

Theme Issue Article

Resolution of inflammation: Intracellular feedback loops in the endothelium

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Summary

Timely termination of the inflammatory reaction is equally important as its elicitation, since a persistent or exaggerated response may lead to detrimental effects in the affected tissues and organs. Therefore, and in accordance with the complex and highly coordinated activation phase, negative regulatory mechanisms have evolved which function on multiple levels to ensure the appropriate termination of the inflammatory response. This review will focus on the mechanisms that are operative in en-

dothelial cells to shut down the activity of specific signaling pathways and transcription factors that have been activated in response to pro-inflammatory mediators, and provide evidence that the stage for resolution is set already early in the activation phase of the inflammatory response. The elucidation of these feedback mechanisms is of importance for the understanding of acute versus chronic inflammation, and for novel strategies for therapeutic intervention.

Keywords

Endothelial cells, signal transduction, inflammation

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Introduction

Inflammation, a response to traumatic, toxic, infectious, ischaemic, or autoimmune injury, was characterized already approximately 2000 years ago by the four cardinal signs rubor, tumor, calor, and dolor by the Roman Celsus. Virchow added the fifth sign, *functio laesa*. Since then, our understanding of the inflammatory response has extended deeply into the cellular and molecular levels, but these early depiction remains still valid. Equally legitimate remains Galan's view of inflammation as an originally "beneficial response" (1) that, only when exaggerated, turns detrimental. Chronic inflammation is today considered a hallmark of many diseases, including diseases affecting the skin (psoriasis, eczema), intestine (Crohn's disease, ulcerative colitis), central nervous system (Alzheimer, multiple sclerosis), rheumatoid arthritis, asthma, arteriosclerosis, preeclampsia and juvenile diabetes (2–6).

The inflammatory response comprises a complex interplay between different cells (such as endothelial cells, neutrophils, monocytes), cytokines (including chemoattractants and growth factors), cell adhesion molecules, as well as low-molecular weight mediators (e.g. prostaglandins, leukotriens, phospholi-

pids). As such, also its resolution has to occur on different levels. A recent series of reviews ("Dampening Inflammation"[7]), has given an illustrative overview over the events that take place during the switch from the initiation to the resolution phase of the inflammatory response. Briefly, switches occur both on the level of proinflammatory mediators, e.g. from prostaglandins and leukotriens to anti-inflammatory lipoxins and products of the omega-3-polyunsaturates fatty acids (resolvins and protectins), and on the cellular level, from the initial invasion by granulocytes, their apoptosis, and subsequent clearance by monocytes. The latter also secrete anti-inflammatory and reparative mediators, e.g. transforming growth factor(TGF)- β , and the episode is completed by their exit through the lymphatic system (8).

The endothelium is one of the central players in the inflammatory reaction, as it forms a barrier between the blood stream containing immune cells and the underlying tissue where, e.g. an infection takes place. Evidently, everything that is exchanged between these two compartments needs to pass through the endothelium. It has become common knowledge that in the regulation of these processes endothelial cells (EC) are not just passive bystanders but play a very active part. In response to pro-inflammatory mediators, EC express genes encoding cytokines,

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growth factors and chemokines (e.g. interleukin[IL]-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, RANTES), and cell adhesion molecules (e.g. E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule[VCAM]-1) that mediate the chemoattraction, tethering, rolling, adhesion and transmigration of immune cells at the respective site. The response can be tuned by additional factors, such as the angiopoietin system recently described in this regard (9). Moreover, it is important to note that pro-inflammatory stimulation evokes responses that exceed the inflammatory response *per se*, and include expression of genes associated with aspects of apoptosis/survival (A20, A1, inhibitor of apoptosis protein family members), coagulation (tissue factor, plasminogen activator inhibitor-1), proliferation, migration/cell and tissue dynamics, metabolic changes, and others (e.g. inducible nitric oxide synthase, matrix metalloproteinases, pentraxins) (10–12).

Within this overall picture of the inflammatory response, one important point is the view that “the beginning programs the end”, i.e. that already at an early phase of the inflammatory response, the stage for its resolution is set. This route to termination appears to be an active process, as opposed to a simple cessation due to e.g. consumption of crucial components. This view seems to hold true also for the events taking place in the endothelium: Among the plethora of pro-inflammatory genes that are expressed in response to mediators such as IL-1, tumor necrosis factor- α (TNF α) or lipopolysaccharide (LPS), a substantial number with inhibitory function is up-regulated (11). The nature of these genes suggest that they specifically inhibit certain transcription factors, signaling pathways, and the associated cellular functions. This review will focus on these inhibitory mechanisms that are operative (mostly) on the level of gene expression in endothelial cells.

