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Gene-specific control of the TLR-induced inflammatory response

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Abstract

Toll-like receptors (TLRs) induce a complex inflammatory response that functions to alert the body to infection, neutralize pathogens, and repair damaged tissues. An excessive or persistent inflammatory response can be fatal, so multiple regulatory mechanisms have evolved to control the extent and duration of inflammation. Our current understanding of the control of inflammation is based on negative regulation of TLR signaling. However, TLR-induced genes have diverse functions, and control of signaling pathways does not allow for groups of genes with distinct functions to be differentially regulated. Recent evidence suggests many inflammatory genes are instead regulated by epigenetic modifications to individual promoters. This level of control allows a single gene to be expressed or silenced according to its function, irrespective of other genes induced by the same receptor, and therefore is “gene-specific.” Gene-specific control of the TLR-induced inflammatory response is an emerging paradigm in the study of inflammation, and may provide the basis for selective modulation of the inflammatory response.

Overview

Inflammation is a complex response to infection, trauma, and other conditions of homeostatic imbalance[1]. Because this response can cause dramatic changes in host physiology, dysregulated inflammation leads to tissue pathology and underlies many human diseases[2]. Current treatments for the diseases caused by persistent or unchecked inflammation often don’t work, have systemic side-effects, or leave patients susceptible to infection. Therefore, it is essential to find ways to safely modulate the inflammatory response. This review will discuss the concept of gene-specific control of the inflammatory response, specifically in the context of the phenomenon of lipopolysaccharide (LPS) tolerance—the altered responsiveness of cells or organisms to repeated doses of bacterial LPS. We suggest that epigenetic modifications are the basis for selective control of different aspects of inflammation.

Inflammation: a complex response initiated by the innate immune system

The inflammatory response to infection consists of multiple components with distinct functions, including pro-inflammatory mediators, which coordinate the immune response, and antimicrobial effectors, which directly target pathogens. Toll-like Receptors (TLRs) are the most well-studied of a growing group of innate immune receptors responsible for

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initiating inflammation[3]. These receptors are expressed by multiple cell-types in the immune system, and recognize structures present on microbes that are not found in the host[3]. Almost immediately after microbes invade, microbial products signal through TLRs on tissue-resident mast cells and macrophages, activating these cells to produce histamine and proinflammatory cytokines. The proinflammatory mediators activate endothelial cells, which then recruit leukocytes, and leak plasma proteins, clotting factors, and complement into the infected tissue. Complement factors both directly target microbes and further activate leukocytes and endothelial cells. Clotting factors stop bleeding and section off the infected area, preventing microbes from spreading. Antimicrobial peptides and proteins (AMP) are also secreted at the site of infection. In contrast to the inflammatory mediators mentioned above, these antimicrobial factors recognize components of bacterial cell walls that are not present in mammalian cell membranes, and therefore target pathogens specifically while having minimal, if any, affect on host tissue physiology[4].

If the pathogen cannot be contained by the initial local response, a systemic response is activated. IL-1 and TNF α produced by macrophages induce fever, presumably making the body temperature inhospitable to pathogens. Macrophages also produce IL-6, which activates the acute phase response (APR) of the liver. The APR induces multiple metabolic and neuroendocrine changes in the body[5]. APR effector proteins include metabolic modulators, additional coagulation factors, anti-inflammatory factors, and opsonins[5]. In addition, peptide hormones, steroid hormones, and endorphins are suppressed or increased, depending on the hormone[5]. Thus, the body undergoes profound alterations in systemic homeostasis to support host defense.

Consequences of dysregulated inflammation

Because the inflammatory response causes dramatic changes in tissue physiology, dysregulated inflammation can lead to a variety of pathological conditions, including septic shock, autoimmunity, atherosclerosis and metabolic syndrome[2]. Recently, many diseases have become linked to a low-grade, chronic inflammatory response. Some of these diseases have been definitively tied to chronic infection. For example, infection with *Helicobacter pylori* can lead to gastric cancer[2]. Other inflammatory etiologies are more cryptic in nature: no infectious cause has been found for the excessive production of SAA (serum amyloid A) in systemic amyloidoses[6]. Chronic inflammation is characterized by an escalating cycle of tissue damage followed by unproductive tissue repair, leading to breaks in self-tolerance (autoimmunity), malignant transformation (tumors), or deleterious changes in tissue morphology and function (fibrotic change)[2].

