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Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways

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Summary

Activation of Toll-like receptor (TLR) signaling and related pathways by microbial products drives inflammatory responses, host defense pathways and adaptive immunity. The cost of excessive inflammation is cell and tissue damage, an underlying cause of many acute and chronic diseases. Coincident with activation of TLR signaling, a plethora of anti-inflammatory pathways and mechanisms begin to modulate inflammation until tissue repair is complete. Whereas most studies have focused on the signaling components immediately downstream of the TLRs, this review summarizes the different levels of anti-inflammatory pathways that have evolved to abate TLR signaling and how they are integrated to prevent cell and tissue destruction.

The inflammatory response must be constantly constrained to prevent molecular, cellular and organ damage. The consequences of unregulated inflammation are associated with, or directly underpin, a substantial fraction of diseases that plague us, including autoimmune and metabolic diseases, infectious diseases caused by large macroparasites to viruses, chronic neurological diseases, malignancy and life-threatening acute responses to pathogen products such as sepsis and shock. Correspondingly, a proportionate percentage of the modern pharmacopoeia is devoted to blocking inflammation, from widely used drugs such as aspirin and the non-steroidal anti-inflammatory medication to humanized anti-cytokine antibodies.

Given that the inflammatory response is essential to constrain infection, recruit and activate lymphocytes and then promote wound healing and repair, how are these processes regulated such that horror autotoxicus is mitigated and organ systems return to homeostasis? The integration of inflammatory inhibition and homeostasis is especially important in large animals that must live for decades to successfully reproduce and raise the next generation. It should not be surprising therefore that elaborate mechanisms to regulate inflammation have co-evolved with pro-inflammatory pathways, that non-resolving or chronic inflammation is linked to the chronic maladies of aging and that older organisms are especially sensitive to inflammatory perturbation[1].

Before the discovery of 'innate' detection systems such as the Toll-like receptors (TLRs), Nod-like receptors (NLRs), C-type lectin receptors (CLRs) and the diverse nucleic acid detectors, 'negative' pathways had been recognized to mediate multiple layers of the

inflammatory response. Subsequent to the notion that innate receptors have preeminent roles in pathogen detection and the initiation of inflammation, a massive literature has established additional layers of regulatory control over inflammation. In this overview, we cannot cover the primary literature on the fine details of each molecule attributed to have negative regulatory influence on inflammation. Instead, we first propose that modulation of inflammation involves inter-connected layers or strata that begins with the anatomy of mammals and extends to the precise control of the metabolic state of inflammatory cells. From these regulatory strata, we will focus on three interrelated pathways whose mechanistic details are emerging and represent new strategies to manipulate and interrupt excessive responses initiated by TLR signaling and related pathways that activate inflammation. As other recent reviews have covered aspects of inflammatory modulation including TLR signaling components and post-transcriptional pathways[2–7] we will focus on the wider context of inflammatory regulation.

Discovery of anti-inflammatory pathways

Three types of investigation have uncovered negative regulators of inflammation. First, observation of unanticipated phenotypes in humans and mutant mice with inflammatory disease have been the starting point for the discovery of numerous key pathways including the interleukin 10 (IL-10) and TGF-β pathways[8–10]. Second, standard experimental models of inflammation including cecal ligation and puncture, endotoxin challenge, bleomycin-mediated lung injury, graft versus host disease, airway challenges of mice with allergens and TLR agonists and infection models that include acute and chronic inflammation-associated damage have led to the definition of many anti-inflammatory pathways. Third, *in vitro* systems to measure the TLR- and NLR-activated pathways on primary cultures of macrophages and dendritic cells from knockout and transgenic mice have been instrumental in pinpointing where a given regulatory factor fits into a signaling pathway. Coupled with complementary approaches such as forward genetics and biochemical reconstitution experiments, many anti-inflammatory factors been discovered and their activities defined to differing extents (Table 1). Nevertheless, caveats apply with these approaches that center on the model systems used and their context. First, many types of experiments in mice cannot be applied to humans. Murine models of inflammation can amplify the preeminence of pathways that are subject to compensatory or redundant effects in people. For example, MyD88-deficient humans have a narrow range of infection phenotypes even in adulthood, while MyD88-deficient mice have broad phenotypes consistent with MyD88's key role in all TLR signaling pathways other than TLR3[11]. These observations are best reconciled by considering that humans are not exposed to the same experimental pressure as would be found in a procedure such as cecal ligation and puncture[12]. Future studies on humans, mice and new model organisms such as the pig[13,14], will likely refine our understanding of the existing anti-inflammatory pathways and uncover new regulatory layers.

A second caveat concerns the interpretation of links between molecules that have multiple functions and whose disruption causes excessive inflammation. Tracing the connections between pathways has proven difficult when the starting point is a whole animal experiment. An informative example is the whole organism disruption of SOCS1, an inducible inhibitor of the type I and II interferon (IFN) receptors[15]. Deletion of *Socs1* leads to death a few

weeks after birth, a phenotype that can be rescued by crossing to mice into *Ifng*$^{-/-}$ or *Rag1*$^{-/-}$ backgrounds. However, *Socs1*$^{-/-}$ *Ifng*$^{-/-}$ mice (as well as juvenile *Socs1*$^{-/-}$ mice) are extraordinarily sensitive to systemic endotoxin administration[16,17]. Several models have been proposed to account for this phenotype, including absence of regulation of TLR signaling by direct binding of SOCS1 to TIRAP, IRAK1 and NF-κB, and excessive IFN-α/IFN-β signaling in the *Socs1*$^{-/-}$ *Ifng*$^{-/-}$ mice[16–20]. Collectively these data illustrate that for a protein like SOCS1 that has multiple effects in multiple cell types and contexts, probing the inflammatory strata to assign a relative hierarchy of negative regulation is complex.