NF- κ B signaling and feedback mechanisms

The analysis of genes that are expressed in EC in response to pro-inflammatory mediators such as IL-1, TNF α , and LPS has revealed that the vast majority is regulated through NF- κ B. After binding to their respective receptors, signals are propagated first through the recruitment of receptor-specific cytoplasmic signaling proteins (such as receptor-interacting proteins [RIP]), TNF α receptor (TNFR) associated factors (TRAF) -1 and -2 for the TNFRs, TRAF6 for IL-1 receptor, and TRAF6 and myeloid differentiation factor 88 (MyD88) for the Toll-like receptors (TLRs), to different mitogen activated protein (MAP) kinases (e.g. MEKK1, -2, -3, TAK1), and further to the NF- κ B signaling complex. At this protein complex, consisting mainly of the inhibitor of NF- κ B (I κ B) kinases (IKK) -1 and -2, as well as the non-catalytic subunit IKK γ (NEMO), these different signaling pathways converge and proceed on a common track towards phosphorylation by the IKKs of I κ B α , the predominant inhibitor of NF- κ B. The latter forms a complex with NF- κ B, thereby retaining the transcription factor in the cytoplasm. Phosphorylation of I κ B α occurs on two serine residues, Ser 32 and 36, followed by recognition by the β -TrCP-like component of an E3 ubiquitin ligase complex (Skp1/Cul1/ROC1/F-box protein FWD1), ubiquitination and degradation via the 26S proteasome

(13). Thereby the nuclear localization signal in NF- κ B becomes exposed, allowing its translocation to the nucleus. An alternative pathway originates from e.g. the lymphotoxin- β receptor and involves NF- κ B-inducing kinase (NIK) and IKK-1, leading to the proteolytic degradation of p105, the precursor of the NF- κ B1/p50 subunit. In addition, phosphorylation of NF- κ B/RelA on several residues mainly in the transactivation domain has been demonstrated to be essential for its activity (for reviews, see [10, 14–16]).

Feedback on the level of gene expression

The first described feedback circuit in the NF- κ B system is the NF- κ B-dependent resynthesis of I κ B α that follows its proteolytic degradation. Newly synthesized I κ B α can lead to the disengagement of NF- κ B from its DNA binding site and shutdown of the transcription factor's activity (17). We and others have demonstrated that ectopic expression of I κ B α , e.g. using recombinant adenoviral vectors, leads to inhibition of a broad spectrum of inflammatory responses in EC, as characterized by down-regulation of IL-1, IL-6, IL-8, VCAM-1, pro-coagulant activity (18), as well as monocyte adhesion and transmigration (19). Using animal models (of restenosis), we could also demonstrate the relevance of this finding *in vivo* (20, 21). The same adenoviral constructs were also shown to be effective in other settings, including rheumatoid arthritis, airway inflammation (22, 23), and postinfarct remodeling in the heart (24). These experiments support the notion that in a situation where the introduction of genetic material is possible (e.g. balloon angioplasty, or the genetic engineering of xenografts), the ectopic expression of a feedback mediator could be utilized to prevent at least parts of the inflammatory response of the endothelium.

The anti-apoptotic gene A20 represents another example. A20 is a NF- κ B-inducible gene in EC (25), and when overexpressed, it inhibits NF- κ B activation and pro-inflammatory gene expression (26). A20 encodes a protein with dual function in regard to ubiquitination: First, it acts as a deubiquitinating enzyme to remove the activating K63-linked ubiquitin chains from RIP1, and second, it catalyzes the attachment of K48-linked ubiquitin, thereby targeting RIP1 for proteosomal degradation (27). Furthermore, it inhibits NF- κ B activation on the level of the IKKs by deubiquitinating IKK γ involving A20-binding inhibitor of NF- κ B (ABIN-1), which physically links A20 to IKK γ (28).

A20 is not the only deubiquitinating enzyme that controls NF- κ B signaling. The tumor suppressor CYLD, the loss of which causes a benign human syndrome called cylindromatosis (29), specifically removes K63-linked polyubiquitin chains from TRAF2, -6, and IKK γ (30–32). Expression of CYLD itself is induced by proinflammatory stimuli (TNF α , IL-1, bacteria) and is NF- κ B dependent (33). In addition to its positive regulation on the transcriptional level, the deubiquitinating function of CYLD is in turn suppressed by phosphorylation, which involves the IKKs (34).