In contrast to the “smoldering” diseases of chronic inflammation, excessive acute inflammation can quickly be lethal. One of the most dramatic consequences of overwhelming acute inflammation is septic shock due to bacterial LPS, the most potent inducer of the inflammatory response. Too much LPS in the blood leads to excessive production of proinflammatory cytokines including IL-1, TNF α , and IL-6[7]. These cytokines activate endothelium systemically, leading to vascular instability and leakiness[8]. At the same time, clotting factors are induced and anti-clotting factors suppressed, leading to an enhanced production of fibrin clots in small blood vessels[9]. Together with the leakage of fluid from capillaries, this excessive clotting stops blood supply to tissues, and eventually leads to multiple organ dysfunction and death; up to 50% of patients diagnosed with septic shock die, even when given the best supportive care and antibiotics[9].

Patients who survive the acute phase of septic shock often become immunocompromised and susceptible to superinfections[7]. This state is known as immunoparalysis, and is thought to be caused by an overproduction of anti-inflammatory mediators compensating for

the initial overproduction of pro-inflammatory mediators[7]. This compensatory anti-inflammatory state may account for some of the lethality of septic shock[7]. Thus pro-inflammatory cytokines and coagulation factors are essential for initiating host defense, but their persistent production is not necessary for protection. Furthermore, when their production is prolonged or excessive, they can cause systemic host damage.

Regulation of the TLR-induced inflammatory response

Accordingly, to avoid host damage, the inflammatory response must be highly regulated at multiple levels. However, the current understanding of the regulatory mechanisms of inflammation is limited primarily to the control of TLR signaling pathways. Toll-like Receptor 4 (TLR4), the prototypical member of the TLR family, is the receptor for LPS[3]. TLR4 activates several distinct signaling modules, including the MAP kinases, NF κ B, and IRFs (Fig. 1A)[3]. This occurs through two pathways: one that depends on the adaptor protein MyD88, and one that is MyD88 independent. In the MyD88 dependent pathway, IRAK1 and IRAK4 are phosphorylated and dissociate from the complex, leading to the activation of TRAF6, which is then ubiquitinated and activates MAPKs (ERK, JNK, and p38), and the IKK complex, leading to degradation of I κ B α and activation of NF κ B. The MyD88 independent pathway activates the adaptor protein TRIF, which complexes with IRF3, leads to IRF3 phosphorylation by TBK-1 and induction of type 1 IFN-inducible genes. The TRIF pathway also induces a delayed NF κ B response, which, like the MyD88 pathway, is required for almost all gene transcription downstream of TLR4.

Control of inflammation initiated through TLR4 signaling has been intensely studied, leading to the discovery of multiple feedback loops that act on this pathway. Following stimulation, the TLR4-MD2 complex was reported to be removed from the surface[10; 11]. In addition, LPS induces several negative regulators of signaling, including A20, IRAK-M, SIGIRR, ST2, SOCS1, SHIP, and MyD88s (Fig. 1B). A20 is a deubiquitinase for TRAF6, IRAKM may inhibit dissociation of IRAK1 from the receptor complex, SIGIRR and ST2 are orphan receptors that may sequester adaptor molecules, SOCS1 inhibits IFNR signaling, SHIP antagonizes PI3K, and MyD88s is a non-functional splice variant of MyD88[12]. LPS also induces production of the anti-inflammatory cytokines IL-10 and TGF- β [13]. Systemically, LPS-induced proinflammatory cytokines activate the hypothalamus-pituitary-axis (HPA)[5]. The HPA initiates cholinergic anti-inflammatory loops through stimulation of the vagus nerve, and induces corticosteroid release from the adrenal gland[5]. Both of these pathways act on macrophages to limit proinflammatory cytokine production[5; 14].

The cumulative effect of these negative regulators is the phenomenon of “LPS tolerance[13].” LPS tolerance was first described over 100 years ago when fever therapy was used to treat diseases such as manic depression, rheumatoid arthritis, and neurosyphilis[5]. Over time, a patient injected with bacteria or bacterial-LPS-containing vaccines would become progressively less responsive or “tolerant,” and would require greater doses—as much as 250ml of typhoid vaccine—to become febrile[15]. In 1947, Dr. Paul Beeson induced LPS tolerance in rabbits by repeatedly injecting them with LPS, and found that “pyrogen tolerance” was mediated by cells of the reticular endothelial system—macrophages[16]. He hypothesized that the RES cells were detoxifying the LPS, protecting the body from its pyrogenic effect[16]. Surprisingly, the LPS-tolerant rabbits were healthy: “Animals which received daily injections of pyrogens...for periods of several weeks showed no sign of deterioration in general health. They tended to gain weight, their coats remained sleek, and there was no special tendency to develop intercurrent infections[16].”