Inflammation has a clock

In vivo, inhibition of inflammatory pathways occurs across a time frame that extends from seconds to years in wound healing and tissue repair, or is continuously ongoing in chronic inflammation. For productive immunity to pathogens to emerge, the initial inflammatory insult needs to be sufficient to trigger a response beyond the homeostatic anti-inflammatory threshold[21]. For example, in the gut, IL-10 constitutively dampens TLR and NLR signaling from the gut flora to maintain normal intestinal functions. Infection with a pathogen like *Shigella flexneri*, that invades the mucosal layers, triggers a response that exceeds the homeostatic threshold[21], and causes massive inflammation[22]. The signals to repair the gut likely begin once the infection is ‘controlled’ such that bacteria may still be present but are no longer proliferating. In the gut, repair mechanisms to restore the epithelia and mucosa must work very quickly as all animals need to acquire nutrients to survive. By contrast, the wound healing and tissue restoration process for bones, deep tissue injuries and muscle takes months to years to restore tissue strength. Regardless of the time frame of tissue repair, negative regulation of inflammation has to be continuously engaged. Thus, productive regulatory pathways are induced proportionally to the inflammatory insult and are themselves subject to additional layers of regulation[23]. An informative example of the latter is the production of IL-10 which is essential to inhibit inflammation at multiple layers, but can also promote an immune environment permissive for multiple pathogens[24]. Obviously, lack of engagement of counter regulation at the correct time and place underlies a plethora of inflammatory diseases touched upon here.

Cell intrinsic and extrinsic anti-inflammation strata

It is plausible to consider that for every pro-inflammatory pathway activated by the microbial and cell damage sensing systems, there are at least as many anti-inflammatory pathways. Anti-inflammatory pathways can be crudely divided into cell intrinsic and extrinsic mechanisms, many of which have been uncovered by unexpected outcomes of the detailed examination of genetically-modified mice that manifest inflammatory diseases. Examples of cell intrinsic pathways include co-regulated inhibitors of TLR signaling such as IκBα, IRAK-M, A20 and ABIN1 (Table 1). Deletion of each of these factors in mice leads to complex inflammatory diseases because of failure to attenuate inflammatory signal after it has been initiated by a microbial product or pro-inflammatory cytokine such as TNF [25–29]. Cell extrinsic mechanisms include anti-inflammatory cytokines such as IL-10 and TGF-β, as well as a myriad of factors that function to block inflammation through the sequential ‘repair’ process outlined above. Another way to define anti-inflammatory pathways is to

break down each step from inflammatory initiation to resolution. These strata constitute an integrated system to mitigate the negative effects of inflammation through the span of an inflammatory response. Table 1 summarizes these strata as a 'snapshot' of the breadth of inflammatory modulation and provides select examples of gene disruption experiments in the mouse that lead to excessive inflammation.

Strata 1 and 2: anatomy and mucosa

Many papers concerned with inflammation begin with a statement concerning the ‘first line’ functions of the innate immune response. However, the first line of defense against pathogens is mammalian anatomy (Stratum 1) and its associated mucosal system (Stratum 2). The lungs, gut and skin receive constant stimulation by commensals and pathogens[30]. However, it is rare that inflammatory responses are ever sufficiently troublesome to send someone to the doctor. Our barrier systems have evolved to mechanically repel or constrain microorganisms that could trigger inflammation. A key example is the mucous lining of the gut where both the viscosity and forward motion ensures that only a fraction of the gut flora encounters the underlying immune cell-rich mucosa. Similarly, the lungs are bathed in surfactant to restrain colonization by microorganisms; an effect complemented by the cough reflex to continuously propel mucus and debris from the lungs upward. The effects of disruption of the lung mucosal lining and failure of coughing are key elements of cystic fibrosis and bacterial overgrowth observed in the terminally ill. Similarly, defects in intestinal mucus production or flow are associated with dramatic inflammatory responses[31,32]. The gut, liver, skin, spleen and lungs are also endowed with sentinel immune cells programmed not to overreact to pathogens or their products[30,33,34]. The spleen is also the target of the neural reflex anti-inflammatory pathway[35]. Collectively, the anatomy of mammals contributes the bulk of 'defense' against pathogen challenge and initiation of inflammation.

Stratum 3: PAMP sequestration

Removal of microbial products including cell wall components before they ever interact with TLRs and NLRs is a key innate mode of inflammatory regulation. When considered with anatomical restriction of TLR ligand exposure via strata 1 and 2, sequestration of PAMPs operates continuously. The complexity of the pathways in strata 3 has been reviewed[5]. Two other ‘innate’ pathways that interface with anatomy, mucous membranes and the circulatory system are the complement and coagulation pathways. The effect of these two pathways on pro-and anti-inflammatory TLR signaling modulation is an emerging area of investigation, along with modulation of the extracellular matrix, and has been discussed in other reviews[36–38].

Stratum 4: signaling down-regulation

A myriad of cytoplasmic proteins work together to regulate cell autonomous TLR signaling[3,39]. These proteins fall into diverse structural and functional groups including sentinel proteins that rapidly inhibit signaling (IRAK-M, A20, ABIN1, IκBα, Tollip, DAP12), proteins that are further induced at the gene expression level to reinforce signaling

down-regulation (A20, IκBα, DUSP family phosphatases), kinases that mediate downstream inhibitory pathways (MSK1, MSK2) and signaling proteins that initiate the production of cytokines that act through autocrine and paracrine pathways to block signaling (TRAF3). Other proteins such as the alanine-rich myristoylated protein MARCKS as one example of many, have been discovered to inhibit TLR signaling through unknown mechanisms[40]. The integration of each of these proteins into greater signaling networks is central to each TLR-responsive cell's decision to terminate or perpetuate an inflammatory response.

