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# The Resolution of Inflammation

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# Preface

It was with tremendous enthusiasm that we endeavoured to compile and edit this volume for *Progress in Inflammation Research* describing novel findings and developments pertaining to the processes governing the resolution of inflammation. It is perhaps surprising that this topic had, to our knowledge, not previously been covered as a separate subject area in a dedicated monograph given what now seems such an obvious thing to do. Historically, researchers have focussed and have made great advances on the initiation and propagation of inflammation. Little attention had been specifically devoted to elucidating the mechanisms orchestrating the resolution of inflammation, although a variety of mechanisms that limit the inflammatory response had been described (e.g., mediator dissipation and deactivation; exogenous mediator removal or reduction; receptor, cell and tissue desensitisation to mediators; identification of agents with anti-inflammatory potential such as IL-10, IL-1 receptor antagonists, TGF-β, etc).

It is now believed that manipulation of more recently described processes, recognised as being actively involved in resolution, are therapeutically manipulatable for the treatment of inflammatory diseases. Indeed, patients with chronic inflammatory diseases are by necessity treated in order to reduce established and persistent inflammation with the added hope of preventing further progression of the inflammatory response. It has recently become evident that many of the anti-inflammatory agents currently used in the clinical setting influence inflammatory resolution. For example, glucocorticoids have been shown to influence processes now recognised as being important mechanisms allowing resolution to occur; namely glucocorticoids trigger apoptosis (programmed cell death) in most leukocytes (the neutrophil however is a notable exception) and augment apoptotic cell clearance by phagocytes. Similarly, aspirin, the most widely used NSAID, is involved in an unorthodox biosynthetic pathway yielding important lipid mediators (e.g., 15-epi-lipoxin A4 and 15-epi-lipoxin B4) actively involved in the resolution process.

This volume contains major contributions from an international panel of experts who describe the basic processes regulating the resolution of inflammation including apoptosis, macrophage clearance of apoptotic cells and novel pro-resolution lipid

mediators. In addition, there are sections that describe how existing anti-inflammatory drugs such as aspirin and glucocorticoids may influence these resolution processes. There are three chapters devoted to describing fine examples of clinically relevant inflammatory disease areas where much progress has been made in understanding resolution. We feel that we are at the beginning of a rapidly burgeoning and exciting area of inflammation research where new advances are being made in understanding the resolution of inflammation. It is without doubt that continued research will fully elucidate the mechanisms whereby existing anti-inflammatory drugs influence resolution. Furthermore, there is now emerging experimental *in vivo* evidence indicating that by pharmacologically and selectively inducing apoptosis of inflammatory cells, specifically enhancing non-phlogistic clearance of apoptotic cells by phagocytes, and administration of pro-resolution lipids (e.g., lipoxins, resolvins and protectins), inflammatory resolution is achievable. Consequently, we believe that better designed and novel classes of drugs that specifically target resolution processes will be forthcoming in the not too distant future.

October 2007

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# The resolution of acute inflammation: A 'tipping point' in the development of chronic inflammatory diseases

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## The scope of this chapter

Evolution has given us inflammation, a formidable ally in the constant battle against infection, cancer and tissue injury. It is a primordial response that protects against injury and restores damaged tissue to its normal physiological function. In fact, our well-being and survival depends upon its efficiency and carefully balanced control. In general, the innate inflammatory response initiates within minutes and, if all is well, resolves within hours. In contrast, chronic inflammation persists for weeks, months or even years. Here, we are going to discuss the key endogenous checkpoints necessary for mounting an effective, yet limited, inflammatory response and the crucial biochemical pathways necessary to prevent its persistence. Figure 1 depicts what we understand today about the endogenous soluble mediators that control the severity of inflammatory onset as well as its longevity. In doing so, we wish to underline the consequence to the host of failing to adequately control inflammatory resolution.

Acute inflammation is characterised by leukocyte recruitment from the circulation, classically defined by the initial trafficking of polymorphonuclear granulocytes, followed by monocytes, which differentiate locally into macrophages [1]. Invariably, this response is triggered by tissue mast cells and resident macrophages, whose degranulation and activation sequentially release a battery of inflammatory mediators, including bioactive amines (histamine and 5-HT), cytokines, chemokines as well as lipid mediators that collectively recruit and activate inflammatory cells, which also results in oedema formation. While this system has an enormous capacity for synergy and redundancy, over the years it has served as the stable basis for the development of anti-inflammatory drug discovery, typified by the development of nonsteroidal anti-inflammatory inhibitors of eicosanoid synthesis beginning in the 1960s to more recent times with the inhibition of the actions of TNF-α. These early days of inflammation research that focused on elucidating the nature of soluble pro-inflammatory mediators have now given way to the view that inflammation is far more complex and sophisticated than originally appreciated, not least muddied by

