin chains, A20 has been shown to inhibit ubiquitin chain synthesis by interfering with E2–E3 binding[81]. A20 inhibits the interactions of the E2 enzymes UBCH5C and UBC13 with TRAF2 and cellular inhibitor of apoptosis (cIAP) proteins, which are RING-finger E3 ubiquitin ligases[81]. These findings suggest that A20 could inhibit multiple E2–E3 combinations, and hence a broad array of ubiquitylation events. Whether A20 restricts substrate ubiquitylation by cleaving polyubiquitin chains or by inhibiting ubiquitin ligase activity, the restriction of the ubiquitylation of signalling proteins appears to be an important mechanism by which A20 regulates immune signals.

Several questions concerning the DUB activity of A20 remain. First, A20 selectively cleaves unanchored K48-linked polyubiquitin chains (that is, free chains that are not covalently attached to signalling proteins), but not K63-linked polyubiquitin chains, to monoubiquitin[20,21]. However, A20 removes K63-linked polyubiquitin chains from TRAF6. It does this without disassembling the chains, but rather by cleaving the entire polyubiquitin chain at the ubiquitin–TRAF6 junction[20]. Although recombinant A20 exhibits these specificities *in vitro*, the physiological targets of A20 in cells are incompletely defined, and it may collaborate with other proteins to define target specificity. Second, the C103 motif of A20 appears to be required for A20 to destabilize E2 enzymes and inhibit E2–E3 interactions[81]. However, it remains to be determined how the DUB activity of A20 would support the ubiquitylation and degradation of E2 enzymes. Another OTU domain-containing protein, otubain 1, uses residues other than the catalytic cysteine to inhibit the function of E2 enzymes, so it is possible that A20 utilizes its OTU domain in this manner[87]. Third, it is currently unclear how the DUB activity of A20 overlaps with that of other DUBs (such as CYLD) that target similar substrates. Such DUBs could remove different types of ubiquitylation chain from common substrates. Alternatively, distinct DUBs could target the same ubiquitylated substrates in distinct cell types, after distinct stimuli or at different times after ligand binding. Future biochemical studies aimed at better understanding how different ubiquitin chains are targeted for deubiquitylation, together with genetic studies aimed at understanding the epistatic relationships between distinct DUBs, could begin to shed light on this issue. Finally, as the DUB activity of A20 clearly depends on its catalytic C103 residue, the physiological role of this DUB activity could be clarified by using knock-in mice expressing A20 proteins mutated at C103.

A20 also regulates cell death. The roles of A20 in regulating cell survival are complex, and may be cell type specific, as A20 inhibits TNF-induced death in fibroblasts but supports FAS-mediated death in activated B cells[14,18]. The anti-apoptotic functions of A20 are not likely to be secondary to decreased NF-κB signalling, as more A20-deficient fibroblasts die in the presence of cycloheximide (which abrogates NF-κB-dependent protein synthesis), and NF-κB-dependent genes tend to have anti-apoptotic functions. The molecular mechanisms by which A20 inhibits apoptosis are incompletely understood but may involve A20 de-ubiquitylating an activated, ubiquitylated form of caspase 8 (REF. 88). By contrast, the mechanism by which A20 supports cell death in activated B cells may involve A20 inhibiting NF-κB-dependent expression of the anti-apoptotic protein BCL-X. The cell death-regulating functions of A20 add an important additional dimension to the A20-mediated regulation of pro-inflammatory NF-κB

Cellular inhibitor of apoptosis
A family of proteins (comprising cIAP1, cIAP2, XIAP and NAIP) that contain a BIR domain and that can act as E3 ubiquitin ligases and as inhibitors of caspases.

signals, as the survival of immune cells and stromal cells has distinct effects in inflammatory, autoimmune and infectious diseases. How the anti-inflammatory and cell death-regulating functions of A20 are integrated *in vivo* and in specific cell types will be an important area of future investigation.