Soon after Beeson’s seminal work, it was found that after repeated or prolonged exposure to non-lethal doses of LPS, animals become resistant to shock induced by a lethal dose[13].

LPS tolerance is thus an adaptive mechanism of the innate immune system which protects the body against septic shock[13]. In the 1960s, Greissman confirmed that macrophages were directly responsible for both the initial pyrogenic effect, and the later tolerance[17]. In vitro experiments recapitulated the results from animal studies. Following initial treatment with LPS, macrophages become transiently unresponsive, or tolerant, to subsequent stimulations with LPS, as indicated by reduced or abolished induction of pro-inflammatory mediators such as IL-6, IL-1, and TNF α [17; 18].

Macrophage tolerance can be induced by stimulation through other TLRs and reflects a general regulatory strategy that evolved to control TLR-induced inflammation[13; 19]. LPS-induced tolerance, however, has been studied most extensively and is perhaps the most clinically relevant model used to characterize this complex phenomenon. Multiple mechanisms have been proposed to explain in vitro LPS tolerance[10]. As a result of the negative regulators of TLR4 signaling, NF- κ B and MAP kinase activation are decreased in tolerant macrophages[10]. In the nucleus, NF κ B p65/p50 dimers show decreased DNA binding activity, while negative regulators of NF κ B activity are upregulated, including p50/p50 homodimers[10; 20; 21]. IL-10 and TGF β may also induce a tolerant-like state[22; 23; 24]. However, none of the known negative regulators of TLR signaling can individually account for LPS tolerance.

Rationale for gene-specific regulation of the inflammatory response

In general, current understanding of the control of inflammation is confined to “go” signals (microbial products, signs of tissue injury, proinflammatory mediators) that induce “stop” signals (anti-inflammatory mediators, negative regulators of signaling)[1]. So inflammation is primarily considered to be regulated through negative feedback[1; 14]. In the specific case of LPS tolerance, the stop signals downregulate all known signaling pathways downstream of TLR4, and therefore are thought to limit all aspects of the TLR4-induced inflammatory response[10]. LPS tolerance has thus traditionally been viewed as a hyporesponsive state of macrophages resulting from receptor desensitization[10].

However, TLR signaling induces not just proinflammatory cytokines and anti-inflammatory mediators, but also chemokines, antimicrobial proteins, tissue repair factors, metabolic regulators, and controllers of adaptive immunity. The TLR-induced response includes literally hundreds of gene products with very different functions and therefore, different regulatory requirements (Fig. 2)[25]. Thus, it is unlikely that all TLR-induced genes are controlled solely at the signaling level, as this method of regulation would not discriminate between subsets of genes with distinct functions. For example, not all TLR-induced genes have the same potential to disturb tissue physiology. While persistent or excessive production of proinflammatory cytokines leads to the pathologies of chronic inflammatory disease and acute septic shock, excessive production of antimicrobial proteins has minimal effect on systemic homeostasis. Indeed, antimicrobial proteins need to be produced constitutively in many compartments to protect us from opportunistic pathogens—even a transient disruption of antimicrobial activity could provide a window of opportunity for microbes to invade.

Clearly, proinflammatory cytokines and antimicrobial proteins are both essential for a protective immune response, but have vastly different potentials to disturb systemic homeostasis. Therefore, these two distinct functional modules of inflammation should be differentially regulated during a normal inflammatory response. Other modules of inflammation, such as tissue repair, may also require distinct regulation. Since these different functional groups of genes are induced by the same receptor (TLR4), and in the same cell type (macrophage), regulation must be at the level of individual promoters. This

level of control allows a single gene to be expressed or silenced according to its function, irrespective of other genes induced by the same receptor, and therefore is “gene-specific.”