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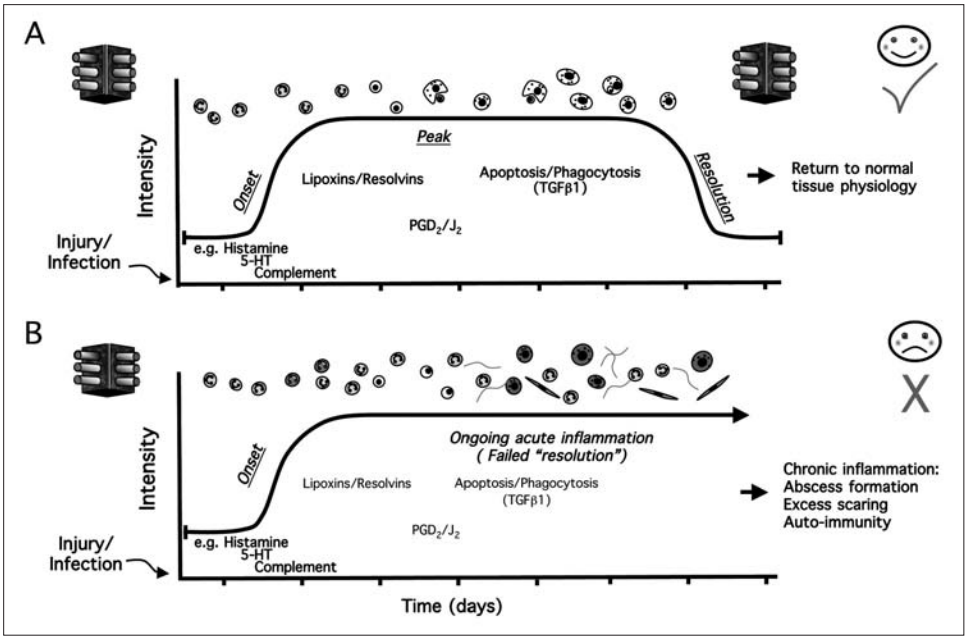


*Figure 1*
*Schematic depicting the cellular and molecular components of resolving inflammation. Acute inflammation is characterised by the accumulation of neutrophils and oedema early in the response. Later, mononuclear cells and macrophages accumulate and help prepare the tissue for resolution. In both (A) and (B) we depict the role that specific molecular mediators play in these events. In (A), sequentially released pro-inflammatory mediators are released very early in response to injury/infection, which initiate and augment the acute-phase of the response (green lights). However, this is counterbalanced by endogenous anti- inflammatory signals such as corticosterone, which serve to temper the severity and limit the duration of this early onset phase. As inflammation progresses, certain „stop signals" prevent further leukocyte traffic into tissue. These stop signals include the lipoxins, resolvins and prostaglandins (PGs) of the D series, and pave the way for monocyte migration and their differentiation to phagocytosing macrophages. These remove dead cells and then exit the site of inflammation. Stromal cells such as fibroblasts also contribute to the resolution of inflammation by the withdrawal of survival signals and the normalisation of chemokine gradients, thereby allowing infiltrating leukocytes to undergo apoptosis or leave the tissue through the draining lymphatics. This sequential set of responses leads to complete resolution and, importantly, the restoration of the inflamed tissue to its prior physiological functioning. This is the ideal sequence of events in physiological inflammation, which contrast to the situation in pathological inflammation (B), where some of the factors that initiate the resolution program lead to the inappropriate accumulation of leukocytes in the wrong place at the wrong time (from [69]).*

the multiple protective and destructive roles the eicosanoids, for instance, that are now known to play in orchestrating the inflammatory response. Such clear diversity is not the preserve of lipid mediators but it extends to cytokines, chemokines and the expression of both activating and inhibitory receptors by inflammatory cells. On this theme of biological diversity, recent evidence suggests that alternative pattern recognition receptors of the scavenger receptor and C-type lectin families may play equally important roles in the recognition of microbes and the regulation of the host inflammatory response. Thus, the C-type lectin, Dectin-1 [2], was recently shown to act in concert with the Toll-like receptor (TLR)-2 to activate macrophages exposed to β-glucans from the yeast *Candida albicans* [3]. A number of these receptors also recognise endogenous inflammatory ligands including the scavenger receptors SR-A and CD36, both of which have been described to mediate the phagocytosis of apoptotic cells, leading to a down-regulation of macrophage activation [4–6] (see the chapter by Dransfield et al.). Thus, many of the factors that drive inflammation also double-up in bringing about its resolution and it is this theme of inflammatory resolution that is going to be the focus of this chapter. In current day inflammation research, one of our objectives must be to understand whether known inflammatory mediators that ignite inflammation also trigger its resolution as well as highlighting resolution. In addition, resolution must be highlighted as a critical facet of the inflammatory response and, at the very least, to underline the importance of not altering its normal course of action when developing novel anti-inflammatory drugs. Ultimately, it is proposed here that resolution is controlled by endogenous pro-resolution factors, which may represent new possibilities for drug discovery in terms of designing modalities that mimic their mode of action or enhance their synthesis. In the course of doing so, we hope to argue that resolution is as active process, whose failure may predispose the host to chronic inflammatory diseases and autoimmunity, such as that typified by rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus and asthma.