SINK (35) is a member of a small protein family that shares features of serine/threonine kinases; however, it contains only five of the 12 subdomains found in most protein kinases and lacks a conserved ATP-binding site. This suggests that it may act as a dominant-negative molecule. Indeed, SINK has been found to interact with the p65 subunit of NF- κ B and to inhibit its

cAMP-dependent protein kinase-mediated phosphorylation that is required for transactivation. Another homologue, SINK-homologous serine/threonine kinase, serves a similar function (36). SINK is TNF α -inducible, at least in human embryonic kidney 293 cells, and it remains to be tested whether it, or other members of its family such as C8FW, are operative in EC.

Regulation of IKK activity

The activity of the IKK complex is regulated through distinct phosphorylation events. Phosphorylation of two residues in the activation loop is necessary for IKK activity. However, at later stages additional phosphorylation in the helix-loop-helix (HLH) domain occurs, preventing interaction of the HLH with the kinase domain, an event necessary for IKK activity (37). Therefore, a series of regulatory phosphorylations first activates, and then terminates IKK activity. However, whereas the activating phosphorylation is thought to occur by auto- or cross-phosphorylation within the IKK complex, it is presently unknown how and by which kinases the inhibitory phosphorylation takes place.

Generation of anti-inflammatory mediators

NF- κ B-dependent expression of cyclooxygenase-2 (COX-2; prostaglandin synthase 2) leads to the generation of pro-inflammatory prostaglandins, mainly prostaglandin E. At later stages COX-2 directs the synthesis of anti-inflammatory cyclopentane prostaglandins. These molecules, e.g. 15dPGJ₂, inhibit the activity of IKK2 through modification of a critical cysteine residue in the activation loop, suggesting a role for these mediators in the resolution of inflammation (38). However, the mechanisms that mediate the switch in the synthesis from pro- to anti-inflammatory prostaglandins by COX-2 remain to be established.

Inhibition of NF-AT

The transcription factor nuclear factor of activated T cells (NF-AT) is expressed, besides in its name-giving cell type, also in EC (39). Its activation involves the calcineurin signaling pathway and results in nuclear translocation of the transcription factor. Down Syndrome Critical Region 1 (DSCR1; calcipressin-1) is an inhibitor of the calcineurin signaling pathway and is induced in EC in response to both inflammatory and angiogenic stimulation, resulting in inhibition of NF-AT (40). Therefore, its inducible expression parallels the situation on the NF- κ B/I κ B α regulatory circuit, and can be predicted to shut down specifically those genes that are dependent on NF-AT, including the associated biological responses. Candidate genes include VCAM-1, intercellular adhesion molecule-1 and tissue factor, which can be regulated both by NF-AT and NF- κ B (39, 41, 42).

Feedback on the level of mRNA stability

Zinc finger protein 36 (Zfp36) is expressed in EC in response to inflammatory mediators, as well as in many other cell types following a broad range of stimuli. Zfp36 has been demonstrated to increase mRNA decay through binding to AU-rich elements (AUUUA) in the 3'-region of many short-lived mRNAs, resulting in their (probably indirect) deadenylation and degradation. Examples for published target mRNAs that are expressed in EC include TNF α , granulocyte-macrophage colony-stimulating factor, COX-2, as well as Zfp36 itself (43–45). Additional targets have been identified in fibroblasts (46). In support of its function, a polymorphism has been found in its coding region that is significantly associated with rheumatoid arthritis (47). Phosphorylation of Zfp36 by the kinase MK2 (which is in turn acti-