In addition to the N-terminal DUB motif, the C-terminal domain of A20 probably has crucial roles in regulating ubiquitin-dependent signals (FIG. 2). The C-terminal domain contains seven zinc fingers, and early studies showed that enforced expression of this domain inhibits NF-κB signalling[89]. More recent studies

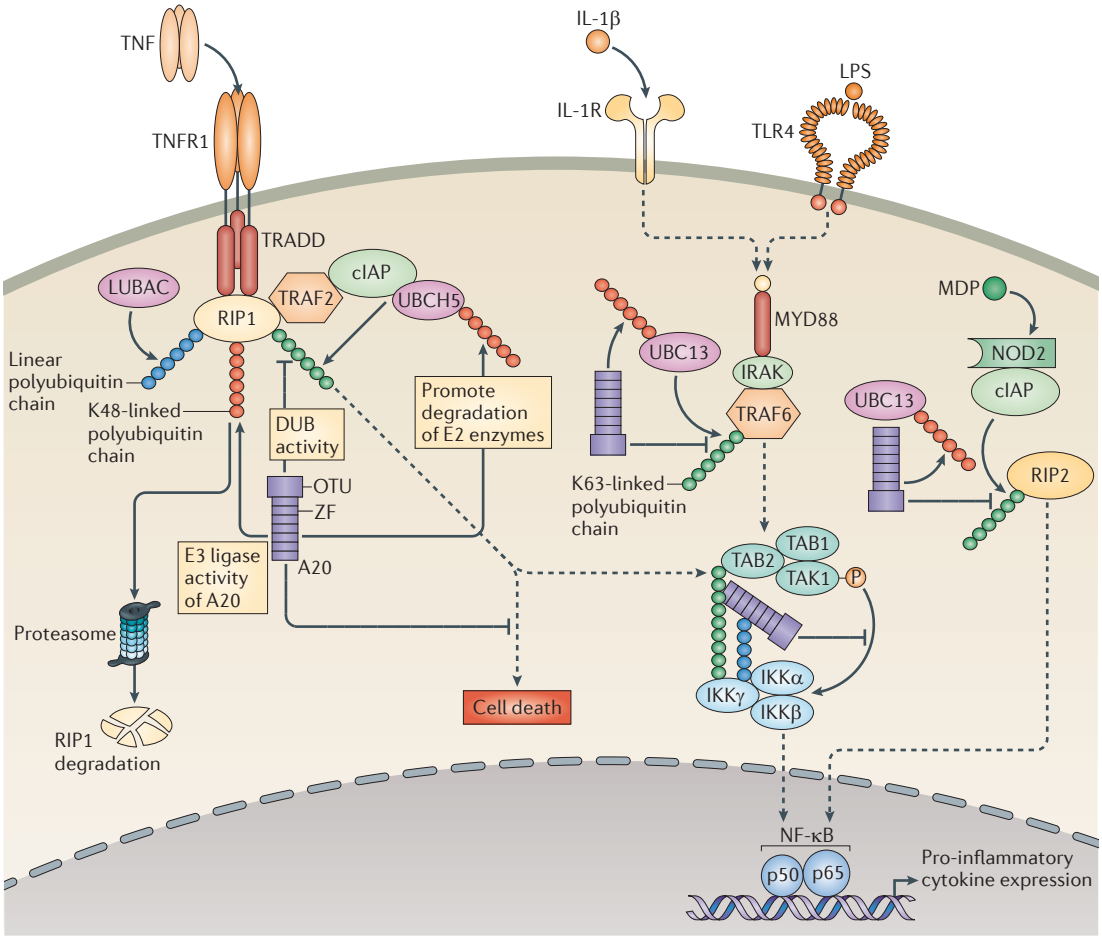

Figure 3 | **A20-dependent regulation of ubiquitin-dependent signalling pathways.** A20 regulates multiple ubiquitin-dependent innate immune signalling cascades, including those downstream of tumour necrosis factor receptor 1 (TNFR1), interleukin-1 receptor (IL-1R), Toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain (NOD) proteins. During these signalling cascades, E2 enzymes, such as UBCH5 and UBC13, and E3 ligases, such as cellular inhibitor of apoptosis (cIAP) proteins, TNFR-associated factor 6 (TRAF6), and perhaps TRAF2, collaborate to build polyubiquitin chains. These polyubiquitin chains can be K48-linked, K63-linked or joined by other linkages, and are attached to substrates such as receptor-interacting protein 1 (RIP1), TRAF6, IκB kinase-γ (IKKγ) and RIP2. A distinct enzymatic complex known as LUBAC (linear ubiquitin chain assembly complex) builds linear polyubiquitin chains on RIP1 and IKKγ[116,117]. In addition, unanchored ubiquitin chains (that is, chains that are not covalently attached to signalling proteins) are integral to these cascades. A20 may regulate these signalling complexes by cleaving K63-linked ubiquitin chains from RIP1, TRAF6 and/or IKKγ through the de-ubiquitylating (DUB) activity of its OTU domain. A20 can also regulate these signalling pathways by supporting the degradation of the E2 enzymes UBCH5 and UBC13, thereby inhibiting E3 ligase activity that is dependent on these E2 enzymes. Finally, A20 can build K48-linked polyubiquitin chains on RIP1, which leads to its degradation. In addition, the ZF4 and ZF7 domains of A20 have been shown *in vitro* to bind to K63-linked ubiquitin chains and linear ubiquitin chains, respectively, on IKK complexes and TNFR complexes and may use these interactions to facilitate the inhibition of these signalling complexes. Although it is not shown in the figure, the ubiquitin-binding activities of A20 might also compete with those of other ubiquitin-binding proteins