## Resolution of acute inflammation

The receptors and signalling pathways that initiate and promote the inflammatory response have become increasingly well characterised; however, relatively little is known about how acute inflammation resolves to prevent chronic inflammatory diseases. We have discussed above the intracellular checkpoints that limit the activation of inflammatory cells either directly in response to infection or tissue injury or through paracrine activation by proinflammatory cytokines. If we were to define the fundamental requirements for the successful resolution of inflammation it is becoming increasingly clear that the most simple but absolutely critical determinant for the inflammatory response to switch off is the neutralisation and elimination of the injurious agents that initiated it. Failure to achieve this first step will invariably lead

to chronic inflammation, with the nature of the agent in question almost certainly dictating the aetiology of the developing chronic immune response. For example, chronic granulomatous disease is characterised by severe, protracted and often fatal infection, which results from a failure of the phagocytic NADPH oxidase enzyme system to produce superoxide and kill invading infections, leading to a predisposition to recurrent bacterial and fungal infections and the development of inflammatory granulomas [7]. Successfully dispensing with the inciting stimulus will signal a cessation to pro-inflammatory mediator synthesis (eicosanoids, chemokines, cytokines, cell adhesion molecules, etc.) and lead to their catabolism. This would halt further leukocyte recruitment and oedema formation. These are probably the very earliest determinants for the resolution of acute inflammation, the outcome of which signals the next stage of cell clearance. The clearance phase of resolution, be it innate immunity [polymorphonuclear leukocyte (PMN) or eosinophil driven] or adaptive immunity (lymphocyte mediated), also has a number of mutually dependent steps. The clearance routes available to inflammatory leukocytes include systemic recirculation [8] or local death of influxed PMNs, eosinophils or lymphocytes followed by their phagocytosis by recruited monocyte-derived macrophages [9]. Once phagocytosis is complete, macrophages can leave the inflamed site by lymphatic drainage [10] with evidence that a small population may die locally by apoptosis [11]. If all of these pathways are strictly followed then acute inflammation will resolve without causing excessive tissue damage and give little opportunity for the development of chronic, non-resolving inflammation. As with the onset phase of the acute inflammatory response, which is driven by a cohort of well-described endogenous factors, the resolution phase of the response is also highly coordinated and under the tight control of what may be called "pro-resolution" factors. In contrast to onset, however, these resolution phase factors are less well described.

## Controlling the early phase of acute inflammation

Inflammation is a reaction of the microcirculation that is characterised by the movement of serum proteins and leukocytes from the blood to the extravascular tissue with PMNs or eosinophils predominating at the early onset phase, giving way to phagocytosing macrophages leading to resolution. One well-described event in the transition towards resolution is the replacement of PMNs or eosinophils by monocytes and phagocytosing macrophages. However, until recently our understanding of the signals that control this cell profile switch was unclear. Studies addressing this issue of leukocyte infiltration in peritoneal inflammation have suggested that the interaction between interleukin (IL)-6 and its soluble receptor, sIL-6R, forms one of the major determinants of this switch from PMNs to monocytes [12, 13]. It was shown that sIL-6R, produced by the infiltrating PMNs, forms a complex with IL-6, which in turn, directly modulates CC and CXC chemokine expression. Thus, CXC

chemokine synthesis, induced by IL-1 and TNF-α, was suppressed, whereas the CC chemokine CCL2 (MCP-1) was promoted. This chemokine shift suppresses further neutrophil recruitment in favour of sustained mononuclear cell influx. In addition to chemokines, the eicosanoids also orchestrate the early transition to resolution in acute inflammation. Transcellular metabolism of arachidonic acid by lipoxygenase/lipoxygenase interaction pathways gives rise to the lipoxin (LX) family of eicosanoid metabolites [14]. LXs display selective actions on leukocytes that include inhibition of PMN chemotaxis [15], PMN adhesion to and transmigration through endothelial cells [16], as well as PMN-mediated increases in vascular leakage [17]. It is unclear at this point whether there is any cross-talk between the LXs and IL-6/sIL-6R complex signalling in the control of leukocyte profile switching. Nonetheless, it seems that when acute inflammation needs to resolve the IL-6/sIL-6R, chemokines and LXs represent some of the earliest signals that control the switch from very early PMNs to monocyte/macrophage.