Stratum 5: transcriptional regulation

After the integration of initial TLR signaling pathways in the cytoplasm, a large cohort of positively and negatively acting transcription factors control the thousands of genes regulated by TLR signaling[41]. Negative regulation of TLR-mediated transcription can be described by at least four phenomena: specific negative feedback mechanisms to suppress the activities of factors that activate inflammatory gene transcription, such as NF-κB[42]; acquired resistance to transcriptional activation following chronic exposure to stimuli such as LPS and TNF[43], usually referred to as endotoxin tolerance[44]; gene-specific effects of steroid hormone receptors and their co-factors[45,46], and the autocrine-paracrine effects of IL-10, which signals to suppress many TLR-activated genes[47]. The effects of these pathways are discussed in more depth below. In addition to these four mechanisms, constitutively expressed transcription factors and chromatin proteins have been demonstrated to have negative effects of TLR-regulated gene expression in macrophages, although the relationship between these factors and tolerance, steroid hormone repression, IL-10 signaling and other physiological processes that are related to inflammatory diseases and the resolution of normal inflammatory responses remains unclear[48].

Stratum 6: Post-transcription and translation

Post-transcriptional regulation and translation of mRNAs encoding inflammatory mediators is essential for inflammatory control[7]. Multiple RNA binding proteins such as TTP and HuR, and miRNAs including miR-155 are involved in fine-tuning the post-transcription inflammatory response[49–51]. Although the identity of several components of the post-transcriptional signaling network has been uncovered, further work is needed to pinpoint how proteins and miRNAs with broad mRNA substrate specificity locate their targets, suppress transcription/translation, and are themselves regulated.

Stratum 7: Processing and secretion

Inflammatory stress is coupled to the endoplasmic reticulum (ER) stress response. The ER stress response inhibits the processing and secretion of many proteins, presumably as a means to conserve resources during stress. However, the ER stress response is regulated by TLR signaling such that many secreted inflammatory cytokines and chemokines are allowed to escape the ER[52,53]. This remarkable process is required for the overall inflammatory response, but how is it regulated? Conceivably, numerous anti-inflammatory pathways could converge on the ER to suppress the production of multiple pro-inflammatory mediators that so far remain unknown.

Strata 8,9: decoys, antagonists and hijacked cytokines

These inhibitory pathways are discussed in more detail below.

Stratum 10:metabolic regulation of inflammation

A final stratum for control of inflammation is the impact of metabolic states on immunity. The metabolic control of inflammation encompasses a large variety of linked processes including translation control, metabolic stress responses[54], autophagy[55], and forms of programmed cell death. Emerging information on metabolism and the immune response is discussed in depth by another review in this issue (Ref. to add- Green review, this issue).

An example of the linkages between the strata of inflammatory control is the production, secretion, and bioavailability of IL-1β, which is negatively regulated at the level of at least eight different check points (Fig. 1).

Transcriptional repression of inflammation

As mentioned above, studies of inflammatory gene transcription have uncovered a number of transcription factors, chromatin proteins and other transcription-related mechanisms that contribute to the suppression of inflammation. One notable example is the rapid transcriptional activation of the gene encoding IkBα by most or all inflammatory stimuli. IκBα induction leads to the suppression of NF-κB activity, thereby attenuating the transcription of NF-κB-dependent genes unless the stimulus is capable of circumventing the suppression[40]. The variable consequences of this suppression mechanism are apparent from a comparison of macrophages stimulated with TNF and LPS[56,57]. The initial response to TNF is transient, due to the upregulation of IκBα, the rapid internalization of the TNF receptor and other feedback inhibitory mechanisms. In contrast, LPS stimulates two distinct NF-κB-inducing pathways with different kinetics, the MyD88 and TRIF pathways, thereby allowing sustained NF-κB activation and a transcriptional cascade that differs substantially from that activated by TNF, despite the upregulation of IκBα by both stimuli. Several other mechanisms that have the potential to suppress NF-κB activity have been described and have been summarized in recent reviews[40]. One challenge in the study of these suppression mechanisms is that their precise physiological relevance during the course of a normal inflammatory response and during abnormal responses associated with disease have been difficult to uncover and therefore remain poorly understood.

In addition to the various mechanisms involved in the broad suppression of NF-κB activity, the transcription of specific sets of inflammatory genes is limited by several other proteins and protein complexes. Two examples are the Mi-2-NuRD complex and Bcl-6, both of which are constitutively expressed in macrophages. Mi-2-NuRD is a multiprotein complex containing histone deacetylase and ATP-dependent nucleosome remodeling activities and has been primarily implicated in transcriptional repression[58]. Deletion of this complex in mouse macrophages leads to greatly enhanced expression of a subset of LPS-induced genes in a stimulus-dependent manner[59]. The genes that were sensitive to Mi-2-NuRD knockdown correspond to those that require nucleosome remodeling by another family of ATP-dependent nucleosome remodeling complexes, the SWI/SNF family, for their transcriptional

activation. Many of these remodeling-dependent genes play critical roles in regulating inflammatory and adaptive immunity, such as *Il12b*, *Il6* and *Nos2*.