and/or help A20 to function as an adaptor protein for other regulators, such as A20-binding inhibitor of NF-κB activation 1 (ABIN1), ITCH, RING-finger protein 11 (RNF11) or TAX1-binding protein 1 (TAX1BP1). In addition to restricting TNFR, IL-1R, TLR and NOD signals, A20 regulates signals triggered by the T cell receptor[9] (not shown) and CD40 (not shown). Finally, A20 restricts TNF-induced apoptosis, possibly by restricting the ubiquitylation of caspase 8 (REFS 14,88) (not shown). IRAK, IL-1R-associated kinase; LPS, lipopolysaccharide; MDP, muramyl dipeptide; NF-κB, nuclear factor-κB; TAB, TAK1-binding protein; TAK1, TGFβ-activated kinase 1; TRADD, TNFR1-associated death domain protein; ZF, zinc finger.

revealed that ZF4 functions as an E3 ligase, supporting the K48-linked ubiquitylation and proteasomal degradation of RIP1 (REF. 22) (FIG. 3). The evidence that A20 both disassembles K63-linked polyubiquitin chains and builds K48-linked chains suggests that these two enzymatic functions would result in the de-activation and degradation of RIP1, thereby terminating the role of this signalling molecule in propagating TNF-induced NF-κB signalling. These findings also raise interesting questions regarding whether and how these two biochemical activities might be coordinated. For example, K63-linked polyubiquitin chains might be exchanged for K48-linked chains in a synchronized ubiquitin-editing process on a single lysine residue in RIP1 (FIG. 3).

The ZF4 motif of A20 also binds directly to ubiquitin chains and supports the binding of A20 to ubiquitylated E2 enzymes[23]. Combined with the ability of A20 to bind to RIP1, this interaction could help to explain how ZF4 supports the ubiquitylation of RIP1. Interestingly, the ZF4 motif was also shown to be required for A20-mediated inhibition of E2–E3 interactions in cells[81], implying that the ZF4 motif can inhibit ubiquitylation. Thus, the ZF4 motif might support or restrict ubiquitylation in distinct contexts. It also remains possible that the ZF4 motif of A20 performs an E3 ubiquitin ligase-independent function. As with the catalytic C103 residue, the physiological importance of ZF4 in regulating various cell signals and immune functions should become clearer with genetic interrogation of this motif via targeted mutation of ZF4.