Gene-specific mechanisms for control of inflammation

Gene-specific control of transcription occurs through modifications to chromatin structure[26]. In eukaryotic cells, genomic DNA is packaged with proteins into chromatin. The main unit of chromatin is the nucleosome—DNA wound around an octamer of histone proteins. Nucleosomes are further arranged into higher order chromatin structures, effectively protecting the genome from potential damage, but also making it difficult to transcribe genes. In order for a gene to be transcribed, it has to be unpacked from the chromatin. Thus chromatin structure is the most essential regulator of gene expression in eukaryotes—if chromatin structure around a gene is not accessible, the gene will be silent. However, chromatin alone is not sufficient; in general, transcription is regulated by cooperation between transcription factors and chromatin modifying enzymes[26; 27]. Transcription factors bind specific sequences in DNA, recruit chromatin modifying enzymes, maintain chromatin states (silent or active), and directly turn on genes[26]. The two main classes of chromatin modifying enzymes, chromatin remodeling complexes (CRCs) and histone modifying enzymes often work together to modify chromatin structure[26]. CRCs actively remodel nucleosomes to make DNA accessible, while histone modifying enzymes place covalent marks on histone tails. Histone acetylation is a positive mark that associates with open, transcriptionally active chromatin, while histone deacetylation correlates with closed, silent chromatin[28]. In addition, histones may be methylated, phosphorylated, sumoylated, and ubiquitinated[29; 30].

Over the past decade, chromatin structure has been shown to be an essential regulator of many inflammatory genes. Generally, inducible genes may be classified as primary response (rapidly induced and protein synthesis independent), and secondary response (delayed and protein synthesis dependent). In an elegant study, Smale and colleagues found that nucleosome remodeling determines the differential kinetics of induction for TLR-induced primary and secondary genes[31]. Specifically, they discovered that in LPS-stimulated macrophages, inducible nucleosome remodeling by the SWI/SNF chromatin remodeling complex is required for both secondary and late primary genes to be transcribed. However, early primary response genes were constitutively accessible and did not require further remodeling. Correlating with this data, Saccani *et al.* showed that LPS stimulation of macrophages induces two waves of NFκB recruitment to promoters; the fast wave (10 to 30 minutes) is recruited to primary genes that do not require remodeling, while the slow wave (90 minutes to 2 hours) induces the delayed response genes that require a change in chromatin structure to be activated[32]. These studies imply nucleosome structure is a crucial controller of the kinetics of NFκB-regulated genes. In addition, switching of NFκB dimers can further regulate appropriate timing of inflammatory gene expression in dendritic cells[33]. Rapidly activated p50/p65 dimers are gradually replaced over the period of hours by slowly activated p52/RelB dimers. While these slowly activated dimers maintain expression of some chemokine genes, they inhibit expression of IL-12p40, a cytokine important in regulating adaptive immunity. Lomvardas *et al.* found that a nucleosome positioned over the start site of the IFNβ promoter imposes stringent requirements for activation on IFNβ, so that this gene is only activated in the correct context, for example in response to viral infection, and not in response to TNFα[34]. In contrast, some IFNα target genes are constitutively remodeled so that they can be quickly induced[35]. Thus, gene-specific control mechanisms ensure that TLR-induced genes are expressed with correct timing and in response to the correct stimulus.

Histone modifications also regulate inflammatory genes. A spatio-temporal pattern of histone modifications at the IFN β promoter recruits transcription factors and the remodeling enzyme Brg1[36]. Saccani and others have shown that induction of some groups of early primary response inflammatory genes by TLR signaling are marked by phosphorylation of histone 3 at serine 10 (H3S10), methylation at H3R17, methylation at H3K4, and acetylation at H3K9/H3K14[37; 38; 39]. Silencing of subsets of these genes, on the other hand, is associated with methylation of H3K9[40]. At secondary response genes, the pattern of histone modifications may depend on recruitment of histone modifying enzymes by primary response gene products[39]. Finally, Brogdon *et al.* asked if histone modifications have functional consequences for the immune response by treating dendritic cells and macrophages with the histone deacetylase inhibitor LAQ824[41]. This treatment led to multiple gene-specific effects, including a decrease in transcription of Th1-inducing cytokines and an increase in Th2-inducing cytokines, implying that histone deacetylation may play opposing roles at these two groups of genes.