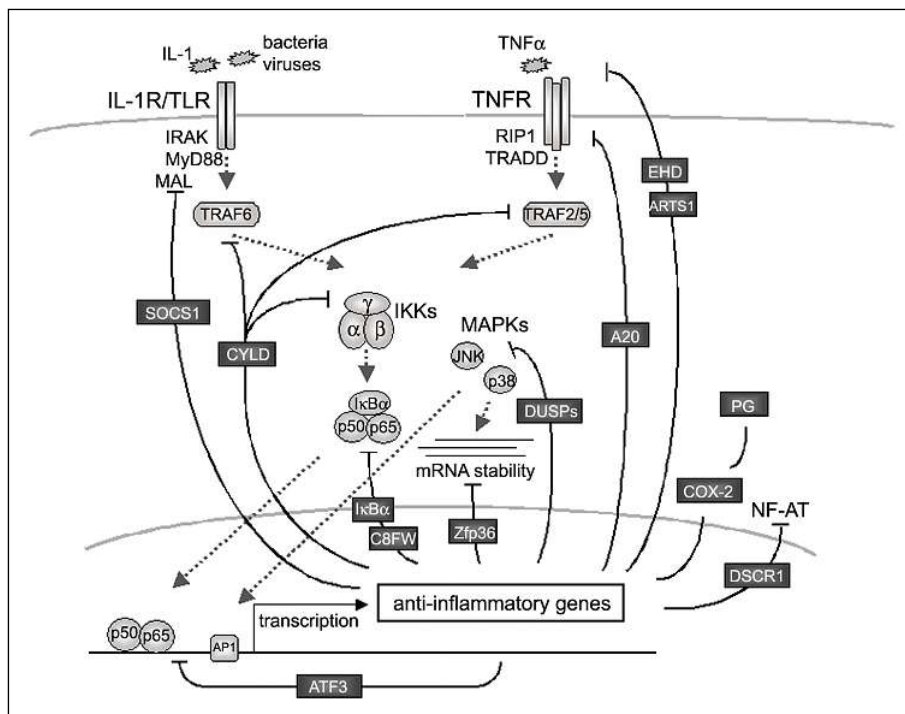


Figure 1: Examples of cognate and potential negative feedback mechanisms in endothelial cells: Following stimulation with pro-inflammatory agents, signaling pathways including, among others, NF- κ B and MAP kinase pathways are activated (dotted lines) that lead to the expression of both pro- and anti-inflammatory genes. The latter (solid lines) act as feedback inhibitors towards these pathways, and exert their action on different levels. Examples include inhibition on the level of gene expression (by ATF3), direct inhibition of a transcription factor (through I κ B α , SINK), inhibition of signaling pathways (DUSPs, DSCR1, SOCS1, CYLD, A20), generation of inhibitory prostaglandins (via COX-2), receptor modification/recycling (ARTS1, EHD) and mRNA destabilization (Zfp36).

vated by p38) increases its stability and its binding to 14–3–3 proteins (48). Further, Zfp36 action depends on its (indirect) association with a specific microRNA, miR-16 (49), which contains sequence-complementarity to AU-rich elements. This opens an emerging aspect of EC research, namely the role of microRNAs in the regulation of the inflammatory response.

TLR signaling

The nine cognate TLRs recognize a wide range of pathogen-derived products (e.g. LPS, methylated DNA, single-stranded RNA, Zymosan). They initiate an innate immune response based on molecular patterns, representing an evolutionary ancient and very early line of defense. Elicited signals include the activation of NF- κ B and expression of interferons. TLR signaling is initiated by dimerization of TLRs, followed by the recruitment of adaptor molecules such as MyD88, Toll-IL1 receptor (TIR) domain-containing adaptor, MyD88 adaptor-like protein, the subsequent association of IL-1 receptor associated kinases (IRAK-1, -4), and the further transduction of the signal via TRAF6 and the TAK1 (TGF- β activated kinase 1)/TAB (activator of TAK1) complex to NF- κ B. Both MyD88 dependent and -independent pathways have been identified (for review, see [50]).

For two of these signaling components, inhibitory versions have been identified which are induced by LPS and thus fulfill the requirements for negative feedback mediators: IRAK-M is a kinase-deficient homologue of IRAK-1 (51), and IRAK-2c encodes a splice variant of IRAK-2 (52). Both interfere with binding, phosphorylation, or dissociation of the functional IRAKs within the cytoplasmic TLR complex. MyD88s (a short form of MyD88) lacks the so-called “interdomain”, and inhibits the ability of IRAK-4 to phosphorylate IRAK-1, most likely through prevention of MyD88-IRAK-4 interaction (53) (see Fig. 1).

Another example of feedback regulation within the TLR signaling pathway is ST2, a membrane protein that can also be expressed in a soluble form (ST2L and sST2, respectively) depending on alternative splicing. ST2 is upregulated by inflammatory cytokines and LPS (54); however, its activity is not regulated on the level of gene expression but through mobilization from intracellular storage sites. ST2L inhibits IL-1, TLR2, -4, and -9, but not TLR3-mediated NF- κ B activation by sequestration of MyD88 and MyD88 adaptor-like (MAL) through its TIR domain, leaving the MyD88 independent pathway unaffected.

Suppressor of cytokine signaling (SOCS)1 is a member of the suppressor of cytokine signaling family. It is LPS- and CpG-inducible in macrophages, and IL-1 inducible in EC, and it appears to be a powerful inhibitor of the inflammatory response on several levels, including signal transducer and activator of transcription (STAT), NF- κ B, p38 and c-Jun N-terminal kinase (JNK) signaling pathways. SOCS1-deficient mice die within three weeks of birth due to multiorgan inflammation depending on interferon- γ signaling (55). Recently, a new target of SOCS1-mediated negative regulation was described: the rapid proteasome-dependent degradation of MAL, which is involved in TLR2 and TLR4 signaling, was found to depend on the E3 ubiquitin ligase activity of SOCS1 (56).