Bcl-6 appears to be a similarly potent suppressor of a subset of inflammatory gene, as many LPS-induced genes were found to be activated at greatly enhanced levels in macrophages from Bcl-6-deficient mice[60]. Bcl-6 directly binds control regions for a large percentage of the affected genes, suggesting that it may suppress transcription of these genes by directly competing with transcriptional activators, possibly leading a repressive chromatin environment. One possibility that has not yet been explored is that Bcl-6 may recruit Mi-2-NuRD to target genes to limit inducible transcription. A careful comparison of the sets of genes suppressed by the two factors may provide insight into this possibility, as regulation of a common set of genes would suggest that the two factors act in concert. Careful delineation of the gene suppressed by these proteins, preferably by RNA sequencing, may also provide clues into the biological reason for the existence of these suppression mechanisms. Several other transcription factors, including IRF4 and ATF3, have also been implicated in the repression of inducible gene transcription[48,61,62]. Although it has been possible to document the importance of these repression mechanisms in the context of mutant mice, the manner in which they are integrated into a normal inflammatory response is unknown and it is not known whether they directly participate in pathways that promote disease.

As discussed above, several factors and mechanisms capable of suppressing or limiting inflammatory gene transcription have been described, but their contributions to normal and abnormal inflammatory responses remain to be elucidated. With this in mind, it is interesting to consider an independent line of investigation that originated with a biological observation that is likely to be of considerable importance, but for which the underlying mechanisms have remained incompletely understood for many years. Specifically, it has long been known that exposure to a potent inflammatory stimulus can lead to acquired resistance to inflammatory gene induction upon subsequent stimulation. This observation was first made with LPS as the stimulus and is referred to as LPS or endotoxin tolerance. However, TNF has similarly been shown to induce tolerance[43].

Multiple molecular mechanisms appear to contribute to tolerance, ranging from mechanisms to suppress the transduction of an inflammatory signal to active repression of inflammatory genes through the assembly of repressive chromatin structures[63–65]. The existence of multiple mechanisms has made it difficult to determine the relative importance of each mechanism that has been described. A few notable studies have provided compelling evidence that changes in chromatin structure contribute to stable suppression of inducible transcription[43,64,66,67]. Repressive histone modifications and chromatin changes that may prevent remodeling by ATP-dependent nucleosome remodeling complexes have been suggested to contribute to resistance to transcriptional activation. Interestingly, only a subset of inducible genes was found to be susceptible to LPS tolerance, with tolerance observed at some genes that contribute to inflammation but not at genes that contribute to anti-microbial immunity[44].

An attractive hypothesis is that the two groups of genes may exhibit distinguishing chromatin characteristics that confer resistance or sensitivity to tolerance. However, initial

efforts to identify these distinguishing characteristics have been unsuccessful, as both sensitive and resistant genes were included within a group of genes found to be dependent on nucleosome remodeling for their activation[44,59,68]. Genes that are sensitive and resistant to tolerance induction were also found in a class of nucleosome remodeling-independent genes. The two classes of genes also cannot be distinguished on the basis of common histone modifications in unstimulated and stimulated cells. Therefore, much remains to be learned about LPS tolerance and the precise mechanisms by which chromatin structure and other events, including signal transduction, contribute to this process.

IL-10 signaling integrates multiple regulatory strata

Genetics teaches that IL-10 is the central anti-inflammatory cytokine that impinges on multiple anti-inflammatory strata. The effects of germline deletion in IL-10- or the IL-10 receptor-encoding genes produce extreme and often lethal inflammatory syndromes in both humans and mice[9,69]. In mice housed in normal or SPF conditions, the effects of IL-10 disruption are first observed in the gut, as noted above. By contrast, germ-free IL-10-deficient mice do not have colitis[70], arguing that the intestinal flora drives the excessive inflammatory response. Furthermore, mice lacking MyD88 and IL-10 do not have colitis, providing conclusive evidence that excessive TLR and IL-1 receptor (IL-1R) signaling in the intestine must be continuously suppressed by IL-10[71,72]. Indeed, depending on the stimulus or infection, pathogenic inflammatory responses are observed in most models of acute and chronic inflammation in IL-10-deficient mice[73]. However, anti-inflammatory effects of IL-10 come at a cost because IL-10 also inhibits productive inflammatory responses against intracellular pathogens, especially *Mycobacteria* and *Leishmania*[74,75]. Thus, the IL-10 anti-inflammatory signal is a trade-off between deleterious and productive inflammatory responses. Another evolutionary curiosity of IL-10 concerns its non-redundant nature in mammals: if IL-10 is so important, why don't we have multiple IL-10-like cytokines? While the answers to this question are speculative, one possibility is that only one IL-10-IL-10R system is required and if it does not function properly then early lethality from excessive inflammation is likely, removing the mutation from the gene pool (similarly, some other essential cytokines like EPO and G-CSF may have the same properties).

IL-10 signaling is dependent on STAT3[47,76] (Fig. 2). The use of STAT3 raises another problematic aspect of deciphering how IL-10 suppresses TLR transcription because STAT3 is activated by numerous cytokines[77,78]. In IL-10-responsive myeloid cells, IL-6 is also a potent activator of STAT3, yet IL-6 mediates none of the suppressive effects of IL-10. The underlying mechanism involved in this dichotomy is mediated by SOCS3 inhibition of the IL-10 'signal' from the IL-6R, while the IL-10R does not bind SOCS3[79]. Furthermore, any cytokine receptor can be engineered into an 'IL-10R' by ensuring STAT3 activation in the absence of SOCS3 inhibition[80]. Thus, the IL-10-mediated anti-inflammatory response is 'generic' in that it depends on a specific way of activating STAT3, independent of the receptor. The underlying mechanisms involved in the 'IL-10 type' of STAT3 activation in comparison to other STAT3-activating receptors remain unknown.