The above data suggest that distinct sets of genes downstream of TLR4 can be regulated at the level of individual promoters by modifications to chromatin. Can chromatin modifications allow selective control of different functional modules of the TLR-induced inflammatory response? LPS tolerance is an ideal system for addressing this question, as we know that the proinflammatory cytokine module becomes silenced in tolerant cells, presumably to prevent tissue damage. Indeed, McCall's group has found that silencing of IL-1 β and TNF α in an LPS tolerant macrophage-like cell line is due to chromatin modifications at the promoters of these genes[42; 43]. Therefore, we can use the phenomenon of tolerance to look at what happens to other functional groups of genes, specifically antimicrobial effectors. Although proinflammatory cytokines are silenced, genes encoding antimicrobial proteins should remain inducible in tolerant macrophages to protect the host from infection. So we asked whether macrophage tolerance reflects selective silencing of pro-inflammatory, but not antimicrobial genes, or other genes that have limited potential to disrupt tissue physiology[44]. We found that TLR-induced genes fall into at least two categories based on their functions and regulatory requirements. The first class of genes, the tolerizeable (T) genes, is not reinduced by LPS stimulation of tolerant macrophages, whereas the second class, the non-tolerizeable (NT) genes, remains inducible in tolerant macrophages. As we had hypothesized, the first class includes many proinflammatory mediators, while the second class includes antimicrobial effectors, pathogen recognition receptors, chemokines, and other genes that don't directly cause tissue damage.

Representatives from the two classes acquire distinct patterns of TLR-induced chromatin modifications (Fig. 3). Specifically, we found that LPS stimulation of naïve macrophages induces positive chromatin modifications at several representative class T and class NT genes. In tolerant macrophages, however, the LPS signal selectively induces positive histone marks and chromatin remodeling at several class NT, but not at class T promoters. We further found that gene products induced during the first stimulation set up the chromatin modifications that allow silencing of the class T genes and priming of the class NT genes. Collectively, these results clearly demonstrate that LPS tolerance reflects primarily gene-specific silencing mechanisms rather than downregulation of TLR signaling.

Interestingly, the induction of class T and NT genes is qualitatively and quantitatively different in naïve and tolerant macrophages. While class T genes are suppressed after an initial LPS stimulation, the induction of many class NT genes by the second LPS stimulation occurs with faster kinetics and increased magnitude. These changes in gene expression are induced by a transient signal, and are associated with epigenetic modifications. In effect, these chromatin modifications hold transcriptional memory of TLR signaling. Thus, TLR-

induced chromatin modifications provide the molecular basis for the transcriptional memory established during macrophage exposure to microbial products. Such transcriptional memory may enable the innate immune system to deal more efficiently with previously encountered pathogens, and, conversely, to prevent non-specific tissue damage in the face of continuous or repeated exposure to microbes[45].

In vivo evidence for component-specific control of inflammation

Do these findings have significance in vivo? First of all, it is controversial whether or not LPS tolerance in vivo leads to increased susceptibility to infection or to increased resistance to infection[5; 13; 46]. The answer most likely depends on the degree, route, and microbial cause of infection. If the response is overwhelming and systemic, as in patients with severe septic shock, systemic compensatory tolerance does indeed lead to a sort of global anergy[7]. This state is distinct, however, from normal tolerance. Physiological LPS tolerance, where the dose does not have excessive systemic effects, may actually enhance microbicidal activity. For example, LPS tolerant mice are more resistant to gram-negative bacteria and *Cryptococcus*, and show increased phagocytosis and killing of *Candida*[13; 47; 48]. Indeed, as noted above, even after Beeson injected his rabbits with LPS every day for several weeks, the animals had "...no special tendency to develop intercurrent infections[15]."

In addition, some compartments in the body are naturally tolerant. For example, the gut mucosa is the largest body surface in contact with the external environment, and hosts billions of microbes. Improper inflammation in the gut leads to inflammatory bowel disease (IBD)[2]. Intriguingly, Smythies *et al.* discovered that intestinal macrophages, although they are constantly exposed to LPS, make almost no proinflammatory cytokines[49]. They do, however, retain potent phagocytic and bactericidal activity. The authors propose stromal cell derived factors including TGF β "condition" recruited monocytes to differentiate into this tolerant phenotype. We may speculate that this differentiation includes chromatin modifications that selectively silence proinflammatory genes.

Glucocorticoids (GC), one of the most well-known anti-inflammatory agents, may also differentially regulate modules of the inflammatory response. Given at supraphysiological levels, GC powerfully suppress immune responses and often make patients susceptible to infection[50]. It has been puzzling why GC used to treat asthma do not lead to lung infections. Similarly, it's not known why low dose, but not high dose, GC improve survival in septic shock[9]. Perhaps the answer lies in GC gene-specific effects. In the lung, while GC inhibit proinflammatory cytokines, they may induce antimicrobial proteins such as collectins and defensins, and enhance survival of monocytes and neutrophils[51]. This is proposed to occur through activation of CEBP β in airway epithelia[52]. Consistent with the above data, in the case of LPS tolerance, we found that the glucocorticoid receptor (GR) agonist dexamethasone inhibited several class T genes, but synergistically induced several NT genes[44]. One mechanism by which GR exerts gene-specific effects is by inhibiting genes that use IRF3 as a co-activator for NF κ B dependent transcription[53].