In addition to its negative action on TNF α -induced signaling, A20 also inhibits the transcription factor interferon regulatory factor-3 (IRF-3), which plays a critical role upon TLR3 or TLR4 stimulation. A20 interacts with the IRF-3 kinases NAK/TBK1 and IKK-i/IKK ϵ , suppressing IRF-3 activation and interferon-stimulated response element (ISRE) dependent transcription (57).

Very recently, a novel self-regulatory mechanism in the MyD88-dependent TLR pathways was identified: the activating transcription factor(ATF)-3, so far not implicated in inflammatory responses, was found to be induced by LPS and in turn to in-

Table 1: IL-1 inducible genes in human umbilical vein endothelial cells with inhibitory function (from Mayer et al. [11]; data deposited in the GEO section of the NCBI data base under the accession nr. GDS649). Abbreviations: I κ B, inhibitor of NF- κ B (69); Zfp36 (TTP): zinc finger protein 36/tristetraproline (43); DSCR1: down-syndrome critical region 1 (40); C8FW (TRIB1): tribbles homologue 1 (drosophila) (61); Etr101 (pip92): early TPA response (62); DUSP: dual-specificity phosphatase (59); EHD1: EH domain containing protein 1 (63); TNFRSF1B: TNF super family receptor member 1b (64); OTAP-C (SLCO1B1): solute carrier organic anion transporter family member 1B1 (65); TCF8: transcription factor 8 (66); GG2-1 (TNFAIP8): TNF α -induced protein 8 (67); TBX3: T-box 3 (68); ADAMTS1: ADAM metalloproteinase with thrombospondin type 1 motif (69); TNFSF15 (TL1): TNF (ligand) superfamily member 15 (70); Jag1: jagged 1 (Alagille syndrome) (71).

Gene	Function
I κ B	inhibitor of NF- κ B
A20	inhibitor of NF- κ B, inhibitor of apoptosis
Zfp36/TTP	destabilization of specific ARE-containing mRNAs
DSCR1	inhibitor of NF-AT
C8FW	homologue of SINK and SHIK (involved in NF- κ B p65 transactivation)
Etr101	inhibitor of several signaling pathways
DUSP1	dual-specificity phosphatase, inhibits MAPKs
DUSP5	dual-specificity phosphatase, inhibits MAPKs
SOCS1	suppressor of cytokine signaling (JAK-STAT, TLR pathways)
RGS7	regulator of G-protein signaling
ARTS-1	aminopeptidase regulator, promotes TNFR1 ectodomain shedding
EHD1	endosomal sorting, recycling of GF receptors (?)
TNFRSF1B	osteoprotegerin, soluble decoy receptor for RANKL
OATP-C	Na-independent transport of prostaglandin E2, thromboxane B2, leukotriene C3, leukotriene E4 (clearance of proinflammatory mediators?)
TCF8	transcriptional repressor (for IL-2 in T cells)
GG2-1	negative regulator of T cell signaling
TBX3	transcriptional repressor (in development)
ADAMTS1	disintegrin, metalloproteinase, anti-angiogenic properties
TNFSF15	inhibits EC proliferation
Jag1	activates notch signaling, thereby represses EC proliferation via Rb

hibit LPS-induced NF- κ B-dependent gene expression by association with the histone deacetylase histone deacetylase-1 (58).

MAP kinase activation

Besides NF- κ B, inflammatory stimulation also activates distinct mitogen activated protein kinase (MAPK) signaling pathways, leading to the activation of transcription factors that often cooperate with NF- κ B. MAPK signaling pathways represent three-tiered kinase cascades consisting of a MAPK, a MAPKK (MEK or MAP2K), and a MAPKKK (MEKK or MAP3K) that, together with their activators and scaffolds, form a tightly controlled network. The three most important MAPK pathways in the context of inflammation are extracellular regulating kinase (ERK), JNK, and p38.

Dual-specificity phosphatases (DUSPs) are important regulators of MAPK activity through dephosphorylation of the threonine-X-tyrosine motif, and also control of their subcellular localization. Ten human DUSPs have been identified so far and constitute three subfamilies according to their substrate specificity and cellular localization. At present, the physiological functions of DUSPs are mostly unknown. A negative feedback control has been described for DUSP-1 (MAP kinase phosphatase-1, MAP kinase phosphatase-1), acting on p38 and JNK. DUSP-1 is transcriptionally regulated by LPS in alveolar macrophages (59), and also in EC (Table 1). However, the precise contribution of this and other DUSPs such as DUSP-5 in the negative feedback control of the inflammatory response has to be further investigated.