## The transition to resolution

Once PMNs and eosinophils have done their job and their help is no longer needed, what happens next? At this juncture it must be borne in mind that these are a formidable cell lineage and if left unchecked could do untold damage to an already inflamed site. After all, these cells are designed to combat infection by releasing hydrolytic and proteolytic enzymes as well as generating reactive oxygen species. Therefore, PMNs and eosinophils must be disposed of in a controlled and effective manner. To oversee this, nature has come up with an ingenious way of defusing such potentially explosive cells called programmed cell death or apoptosis. Apoptosis of inflammatory cells is a physiological process for the non-phlogistic removal of cells. During apoptosis, cells maintain an intact membrane and, therefore, do not release their potentially histotoxic agents. Necrosis of inflammatory leukocytes, on the other hand, involves a loss of membrane integrity, leading to the release of potentially toxic intracellular contents [9]. Moreover, apoptotic cells express a repertoire of surface molecules that allow their recognition and phagocytosis by macrophages [18]. Despite stating above that once the injurious agent has been neutralised PMNs and eosinophils are redundant, in fact, the way in which these cells die helps the resolution process enormously. Recognition of these apoptotic cells by macrophages does not liberate pro-inflammatory agents from the macrophages themselves but can release anti-inflammatory signals such as IL-10 and transforming growth factor-β (TGF-β) [19]. Thus, not only is apoptosis a non-inflammatory way of disposing of cells, but this method has the added advantage of conferring upon macrophages an anti-inflammatory phenotype conducive to resolution. It is important to note that if not recognised and disposed of, apoptotic cells will eventually undergo secondary necrosis releasing damaging intracellular contents and amplifying the inflammatory

response. Therefore, increasing the rate of apoptosis, as a potentially anti-inflammatory strategy, must be matched by a mechanism that up-regulates macrophage phagocytic clearance capacity [20]. Thus, the removal process might also be susceptible to selective modulation by pharmacological agents for therapeutic gain.

As mentioned above, the strategy of enhancing leukocyte apoptosis must also be paralleled with enhancing their phagocytosis by macrophages and other non-professional phagocytes. On this theme, there are an increasing number of factors that aid the phagocytic clearance of apoptotic granulocytes. Ligation of the matrix receptor CD44, for instance, results in the rapid and specific internalisation of apoptotic PMNs [21]. Besides controlling PMN trafficking, LXs also stimulate monocyte chemotaxis and adherence. Certainly, this may seem dangerous for inflammation as too many monocyte-derived macrophages can be a bad thing, but these LX-chemoattracted macrophages accelerate resolution by enhancing phagocytosis of apoptotic PMNs in a non-phlogistic manner [22]. In addition to a role in granulocyte apoptosis, glucocorticoids facilitate the phagocytic response. It was recently found that exposure of peripheral blood monocytes to glucocorticoids during the first 24 h of the 5-day culture period induced a highly phagocytic monocyte-derived macrophage phenotype [23]. Functional and morphological homogeneity was matched by cell surface phenotype, including specific induction of expression of the haemoglobin scavenger receptor, CD163 following glucocorticoid treatment. A potentially pro-resolution role for CD163 was demonstrated recently in both *in vitro* and *in vivo* models of resolving inflammation [24]. Here, the authors showed that human peripheral blood monocyte-derived macrophages either in culture medium or in resolving phase cantharidin-induced skin blisters express CD163. These authors also found elevated levels of CD163 on circulating monocytes in cardiac surgical patients during the resolution phase of the systemic inflammatory response to cardiopulmonary bypass surgery. In each case, binding of the haemoglobin–haptoglobin complex to CD163-bearing cells elicited potent IL-10 secretion, which in turn enhanced hemeoxygenase 1, widely shown to have anti-inflammatory and tissue protective properties. Such induction of hemeoxygenase 1 was observed *in vivo* 24–48 h after the onset of cardiopulmonary bypass surgery. This is coincident with the observations of Willis and colleagues [25], who showed that hemeoxygenase 1 was expressed during and essential for the resolution (24–48-h phase) of a rat carrageenin-induced pleurisy. Thus, apoptosis and the phagocytosis of apoptotic cells are crucial to the resolution process, failure of which may predispose, as mentioned earlier, to chronic inflammation and possibly autoimmunity. This has been proposed in the case of systemic lupus erythematosus (SLE); an autoimmune syndrome that is associated with the presence of autoantibodies to endogenous antigens exposed on dead or dying cells, which failed to be cleared and disposed of. Studies in mice, for instance, have established a role for the complement receptor C1q on macrophages in the development of this disease. C1q was discovered to be important for the phagocytic clearance of apoptotic cells; in the absence of this receptor mice devel-