Recent experiments indicate that the ZF7 motif of A20 also regulates NF-κB signalling by directly restricting the TAK1-dependent activation of IKKγ[24]. This activity is mediated via the binding of the ZF7 motif to linear ubiquitin chains[90,91] (FIG. 3). The presence of truncated mutants of A20 lacking ZF7 in B cell lymphomas reinforces the importance of this motif. Thus, A20 may use several biochemical activities to regulate ubiquitylated signalling complexes. These observations raise interesting questions regarding how the functions of the C103, ZF4 and ZF7 motifs collaborate to restrict NF-κB signals. For example, in other proteins, spatially separated ubiquitin-binding motifs recognize distinct conformations of polyubiquitin chains through multipartite interactions[92,93], so the multiple ubiquitin-binding motifs in A20 may collaborate to recognize substrates bearing specific ubiquitin chains. In addition, the other five zinc fingers of A20 may have important functions complementary to those of ZF4 and ZF7. The complex biochemical mechanisms by which A20 regulates ubiquitylated signalling proteins provide clues as to how ubiquitin-dependent signals are generally regulated and also suggest novel approaches for the therapeutic manipulation of these signalling cascades through the targeting of these motifs. For example, selective inhibition of the C103-based DUB activity or the ZF4- or ZF7-based ubiquitin-binding motifs of A20 might preferentially affect the regulation of TNF-induced versus TLR-induced NF-κB signals. In addition, selective inhibition of A20 interactions with K63-linked versus linear ubiquitin chains may favour distinct ubiquitin-dependent signals.

A20 and the collaborative regulation of ubiquitin-dependent signals. Several lines of evidence suggest that A20 may collaborate with other proteins to regulate cell activation and survival signals. A20 physically interacts with several proteins that bind to ubiquitin, including A20 itself, A20-binding inhibitor of NF-κB activation 1 (ABIN1; also known as TNIP1), ABIN2 (also known as TNIP2), TAX1-binding protein 1 (TAX1BP1) and IKKγ. In addition, A20 interacts with various proteins that function directly or collaboratively as E3 enzymes, such as TRAF2, TRAF6, cIAP1, cIAP2, ITCH and RING-finger protein 11 (RNF11)[12,13,94–98] (FIG. 2). The overlapping effects on TNF signalling in cells lacking several of these proteins suggest that these interactions may be physiologically relevant. Complexes containing various combinations of these proteins could recognize diverse ubiquitylated signalling proteins and modify them in tandem with E2 enzymes. How each of these proteins collaborates with A20 to regulate cell signals and immune responses is poorly understood, but biochemical and genetic tools are emerging to address these questions.

ABIN1 was identified as a binding partner of A20 via a yeast two-hybrid experiment, and enforced expression of ABIN1 suppresses TNF-induced NF-κB signals[12]. ABIN1 appears to cooperate with A20 to inhibit TRAF3-dependent signals that lead to type I IFN production[85]. ABIN1 deficiency in mice leads to late embryonic lethality and TNF-dependent cell death, demonstrating that ABIN1, similarly to A20, preserves perinatal survival and restricts TNF-induced cell death[14,99,100]. ABIN1-deficient mice that survive to adulthood develop spontaneous autoimmunity and inflammation, a phenotype grossly resembling that of A20-deficient mice[100]. Thus, ABIN1 and A20 are both crucial for preserving immune homeostasis in adult mice. Part of this anti-inflammatory function may be due to the restriction by ABIN1 of MYD88-dependent TLR signalling and NOD2 signalling — functions that are again shared with A20 (REFS 16,17,100). ABIN1 has also recently been shown to restrict the TLR-induced expression of CCAAT/enhancer-binding protein (C/EBP)-dependent genes rather than NF-κB-dependent genes[101]. Hence, ABIN1 binds to A20 in cells, and ABIN1 deficiency resembles A20 deficiency in several regards. However, it remains to be determined whether and how A20 and ABIN1 collaborate to regulate cell signalling.

Similarly to polymorphisms of *TNFAIP3*, polymorphisms of *TNIP1* (the gene encoding ABIN1) are strongly linked with susceptibility to SLE[28,102] and psoriatic arthritis[103]. Attempts to establish epistatic relationships between human SNPs in the genes encoding A20 and ABIN1 have not yet been successful, but the power to detect such associations may be limited by clinical variables and genetic haplotypes. Additional similarities between the functions of A20 and ABIN1 in human cells are suggested by the presence of somatic mutations in *TNIP1* in human lymphomas[104,105]. Thus, ABIN1 may share the tumour-suppressive function of A20 in human lymphocytes.