Additional genetic regulators

Besides these partially well-characterized mechanisms, Table 1 summarizes the results of screening expression profiling data (human umbilical vein endothelial cells stimulated with IL-1) (11) for inducible genes with inhibitory potential. In addition to those that have been described above, it reveals inhibitors that were previously described mostly in the context of other cells or stimuli, and that act on many different levels; their regulated expression in EC suggests that they fulfill similar functions in this cell type.

Concluding remarks

Two main conclusions can be drawn from the nature and kinetics of expression of genes with inhibitory potential that are expressed

upon pro-inflammatory stimulation in EC: First, several feedback mechanisms are initiated already within the first hours of the inflammatory episode, supporting the concept of “the beginning programs the end”. Second, the identity of these genes show that these feedback mechanisms occur on many different regulatory levels, ranging from receptors (ectodomain shedding, endosomal sorting) to signaling pathways, transcription factors, and to mRNA stability (Table 1). Whether the downregulation of individual regulatory pathways in EC is associated with distinct biological functions remains to be demonstrated. It may be speculated that the biological implication of this scenario could be the ability for a cell to fine-tune the inflammatory response and adapt it in quality and quantity in accordance to the context of the specific patho-physiological situation.

At present, many of these feedback mechanisms have been found on the level of gene expression, probably due to the experimental approaches that have been taken. However, as the use of proteomics and other -omics are becoming increasingly popular, it can be expected that our knowledge will proceed further towards the contribution of protein modifications such as phosphorylation/dephosphorylation, ubiquitination, changes in lipid profiles, or the role of microRNAs in the process of resolution of inflammation. This knowledge should be helpful for the understanding of the molecular mechanisms that regulate the inflammatory response, including acute versus chronic, and the design of therapeutic strategies to interfere with these processes.

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Abbreviations

COX-2, cyclooxygenase-2, prostaglandin synthase 2; DSCR1, Down Syndrome Critical Region 1, calcipressin-1; DUSP, dual-specificity phosphatases; EC, endothelial cells; I κ B, inhibitor of NF- κ B; IKK, I κ B kinase; IL, interleukin; IRAK, IL-1 receptor associated kinase; IRF, interferon regulatory factor; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MyD88, myeloid differentiation factor 88; NEMO, NF- κ B essential modulator; NF-AT, nuclear factor of activated T cells; NF- κ B, nuclear factor- κ B; RIP, receptor-interacting protein; SOCS, suppressor of cytokine signaling; TIR, Toll-IL1 receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, TNF receptor; TRAF, TNFR associated factor; VCAM-1, vascular cell adhesion molecule 1; Zfp36, zinc finger protein 36, tristetraprolin (TTP).

References

1. Mayno G. The healing hand: Man and wound in the ancient world. Harvard University Press, Cambridge, Massachusetts, 1975.
2. Ahmed A, et al. Induction of placental heme oxygenase-1 is protective against TNF α -induced cytotoxicity and promotes vessel relaxation. *Mol Med* 2000; 6: 391–409.
3. Nickoloff BJ, et al. Lessons learned from psoriatic plaques concerning mechanisms of tissue repair, remodeling, and inflammation. *J Invest Dermatol Symp Proc* 2006; 11: 16–29.
4. Schreiber S, et al. Genetics of Crohn disease, an archetypal inflammatory barrier disease. *Nat Rev Genet* 2005; 6: 376–388.
5. Weiner HL, Selkoe DJ. Inflammation and therapeutic vaccination in CNS diseases. *Nature* 2002; 420: 879–884.
6. Yan SF, et al. Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. *Circ Res* 2003; 93: 1159–1169.
7. Henson PM. Dampening inflammation. *Nat Immunol* 2005; 6: 1177–1206.
8. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol* 2005; 6: 1191–1197.
9. Fiedler U, et al. Angiotensin-2 sensitizes endothelial cells to TNF- α and has a crucial role in the induction of inflammation. *Nat Med* 2006; 12: 235–239.
10. de Martin R, et al. The transcription factor NF- κ B and the regulation of vascular cell function. *Arterioscler Thromb Vasc Biol* 2000; 20: E83–88.
11. Mayer H, et al. Deciphering regulatory patterns of inflammatory gene expression from interleukin-