oped a lupus-like syndrome [26]. The persistence of apoptotic cells and necrotic bodies led to the development of an inappropriate immune response to endogenous antigens. Evidence has also been established in human SLE patients for an association between C1q deficiency and disease [27]. The phagocytosis of apoptotic cells has been suggested to play an important role in the negative regulation of macrophage activation, apoptotic leukocytes may well fit into the category of endogenous anti-inflammatory mediators, therefore the mechanisms of apoptosis and the clearance of apoptotic cells may be critical in the development of chronic inflammation (as discussed further by Dransfield et al. in this book).

## Soluble mediators of resolution: Opportunities for drug discovery

Returning to eicosanoids, prostaglandin (PG) $D_2$, a metabolite of the action of haematopoietic $PGD_2$ synthase on COX-derived $PGH_2$, has emerged recently as an eicosanoid with both pro- and anti-inflammatory properties. $PGD_2$ undergoes dehydration *in vivo* and *in vitro* to yield biologically active PGs of the $J_2$ series, including $PGJ_2$, $\Delta^{12,14}$-$PGJ_2$ and 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ (15d-$PGJ_2$). In addition to being a high-affinity natural ligand for anti-inflammatory peroxisome proliferators-activated receptor gamma (PPARγ), 15d-$PGJ_2$ also exerts its effects through PPARγ-dependent as well as -independent mechanisms to suppress pro-inflammatory signalling pathways and the expression of genes that drive the inflammatory response. 15d-$PGJ_2$ also preferentially inhibits monocyte rather than PMN trafficking through the differential regulation of cell-adhesion molecule and chemokine expression [28]. We have shown that COX 2-derived $PGD_2$ metabolites contribute to the resolution of acute inflammation (pleuritis) through the preferential synthesis of $PGD_2$ and 15d-$PGJ_2$ [29], which, along with the alternative DNA-binding p50–p50 homodimers complexes of NF-κB [30] bring about resolution by inducing leukocyte apoptosis [11]. Recently, we extended these studies to examining the role of $PGD_2$ metabolites in the resolution of adaptive immunity and lymphocyte function [31]. Indeed, there is an increasing body of evidence detailing the differential effects of $PGD_2$ metabolites on leukocyte apoptosis as well as the signalling pathways involved [32, 33].

In addition to the well-known eicosanoids, there is a new generation of lipid mediators showing real promise as endogenous anti-inflammatories. Resolvins and docosatrienes are fatty acid metabolites of the COX/lipoxygenase pathways, where the omega-3 fatty-acid constituents of fish oils docosahexaenoic acid and eicosapentaneoic acid are the substrates and not arachidonic acid. These resolvins and docosatrienes were identified in inflammatory exudates during the resolving phase of acute inflammation and shown to be potent inhibitors of PMN transendothelial migration and microglial-cell cytokine expression, and to ameliorate experimental models of dermal inflammation and leukocyte accumulation in peritonitis at nano-

gram doses [34]. These studies on resolvin metabolism are uncovering surprising new avenues in anti-inflammation research, putting fatty acid metabolites right at the forefront of potential drug therapy. These studies are also challenging existing dogma that not all eicosanoids are detrimental to inflammation and are putting a balanced view of their role in pathophysiology. To add fuel this notion, a recent and very surprising paper has shown that eicosanoids of the LXs family, described above, are orally active in models of acute inflammation [35].