The effects of IL-10 (like LPS tolerance and steroid hormone inhibition) on transcription are gene specific: numerous TLR-regulated genes are unaffected by IL-10 (*Nfkbia*, *Tnfaip3*),

while others show varying degrees of inhibition from complete (*Il12b*) to partial (*Tnf*)[47]. The underlying mechanisms involved in the selection of genes for inhibition remain unknown[81,82]. Two mechanisms are possible: a single master regulatory factor could mediate transcriptional repression or multiple factors could work together (Fig. 3). In the case of the former, no unique IL-10-regulated factor has been discovered that would be epistatic to STAT3[47,83,84]. Instead, it seems likely that multiple factors suppress gene expression in a gene-specific way. To date, the best understood of these is NFIL3, a B-ZIP factor induced by IL-10 that regulates *Il12b* (encoding IL-12p40, the common subunit of IL-12 and IL-23) by binding to a distal enhancer ~10 kb upstream of the *Il12b* promoter[85–87]. NFIL3-deficient macrophages overproduce IL-12p40 and IL-12p70 in response to TLR stimulation[87,88]. However, while NFIL3 is necessary to regulate *Il12b* transcription, it is not sufficient because IL-10 retains residual inhibitory effects on *Il12b* transcription in *Nfil3*$^{-/-}$ macrophages[87,88]. Therefore, additional factors induced by IL-10 that are associated with transcription including Bcl-3, Sbno2, Etv3 and IkBNS may work together to suppress TLR-induced genes, along with factors that possibly regulate elongation on actively transcribed TLR-regulated genes[82,87].

Although the majority of the inhibitory effects of IL-10 are focused on the level of transcriptional control, IL-10 also induces additional modifiers of inflammatory signaling that operate at other strata. For example, IL-10, via STAT3, increases the TLR-mediated expression of tristatraprolin (TTP, encoded by *Zfp36*) to enhance degradation of AU-rich 3' UTR target mRNAs targeted for degradation by TTP[89]. In the same time frame, IL-10 synergistically induces DUSP1, which can dephosphorylate p38 MAPK[90]. As p38-mediated phosphorylation of TTP is inactivating, DUSP1 maintains TTP activity, further enhancing the effects of IL-10 on mRNA stability (Fig. 1). Another target of IL-10 is the gene encoding the IL-1R antagonist, whose expression is highly induced by IL-10. Therefore, IL-10 regulates IL-1 signaling at the transcriptional, post-transcriptional and receptor levels in concert with other inhibitory processes (Fig. 1). A final example of the dichotomy of anti-inflammatory effects of IL-10 is the regulation of LPS-induced miRNAs. IL-10 is a potent transcription inhibitor of the pro-inflammatory miR-155 but not of miR-146[91], which has cell-intrinsic anti-inflammatory effects[50]. How the IL-10 signaling pathway makes this discrimination remains unknown.

Decoys, antagonists and hijacked cytokines

Post-production removal of TLR-induced pro-inflammatory cytokines and chemokines coupled with receptor antagonism are key ‘downstream’ anti-inflammatory processes that are often overlooked in deciphering inflammatory control *in vivo*[92]. Post-production removal of cytokines have been harnessed for successful therapies: soluble TNF receptors for rheumatoid arthritis and inflammatory bowel disease (etanercept), and the IL-1R antagonist for treatment of inflammatory cryopathies (anakinra). Soluble pro-inflammatory mediators are removed or inhibited by distinct mechanisms. For example, membrane-bound and soluble cytokine and chemokine receptors act as ‘sinks’ on a variety of different cell types to soak up pro-inflammatory mediators. Another type of inhibitory mechanism involves the active production of decoy receptors that inhibit a select group of cytokines, including IL-1, IL-13 and IL-22. Decoy receptors are non-signaling receptors that have an

equal or higher affinity for their ligand than the signaling receptor. For example, the IL-13Rα2 decoy receptor binds IL-13 and has an essential role in blocking IL-13 and T_H2-mediated inflammation[93]. A final type of inhibition involves the production of cytokine mimics that act as receptor antagonists. The best characterized example of inhibition of IL-1R signaling by the IL-1R antagonist encoded by *Il1ra*. The IL-1R antagonist competes with IL-1α and IL-1β for binding to the IL-1R, blocking signaling[94]. Why do so few cytokines have decoy receptors? While it is conceivable that IL-1, IL-13 and IL-22 have a high potential for inflammatory tissue destruction, other cytokines with known connections to pathogenic inflammation, including IFN-γ, IL-12 and IL-23, lack decoy receptors. Therefore, the decoy receptor system for IL-1, IL-13 and IL-22 likely has a fine-tuning role in inflammation that could be exploited therapeutically for other cytokines. A similar selectivity problem exists when considering the hijacking of cytokines by viruses. IL-10 has been hijacked at least three times (by Epstein-Barr virus, cytomegalovirus and Orf poxvirus) and IL-6 once (Kaposi's sarcoma-associated herpes virus. Yet most viruses that can fit additional genes into their genomes lack virokines, even though these molecules have powerful effects on host immune modulation. Like the cytokine decoy receptors, opportunities exist to engineer new drugs that can modulate acute and chronic inflammation with low toxicity (due to the selectivity for a given receptor).

Perspectives

In this brief overview we have attempted to emphasize that the pathways that restrain inflammation operate at many levels, and over broad time frames. Constitutive and inducible inflammation is regulated by a multitude of cell intrinsic and extrinsic mechanisms that are themselves regulated. Given that a large fraction of clinical medicine and health is concerned with inflammatory diseases, and that many of the most successful drugs target inflammation, it seems likely that new opportunities for disease mitigation can be developed by observing how the body naturally regulates inflammation. To achieve this goal, better tools and techniques are necessary to understand complex signaling pathways. Cell-specific deletions will be also required to assess molecular function in whole animal models of acute and chronic inflammation and these will need to be coupled to more sophisticated and realistic mouse models of inflammation, which will be essential for translation studies to humans. Finally comparative studies between animal models and human tissue samples and *ex vivo* primary cell cultures will be essential to pinpoint the key features inflammatory control relevant to humans.