- 1-stimulated human endothelial cells. *Arterioscler Thromb Vasc Biol* 2004; 24: 1192–1198.
12. Zhao B, et al. Human endothelial cell response to gram-negative lipopolysaccharide assessed with cDNA microarrays. *Am J Physiol Cell Physiol* 2001; 281: C1587–1595.
13. Chen ZJ. Ubiquitin signalling in the NF-kappaB pathway. *Nat Cell Biol* 2005; 7: 758–765.
14. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; 109: S81–96.
15. Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev* 2004; 18: 2195–2224.
16. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005; 5: 749–759.
17. Arenzana-Seisdedos F, et al. Inducible nuclear expression of newly synthesized IκBα negatively regulates DNA-binding and transcriptional activities of NF-kappa B. *Mol Cell Biol* 1995; 15: 2689–2696.
18. Wrighton CJ, et al. Inhibition of endothelial cell activation by adenovirus-mediated expression of I kappa B alpha, an inhibitor of the transcription factor NF-kappa B. *J Exp Med* 1996; 183: 1013–1022.
19. Oitzinger W, et al. Adenovirus-mediated expression of a mutant IκappaB kinase 2 inhibits the response of endothelial cells to inflammatory stimuli. *Blood* 2001; 97: 1611–1617.
20. Breuss JM, et al. Activation of nuclear factor-kappa B significantly contributes to lumen loss in a rabbit iliac artery balloon angioplasty model. *Circulation* 2002; 105: 633–638.
21. Cejna M, et al. Inhibition of neointimal formation after stent placement with adenovirus-mediated gene transfer of IκBα in the hypercholesterolemic rabbit model: initial results. *Radiology* 2002; 223: 702–708.
22. Foxwell B, et al. Efficient adenoviral infection with IκBα reveals that macrophage tumor necrosis factor alpha production in rheumatoid arthritis is NF-kappaB dependent. *Proc Natl Acad Sci USA* 1998; 95: 8211–8215.
23. Griesenbach U, et al. Anti-inflammatory gene therapy directed at the airway epithelium. *Gene Ther* 2000; 7: 306–313.
24. Trescher K, et al. Inflammation and postinfarct remodeling: Overexpression of IκappaB prevents ventricular dilation via increasing TIMP levels. *Cardiovasc Res* 2006; 69: 746–754.
25. Krikos A, et al. Transcriptional activation of the tumor necrosis factor alpha-inducible zinc finger protein, A20, is mediated by kappa B elements. *J Biol Chem* 1992; 267: 17971–17976.
26. Cooper JT, et al. A20 blocks endothelial cell activation through a NF-kappaB-dependent mechanism. *J Biol Chem* 1996; 271: 18068–18073.
27. Wertz IE, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signaling. *Nature* 2004; 430: 694–699.
28. Mauro C, et al. ABIN-1 binds to NEMO/IKKgamma and co-operates with A20 in inhibiting NF-kappa B. *J Biol Chem* 2006; 281: 18482–18488.
29. Bignell GR, et al. Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* 2000; 25: 160–165.
30. Brummelkamp TR, et al. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. *Nature* 2003; 424: 797–801.
31. Kovalenko A, et al. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature* 2003; 424: 801–805.
32. Trompouki E, et al. CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activation by TNFR family members. *Nature* 2003; 424: 793–796.
33. Jono H, et al. NF-kappaB is essential for induction of CYLD, the negative regulator of NF-kappaB: evidence for a novel inducible autoregulatory feedback pathway. *J Biol Chem* 2004; 279: 36171–36174.
34. Reiley W, et al. Regulation of the deubiquitinating enzyme CYLD by IκappaB kinase gamma-dependent phosphorylation. *Mol Cell Biol* 2005; 25: 3886–3895.
35. Wu M, et al. SINK is a p65-interacting negative regulator of NF-kappaB-dependent transcription. *J Biol Chem* 2003; 278: 27072–27079.
36. Huang J, et al. Identification of a novel serine/threonine kinase that inhibits TNF-induced NF-kappaB activation and p53-induced transcription. *Biochem Biophys Res Commun* 2003; 309: 774–778.
37. Rothwarf DM, Karin M. The NF-κB activation pathway: a paradigm in information transfer from membrane to nucleus. *Sci STKE* 1999; RE1.
38. Rossi A, et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκappaB kinase. *Nature* 2000; 403: 103–108.
39. Quinlan KL, et al. Substance P activates coincident NF-AT- and NF-kappa B-dependent adhesion molecule gene expression in microvascular endothelial cells through intracellular calcium mobilization. *J Immunol* 1999; 163: 5656–5665.
40. Hesser BA, et al. Down syndrome critical region protein 1 (DSCR1), a novel VEGF target gene that regulates expression of inflammatory markers on activated endothelial cells. *Blood* 2004; 104: 149–158.