## Signalling pathways that regulate inflammation

The inflammatory response is characterised by coordinated activation of various signalling pathways that regulate expression of both pro- and anti-inflammatory mediators in resident tissue cells and recruited leukocytes (see Fig. 1). Currently most of our knowledge of signalling in inflammation is gained from studying members of IL-1 and TNF receptor families and the Toll-like microbial pattern recognition receptors (TLRs), which in fact belong to the IL-1R family. IL-1 and TNF-α represent the archetypal pro-inflammatory cytokines that are rapidly released upon tissue injury or infection. TLRs recognise microbial molecular patterns, hence the term pattern-recognition receptor (PRR), and therefore TLRs represent a germline-encoded non-self recognition system that is hard-wired to trigger inflammation. However, there is some suggestion that endogenous ligands may trigger TLRs during tissue injury and certain disease states, which may act to promote inflammation in the absence of infection [36]. Although structurally different, these receptors use similar signal transduction mechanisms. Receptor engagement results in recruitment of adaptor proteins that possess either Toll–IL-1 receptor (TIR) domains in the case of TLRs and IL-1R or death domains (DD) in the case of the TNFR family, linked to the regulation of cell survival [37]. Once recruited these adaptors recruit further signalling proteins that belong to the TRAF family [38, 39] and various protein kinases, including IRAK1 and 4 in the case of TIR signalling [40] and RIP kinases in the case of TNFR signalling [41, 42]. These molecules activate several effector pathways, the most important of which lead to activation of mitogen-activated protein kinases (MAPK) [43, 44], including JNK [45] and p38 MAPK [46], as well as IκB kinases (IKK) [47]. The MAPKs lead to direct and indirect phosphorylation and activation of various transcription factors, especially those that belong to the bZIP family: AP-1 [48] and CREB [49], which bind to the promoters of pro-inflammatory genes. MAPKs also regulate pro-inflammatory gene expression through post-transcriptional mechanisms such as mRNA turnover, mRNA transport and translation [50–52]. The IKKs, which form a complex composed of two catalytic subunits-IKKα and IKKβ and a regulatory subunit IKKγ/NEMO, are responsible for activation of the NF-κB transcription factor [47], which has emerged as a central regulator of inflammatory and immune responses [53, 54]. Target genes for the IKK

and MAPK pathways include IL-1 and TNF-α, generating a feed-forward mechanism to amplify the inflammatory response. The pro-inflammatory cytokines IL-6, IL-12 and type I interferons (IFNs), which are also target genes for IKK and MAPK regulation, signal *via* receptor-associated tyrosine kinases (RTKs) that belong to the JAK group, whose activation results in phosphorylation and nuclear translocation of STAT transcription factors [55]. Engagement of cytokine receptors, as well as TLRs, can also lead to activation of phosphoinositide-3-kinases (PI3K), which in turn activate other proteins kinases such as AKT [56]. Collectively, these proteins kinases coordinate the expression of a large number of pro-inflammatory mediators to initiate and maintain the inflammatory response.

## Negative regulation of pro-inflammatory signalling

All of the intracellular signalling pathways described above, which contribute to the onset of innate immunity and inflammation are also subject to negative regulation. PI3K signalling is inhibited by the PTEN phosphatase that belongs to the protein tyrosine phosphatase (PTP) family; some of its other members, for instance SHIP, SHP1/2 and CD45, are responsible for negative regulation of TK signalling [57]. MAPK kinase phosphatases (MKPs), which also belong to the PTP family, control the duration of MAPK activation as recently shown for TNF-α-mediated JNK activation [58]. Inducible suppressors of cytokine signalling (SOCS), which function as ubiquitin ligases, are responsible for the negative feedback control of JAK-STAT signalling [59]. A20 is another inducible ubiquitin ligase, which functions as a negative feedback regulator of TLR and TNFR signalling to IKK and NF-κB. A20 is also a direct target gene for the NF-κB pathway constituting a negative feedback loop for NF-κB activation [60]. Recently, a new pathway for negative regulation of IKK/NF-κB was described from observations made in mice that harbour a variant of IKKα, $IKK\alpha^{AA}$, that can not be activated by upstream regulators. Although $IKK\alpha^{AA}$ mice do not develop "spontaneous" inflammation, they develop an exaggerated inflammatory response when challenged with bacteria, fungal cell wall particles, or even immune complexes [61]. These studies established that, while IKKβ catalytic activity is important for the activation of NF-κB through phosphorylation of endogenous inhibitory (IκB) proteins [62], IKKα is required for termination of NF-κB activation through phosphorylation of the transcription factors RelA (p65) and c-Rel [61]. IKK-mediated phosphorylation results in polyubiquitination of the target protein, leading to its accelerated degradation *via* the 26S proteasome. However, while IκB degradation is essential for NF-κB activation and nuclear translocation, the accelerated degradation of nuclear Rel proteins *via* IKKα-mediated phosphorylation is important for controlling the duration of NF-κB activation. The evolution of two catalytic subunits in the IKK complex with opposing, yet complimentary, activity therefore ensures rapid and transient activation of NF-κB.

As discussed below, pro-inflammatory signalling pathways have the capacity of inducing the parallel expression of anti-inflammatory mediators, such as IL-10. Recent studies reveal that the signalling pathway used by TLRs to activate expression of pro- and anti-inflammatory cytokines diverges at the level of the adaptor proteins TRAF3 and TRAF6, such that TRAF3 is critical for induction of IL-10 expression and in its absence, expression of the TRAF6-dependent pro-inflammatory cytokines IL-6 and IL-12 is dramatically increased [63]. The balance between the TRAF3- and TRAF6- generated signals may therefore play an important role in controlling the inflammatory response and its perturbation may interfere with the proper resolution of inflammation.