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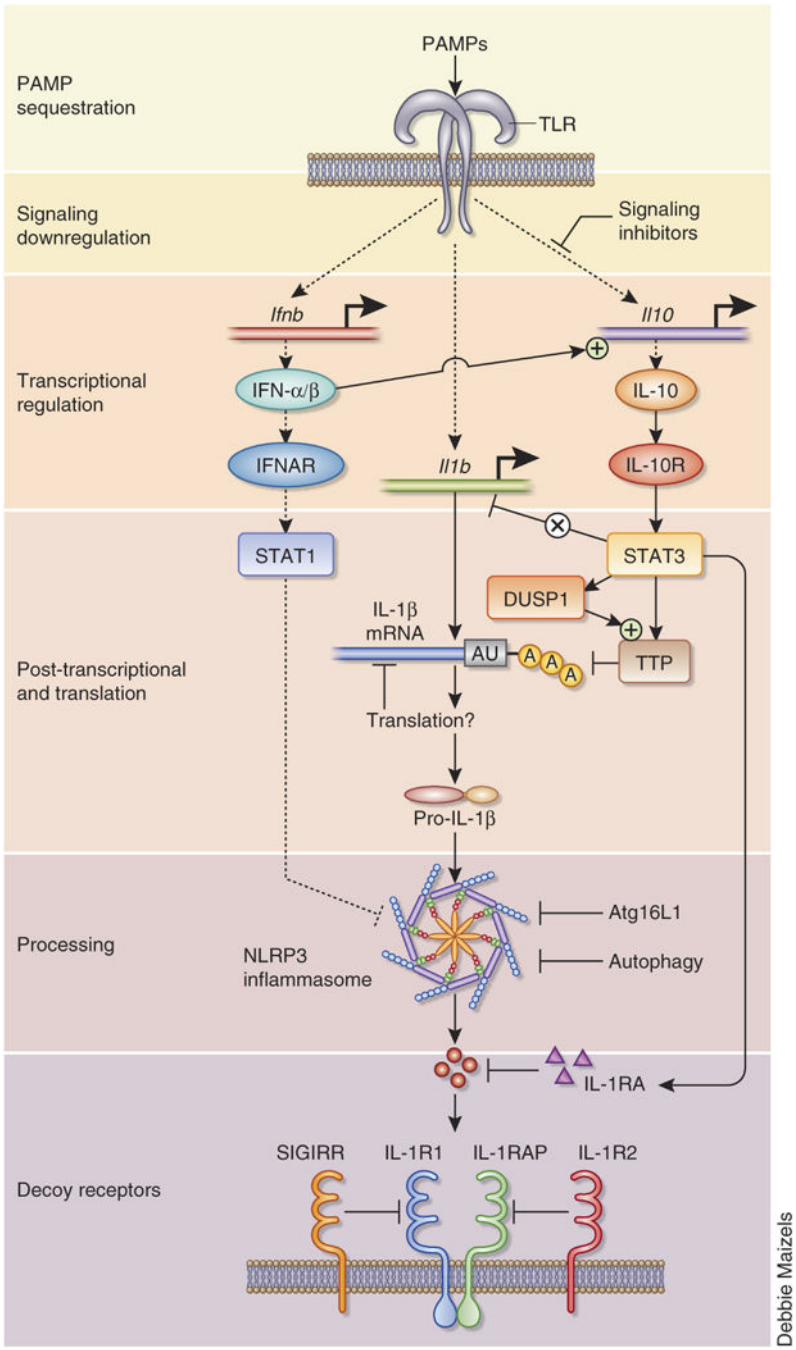

Figure 1.

Negative regulation of IL-1β production, signaling and bioavailability as an example of multi-tiered anti-inflammatory integration. IL-1β is inhibited by at least eight interrelated mechanisms including the initial counter-regulation of TLR signaling, sustained TTP activity via DUSP1, transcription by IL-10, mRNA processing by TTP and related mRNA binding proteins (regulated by IL-10), the type I IFN-mediated inhibition of the NLRP3 inflammasome[116], autophagy regulation by Atg16L1, autophagy-mediated destruction of

inflammasomes[118] and then the signaling and bioavailability of IL-1β itself. Several levels of IL-1β regulation are discussed in detail in the text.