Finally, a recent paper by Scott *et al.* describes development of a small "innate defense-regulator peptide" that is able to inhibit proinflammatory cytokine production while enhancing antimicrobial activity in macrophages and in mice[54]. The mechanism of the peptide's action is not clear.

Conclusions

Safe and effective treatments for many inflammatory diseases currently do not exist; most therapies either target a single proinflammatory mediator or broadly affect multiple body

systems. While the first type of drug only works in very specific contexts, the second type of drug may have unwanted side effects. For example, blocking TNF α doesn't work for people with sepsis, although it is highly effective for people with rheumatoid arthritis[7; 55]. Glucocorticoids, on the other hand, work well to inhibit inflammation in many contexts[50]. However, since they affect multiple systems, GC also have severe side-effects including diabetes, decreased wound healing, skin atrophy, muscle-wasting, adrenal axis insufficiency, cataracts, ulcers, hypertension, metabolic disorders, water imbalance, and osteoporosis[50]. In addition, both TNF α blockers and GC increase susceptibility to infections[55].

Ideally, we would like to inhibit inappropriate proinflammatory responses and leave antimicrobial and other physiological responses intact. In this review, we have discussed the possibility that such selective control of inflammation could be achieved through epigenetic modifications. Gene-specific control of sub-groups or modules of inflammatory genes with distinct functions is an emerging paradigm in the study of the innate immune response to infection. This new conceptual scheme may provide important insight into the nature of the inflammatory response and lead to development of novel treatments for inflammatory disease.

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Figure 1.

The TLR4 signaling pathway is highly regulated. A. Following LPS binding, TLR4 signals through two main adaptor molecules, MyD88 and TRIF. In the MyD88 dependent pathway, IRAK1 and IRAK4 are phosphorylated, then dissociate from the complex and activate TRAF6. TRAF6 activates the MAP kinases ERK, JNK, and p38, as well as the IKK complex. IKK phosphorylates I κ B α , leading to its degradation, releasing NF κ B to enter the nucleus and activate gene transcription. In the TRIF dependent pathway, TBK-1 phosphorylates IRF3, which then dimerizes and enters the nucleus to transcribe Type 1 IFN-inducible genes. TRIF signaling also leads to a delayed NF κ B response. B. TLR4 induces multiple negative regulators. A20 deubiquitinates and deactivates TRAF6. IRAKM may

inhibit dissociation of IRAK1 from the receptor complex. SIGIRR and ST2 are inhibitory cell-surface receptors. SOCS1 inhibits IFNR signaling, SHIP antagonizes PI3K, and MyD88s is a non-functional splice variant of MyD88. An unknown regulator likely inhibits IRF3 phosphorylation. IL-10, TGF β , and cholinergic stimulation also inhibit induction of proinflammatory cytokines. *Schematic adapted with permission of D. Hargreaves.*

Figure 2.

The TLR4-induced inflammatory response has multiple components with distinct regulatory requirements. Upon recognizing bacterial infection, TLR4 signaling initiates the inflammatory response gene program. Inflammatory response genes fall into different functional groups, or components. Each component is regulated according to its role in the immune response and its potential for deleterious effects on the body if produced excessively. For example, proinflammatory cytokines and vasoactive factors (clotting factors, histamine) need only be induced transiently, and require tight regulation to prevent systemic shock. Antimicrobial proteins need to be produced for as long as pathogens persist, have no potential to cause shock, and are not highly regulated. For some components, such as oxidative killing, which have both direct antimicrobial activity and potential to damage tissues, the regulatory requirements are less clear, and must be tissue-type and context-dependent.

Figure 3.

LPS tolerance is an example of gene-specific regulation of the inflammatory response. Following LPS stimulation of naïve macrophages, both class T and class NT promoters recruit transcription factors and display increased histone acetylation, histone H3K4 trimethylation, and accessibility (represented by yellow starburst). In tolerant macrophages stimulated with LPS, class T promoters are deacetylated and inaccessible. Class NT promoters, on the other hand, are inducibly reacetylated, remethylated, and made accessible with faster kinetics.