Some of these signalling pathways and their respective negative regulators are illustrated in Figure 2; a deficiency in any one of these negative regulators may result in either "spontaneous" chronic inflammation, perhaps reflecting host cell activation by PAMPs present in endogenous microflora, or an exaggerated inflammatory response to insult or injury that culminates in severe inflammation and damage to the host. Although all of these negative regulatory mechanisms affect different signalling pathways, genetic studies in mice have shown that even the absence of one negative regulator is sufficient to result in serious inflammatory disorders. Undoubtedly, aberrations in such negative regulatory pathways will be found to contribute to the development of chronic inflammatory diseases.

## Resolution of T cell-driven inflammation

Inflammation has an important role in instructing the adaptive immune response, in particular the maturation and migration of dendritic cells (DCs) from the site of inflammation, where they pick up antigens and traffic to the secondary lymphoid organs where they can prime antigen-specific immune responses. There have been major advances in the study of DC biology in the past few decades that clearly show that both pro-inflammatory cytokines, including TNF-$\alpha$, and microbial products drive DC maturation and migration to draining lymphoid tissue. The immature DC at the site of inflammation has the capacity to efficiently take up and process antigens. Specific signals in the inflammatory environment trigger the expression of chemokines and receptors that promote the migration of antigen-loaded DCs to the local lymphoid organs where they can present their antigens to cells of the adaptive immune system. Although cross-talk between the innate and adaptive immune system is extensively reviewed elsewhere [64], it suffices to underline that the switch from an inflammatory response driven by short-lived granulocytes to a lymphocyte- or macrophage-dominated event is classically associated with chronic inflammation. Various investigators have examined the resolution of lymphocyte-driven adaptive immune responses including Type III hypersensitivity (Arthus reaction) or Type IV delayed-type hypersensitivity (DTH) reactions, which are clinically relevant models

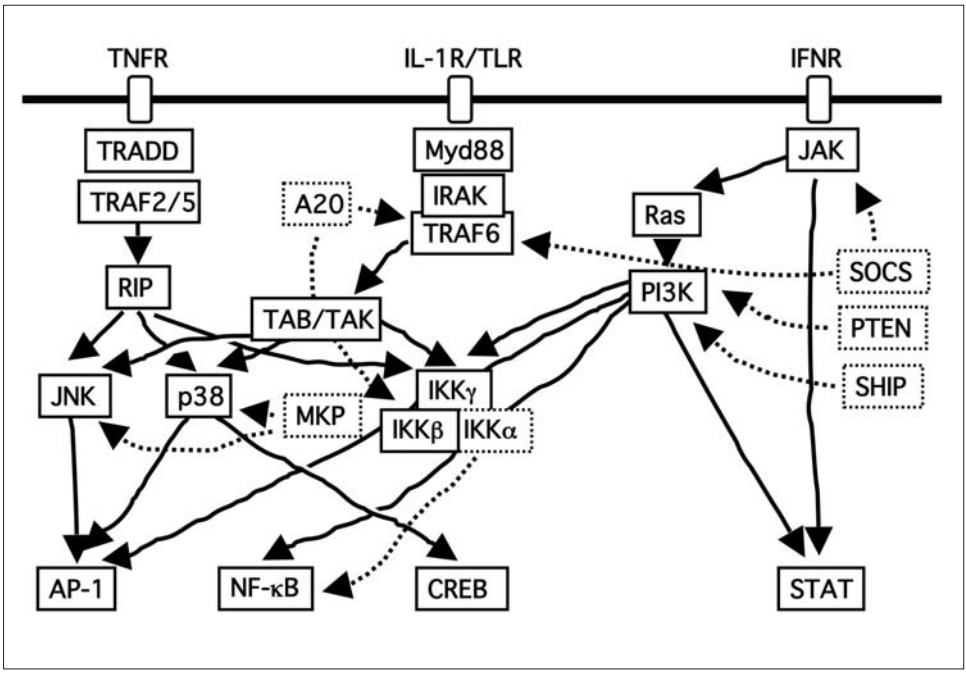


*Figure 2*
*Schematic illustration of the co-ordinated activation of pro-inflammatory signalling pathways by TLR ligands and the pro-inflammatory cytokines TNF-α, IL-1 and IFN.*
*Adaptor molecules (MyD88, TRADD, TRAF) and receptor associated kinases (RIP, IRAK, JAK) couple to downstream kinase cascades (MAPK; JNK, p38; TAB/TAK, IKK), which regulate the activation of transcription factors (AP-1, NF-κB, CREB, STAT) and the expression of pro-inflammatory genes. A number of negative regulatory mechanisms (broken lines) limit the activation of specific signalling pathways; SOCS targets JAK/STAT and TLR signalling; PTEN and SHIP phosphatases block PI3K; A20 and IKKα negatively regulate the NF-κB pathway; the MAPK phosphatase MKP limits activation of JNK and p38.*