Little is known about the biochemical functions of ABIN1. Unlike A20, ABIN1 does not have recognizable DUB or ubiquitin ligase motifs and is not known to exhibit enzymatic ubiquitin-modifying functions. As ABIN1 binds to polyubiquitin chains with high affinity, one model for how A20 and ABIN1 collaborate is that ABIN1 binds to both ubiquitylated signalling proteins and A20, thereby bringing A20 into close proximity with its ubiquitylated targets[101,106]. This adaptor function could provide a biochemical underpinning for the biological similarities observed in A20- and ABIN1-deficient animals, as well as for the correlation of SNPs in the genes encoding A20 and ABIN1 with susceptibility to SLE, psoriasis and other diseases[28,38,102–105]. An alternative model for ABIN1 function is suggested by the observation that ABIN1 and IKKγ contain similar ubiquitin-binding motifs[94,99,106]. ABIN1 might thus compete with IKKγ for binding to polyubiquitin chains in activated signalling complexes. As the recruitment of IKKγ is essential for the propagation of most canonical NF-κB signals, ABIN1-mediated displacement of IKKγ from signalling complexes could inhibit NF-κB signalling. A20 also binds to ubiquitin chains with high affinity, although A20 uses ubiquitin-binding motifs that are distinct from the motif used by ABIN1. Whether and how A20 may also compete with IKKγ for ubiquitin binding, and whether such competition involves ABIN1, remains to be determined. Further definition of the types of ubiquitin chain involved and the specific functions of these ubiquitin-binding motifs are needed to address these issues.

In addition to binding to ABIN proteins, A20 interacts with various other proteins, including TRAF2, TRAF6, ITCH, RNF11 and TAX1BP1 (REFS 12,13,94–98) (FIG. 2). The phenotypes of mice and cells lacking these proteins partially overlap with the phenotypes of A20 and ABIN1 deficiency[14,100,107–111], and TAX1BP1, RNF11 and ITCH have been shown to support A20 function during TNF and TLR signalling[85,99,111,112]. Intriguingly, all of these proteins bind to ubiquitin and/or help to build ubiquitin chains. It remains to be determined how A20 interacts with these proteins, whether they collaborate in a single complex or in multiple complexes, and how they regulate ubiquitylated signalling complexes. Nevertheless, it is likely that A20 functions in larger ubiquitin-editing protein complexes that have a high degree of specificity and regulation.

Therapeutic implications of A20 biology

The broad (and growing) links between polymorphisms in *TNFAIP3* and inflammatory and malignant diseases suggest that aberrant A20-dependent signals are pathogenic in many conditions. Loss of A20 function is directly implicated in the pathogenesis of human lymphomas, and reduced A20 function or expression has been linked with susceptibility to SLE in humans. Reduced or absent expression of A20 also causes spontaneous inflammatory and autoimmune diseases in mice. The direct correlation of hypomorphic A20 expression with both human and experimental diseases argues that such mouse models are excellent models for understanding the role of A20 in human disease. Moreover, this correlation suggests that increasing the expression and/or function of A20 is a promising therapeutic strategy. One indication of the potential of A20 as a buttress against destructive inflammation is the resistance to experimental colitis observed in mice with enforced A20 expression in IECs, which has no apparent effect on normal intestinal function[72]. In the case of B cell lymphomas, re-expression of A20 is directly cytotoxic for human B cell lymphomas bearing *TNFAIP3* mutations but does not affect cell lines with intact A20 loci and normal NF-κB expression[51]. The challenge for finding effective therapies is to devise strategies that enhance A20 expression. As current therapeutic approaches based on antibodies or small molecules are more effective in reducing the functions of proteins rather than restoring them, targeting proteins that inhibit A20 function could be a more tractable, if somewhat indirect, therapeutic approach. Recent studies have identified microRNAs that target *TNFAIP3* mRNA, so inhibition of these microRNAs could enhance A20 expression[113–115]. Regardless of the approach, the genetic links between SNPs in *TNFAIP3* and rheumatoid arthritis, SLE, psoriasis, coeliac disease, Crohn's disease, type 2 diabetes, atherosclerosis and lymphomas — as well as the potential use of A20 modulation in vaccine development and transplantation[63–65,76–78] — suggest that approaches that either enhance or suppress A20 expression may be promising arenas for therapeutic development.

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Competing interests statement
The authors declare no competing financial interests.