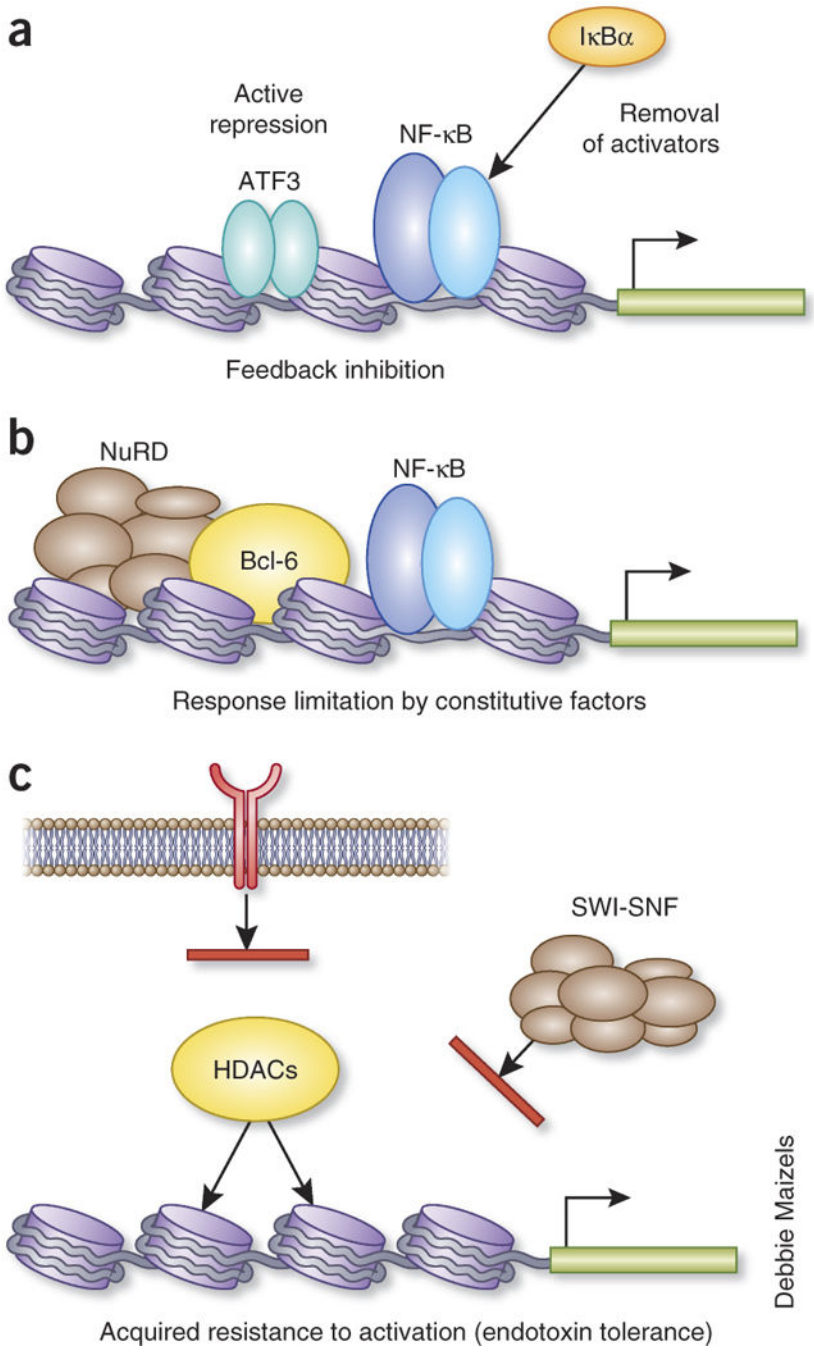

Figure 2.

Three fundamental levels for transcriptional suppression of inflammation (Stratum 5). (**a**) Transcription factors induced during the inflammatory response, such as ATF-3 and IκBα, modulate feedback inhibition on the inducing gene. (**b**) Constitutively expressed factors, like Bcl-6 and NuRD, have sentinel effects on selected genes and limitation the transcriptional response. (**c**) Resistance to activation is acquired by suppressing TLR signaling or through creation of a repressive chromatin structure that may involve histone deacetylation and blockade of SWI/SNF-mediated activation. Secreted factors, such as IL-10, can promote

transcriptional inhibition in different time frames: induced feedback mechanism (a) or a constitutively acting mechanism that limits the potency of activation (b), i.e. in the intestines, depending on context.