of adaptive immune diseases. For instance, in a purified protein derivative-induced DTH response, it was shown that the induction and resolution of this response may depend on the expression of cytokines, such as IL-2 and IL-15, that regulate both proliferation and apoptosis in T cells [65]. Failure to control either of these phases of the reaction may contribute to the chronicity of T lymphocyte-mediated inflammatory reactions. In another important series of studies the endogenous factors that control the longevity of granulomatous autoimmune thyroiditis revealed that the ratio of $CD4^+/CD8^+$ T cells are critical determinants of its resolution. In this disease process, $CD4^+$ T cells outnumber $CD8^+$ T cells when lesions progress to

fibrosis, while $CD8^+$ T cells outnumbered $CD4^+$ T cells in thyroids that resolve [66]. Recently, we found that haematopoietic $PGD_2$ synthase ($hPGD_2S$) transgenic mice, bearing a DTH reaction, display an exaggerated inflammatory response that fails to resolve [67]. While $hPGD_2S$-derived $PGD_2$ and the cyclooxygenase-derived PGs possess potent but diverse biological roles in host defence, the suppressive effects of $hPGD_2S$ on T lymphocyte functioning appears to be mediated by 15d-$PGJ_2$ and its inhibition of NF-κB DNA binding, with no contribution from $PGD_2$ and its actions on either of its receptors namely DP1 or DP2/CRTH2. These findings suggest an important role for $hPGD_2S$ as a checkpoint controller in the progression from acute to resolving inflammation. Whether the absence of $hPGD_2S$ predisposes to chronic inflammation or autoimmunity has yet to be determined. Nonetheless, the clear lack of inflammation in animals that over-expressed $hPGD_2S$ further reinforces the critical role that this down-stream $PGH_2$ metabolising enzyme plays in the aetiology of T lymphocyte-driven immune responses.

## Unblocking the drains!

The role of the lymphatic system in the context of the resolution of acute innate inflammation is enormously understudied given its essential function in draining inflammatory mediators and effete leukocytes away from the inflamed site [68]. We have already discussed the importance of PMN clearance to the resolution of acute inflammation, but it is equally important that phagocytosing inflammatory macrophages are cleared away from the inflamed site to prevent local macrophage-induced tissue damage, potential granuloma tissue damage and the development of chronic inflammation. However, despite the need to understand the endogenous control of macrophage clearance during acute inflammatory resolution, little is known about this field. There is increasing evidence that macrophage clearance from an inflamed site is a highly regulated event. Using an experimental model of acute resolving peritonitis, it was shown that macrophages adhere specifically to mesothelium overlying draining lymphatics and that their emigration rate is regulated by the state of macrophage activation [10, 68] providing the first evidence that macrophage emigration from the inflamed site is controlled by adhesion molecule regulation of macrophage–mesothelial interactions. This report highlights the importance of adhesion molecules controlling clearance of inflammatory macrophages into the draining lymphatic circulation, thus highlighting new pathways in the resolution of acute inflammation.

## Conclusions

In conclusion, it is clear the inflammatory response has a number of built-in checkpoint controls that limit the duration and magnitude of acute inflammation. Defects

in these endogenous anti-inflammatory pathways will undoubtedly predispose to the development of chronic inflammatory diseases. A further understanding and analysis of these pathways in the pathogenesis of chronic inflammation is required, this will allow the pursuit of therapeutic strategies to correct possible defects in these feedback control systems or manipulate these pathways to suppress inflammation. However, it is equally clear that the resolution of inflammation is driven by a complex set of pro-resolution mediators that regulate specific cellular events required to clear inflammatory cells from the site of injury or infection and restore tissue homeostasis. These endogenous anti-inflammatory and pro-resolution mechanisms are clearly intimately linked; however, the true goal in the treatment of chronic inflammatory diseases must be to inhibit persistent inflammation and restore tissue function. To achieve this goal we must improve our understanding of the resolution of inflammation and identify possible approaches to promote this process in combination with anti-inflammatory therapy. Perhaps the, as-yet-unidentified, anti-resolution factors that may prevent the proper resolution of inflammation would represent appropriate targets to achieve this goal.

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