41. Mechtcheriakova D, et al. Specificity, diversity, and convergence in VEGF and TNF-α signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. *FASEB J* 2001; 15: 230–242.
42. Minami T, et al. Thrombin-induced auto-inhibitory factor, down syndrome critical region-1, attenuates NFAT-dependent vascular cell adhesion molecule-1 expression and inflammation in endothelium. *J Biol Chem* 2006; 281: 20503–20520.
43. Carballo E, et al. Feedback inhibition of macrophage tumor necrosis factor-α production by tristetraprolin. *Science* 1998; 281: 1001–1005.
44. Carballo E, et al. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. *Blood* 2000; 95: 1891–1899.
45. Chrestensen CA, et al. MAPKAP kinase 2 phosphorylates tristetraprolin on *in vivo* sites including Ser178, a site required for 14–3–3 binding. *J Biol Chem* 2004; 279: 10176–10184.
46. Lai WS, et al. Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. *Mol Cell Biol* 2006; in press.
47. Carrick DM, et al. Genetic variations in ZFP36 and their possible relationship to autoimmune diseases. *J Autoimmun* 2006; 26: 182–196.
48. Tchen CR, et al. The stability of tristetraprolin mRNA is regulated by mitogen activated protein kinase p38 and by tristetraprolin itself. *J Biol Chem* 2004; 7: 7.
49. Jing Q, et al. Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* 2005; 120: 623–634.
50. O'Neill LA. The role of MyD88-like adapters in Toll-like receptor signal transduction. *Biochem Soc Trans* 2003; 31: 643–647.
51. Wesche H, et al. IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. *J Biol Chem* 1999; 274: 19403–19410.
52. Rao N, et al. A novel splice variant of interleukin-1 receptor (IL-1R)-associated kinase 1 plays a negative regulatory role in Toll/IL-1R-induced inflammatory signaling. *Mol Cell Biol* 2005; 25: 6521–6532.
53. Janssens S, et al. Regulation of interleukin-1- and lipopolysaccharide-induced NF-κB activation by alternative splicing of MyD88. *Curr Biol* 2002; 12: 467–471.
54. Kumar S, et al. Expression of ST2, an interleukin-1 receptor homologue, is induced by proinflammatory stimuli. *Biochem Biophys Res Commun* 1997; 235: 474–478.
55. Chen Y, et al. Severe pancreatitis with exocrine destruction and increased islet neogenesis in mice with suppressor of cytokine signaling-1 deficiency. *Am J Pathol* 2004; 165: 913–921.
56. Mansell A, et al. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat Immunol* 2006; 7: 148–155.
57. Saitoh T, et al. A20 is a negative regulator of IFN regulatory factor 3 signaling. *J Immunol* 2005; 174: 1507–1512.
58. Gilchrist M, et al. Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. *Nature* 2006; 441: 173–178.
59. Zhao Q, et al. The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. *J Biol Chem* 2005; 280: 8101–8108.
60. de Martin R, et al. Cytokine-inducible expression in endothelial cells of an I kappa B alpha-like gene is regulated by NF-κB. *EMBO J* 1993; 12: 2773–2779.
61. Kiss-Toth E, et al. Human tribbles, a protein family controlling mitogen-activated protein kinase cascades. *J Biol Chem* 2004; 279: 42703–42708.
62. Charles CH, et al. Pip92: a short-lived, growth factor-inducible protein in BALB/c 3T3 and PC12 cells. *Mol Cell Biol* 1990; 10: 6769–6774.
63. Haider NB, et al. Evaluation and molecular characterization of EHD1, a candidate gene for Bardet-Biedl syndrome 1 (BBS1). *Gene* 1999; 240: 227–232.
64. Miyashita T, et al. Osteoprotegerin (OPG) acts as an endogenous decoy receptor in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis of fibroblast-like synovial cells. *Clin Exp Immunol* 2004; 137: 430–436.
65. Tamai I, et al. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* 2000; 273: 251–260.
66. Williams TM, et al. Identification of a zinc finger protein that inhibits IL-2 gene expression. *Science* 1991; 254: 1791–1794.
67. Kumar D, et al. Identification of a novel tumor necrosis factor-α-inducible gene, SCC-S2, containing the consensus sequence of a death effector domain of fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein. *J Biol Chem* 2000; 275: 2973–2978.
68. Bamshad M, et al. Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nat Genet* 1997; 16: 311–315.
69. Kuno K, et al. Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. *J Biol Chem* 1997; 272: 556–562.
70. Tan KB, et al. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells. *Gene* 1997; 204: 35–46.
71. Lindsell CE, et al. Jagged: a mammalian ligand that activates Notch1. *Cell* 1995; 80: 909–917.