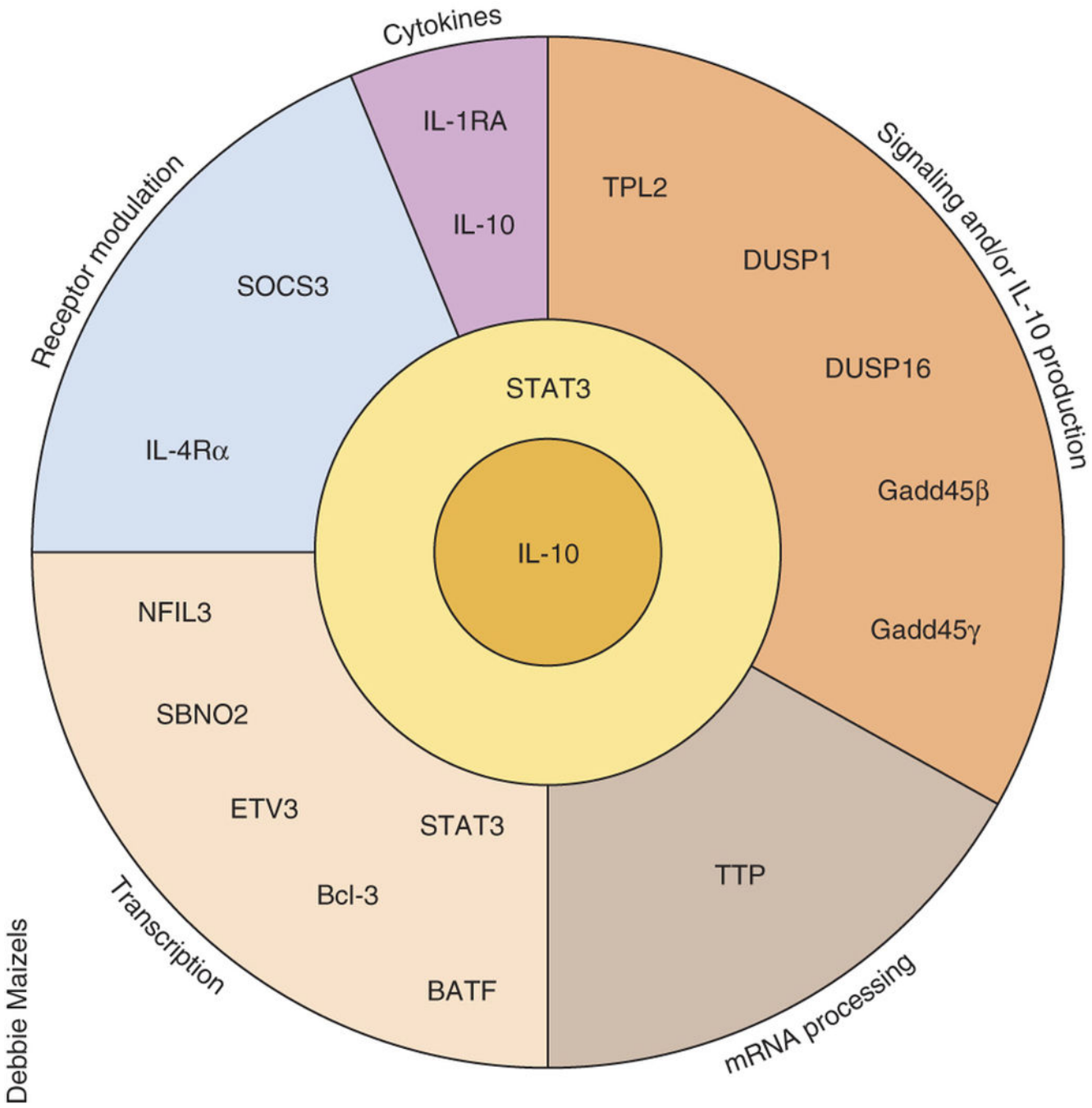

Figure 3.

IL-10 regulates the production of downstream factors that control multiple strata of inflammation. Shown are a subset of known factors induced by IL-10 in a STAT3-dependent way and their known or speculated effects on inflammation. IL-10, via STAT3 also induces further IL-10 production in a self-reinforcing loop. The production of IL-10 by myeloid cells has been described in detail by Saraiva and O'Garra[24], from which this diagram was inspired.

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Table 1

Representative gene deletions that result in enhanced inflammation are shown in this table. Numerous other gene deletions have been linked to negative effects on inflammatory responses. Murine loss-of-function alleles were the primary criteria for inclusion.

STRATA	REPRESENTATIVE EXAMPLES	EXAMPLE GENE DISRUPTION LEADS TO EXCESSIVE INFLAMMATION*
1. Anatomical barriers and Inter-organ communication systems	•Skin	
	•Peristaltic flow of the intestines	
	•Vagus nerve-mediated inhibition of splenic macrophage activation[35]	
	•Sentinel innate B cells that suppress endotoxoc shock[33]	
	•Cytokine-mediated hematopoiesis	
2. Mucosal surfaces and innate wound responses	•Mucous-mediated inhibition of TLR signaling	MUC1 (*Muc1*)[32]
	•Extracellular matrix pathways	CD44 (Cd44)[95]
	•Complement activation	C5aR (*C5ar1*)[96]
	•Coagulation cascade	Anti-thrombin III (*Serpinc1*)[97]
3. PAMP sequestration and repair receptor modification	•PAMP sequestration (soluble TLRs, PAMP binding factors)	sTLR2, sTLR4[5]
	•Receptor competition	SIGIRR (*Sigirr*)[98]
	•Signaling cross-talk	CD11b (*Itgam*)[99]
	•Signal abatement at the membrane	RP105 (*Cd180*)[100], DAP12 (*Tyrobp*)[101]
4. PAMP signaling	•Sentinel inhibitors (can also be induced/regulated)	IRAK-M (*Irak3*)[26], TAK1 (*Map3k7*)[102], CYLD (*Cyld*)[103], Tollip (*Tollip*)[104], TRAF3 (*Traf3*)[105,106]
	•Inhibitors (regulated)	A20 (*Tnfaip3*)[27], ABIN1 (*Tnip1*)[28,29,107], IκBα (*Nfkbia*)[25]
	•Phosphatases	DUSP1 (*Dusp1*)[90,108,109]
	•Kinases	PI3Kp85 (*Pik3r1*)[110,111], MSK1 (*Msk1*)[112], MSK2 (*Msk2*)[112]
5. Transcription	Transcription factors	STAT3 (*Stat3*)[76], IRF4 (*Irf4*)[48], NFIL3 (*Nfil3*)[87,88], ATF3 (*Atf3*)[62]
	Chromatin modification	BCL6 (Bcl6)

STRATA	REPRESENTATIVE EXAMPLES	EXAMPLE GENE DISRUPTION LEADS TO EXCESSIVE INFLAMMATION*
6. Post-transcriptional mRNA regulation	mRNA destabilization	TTP (*Zfp36*)[7,49], HuR (*Elavl1*), AUF1 (*Hnrnpd*), TIA-1 (*Tia1*)[113]
	MicroRNAs	mIR-146a (*Mir146a*)[50]
	Translation	
7. Processing and secretion of inflammatory modifiers		
8. Expression of decoy receptors and receptor antagonists	IL-1 receptor modifiers	IL-1Ra (*Il1rn*), IL-1 decoy receptor (*Il1r2*)[14,115]
	IL-13 decoy receptor	IL-13 decoy receptor (*Il13r2*)[93]
9. Anti-inflammatory cytokines	Sentinel/homeostatis	TGF-β (*Tgfb1*)[8]
	Inducible	Type I IFNs (*Ifnb*)[116]
	Both sentinel and inducible	IL-10 (*Il10*)[9]
10. Metabolic regulators	Global homeostatic mechanisms	
	Autophagy	Atg16L1 (*Atg1611*)[117]