

# Stroma: the forgotten cells of innate immune memory

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## Summary

All organisms are exposed constantly to a variety of infectious and injurious stimuli. These induce inflammatory responses tailored to the threat posed. While the innate immune system is the front line of response to each stimulant, it has been considered traditionally to lack memory, acting in a generic fashion until the adaptive immune arm can take over. This outmoded simplification of the roles of innate and acquired arms of the immune system has been challenged by evidence of myeloid cells altering their response to subsequent encounters based on earlier exposure. This concept of ‘innate immune memory’ has been known for nearly a century, and is accepted among myeloid biologists. In recent years other innate immune cells, such as natural killer cells, have been shown to display memory, suggesting that innate immune memory is a trait common to several cell types. During the last 30 years, evidence has slowly accumulated in favour of not only haematopoietic cells, but also stromal cells, being imbued with memory following inflammatory episodes. A recent publication showing this also to be true in epithelial cells suggests innate immune memory to be widespread, if under-appreciated, in non-haematopoietic cells. In this review, we will examine the evidence supporting the existence of innate immune memory in stromal cells. We will also discuss the ramifications of memory in long-lived tissue-resident cells. Finally, we will pose questions we feel to be important in the understanding of these forgotten cells in the field of innate memory.

**Keywords:** endothelial cell, fibroblast, inflammation, innate immune memory, stromal memory

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## Introduction

In an immune setting, the term ‘memory’ evokes vaccines, memory T cells and the antibody response. Memory in mammals is not, however, solely the remit of lymphocytes. The field of immunology is changing, and evidence in both arms of the immune system is breaking down old concepts of innate and adaptive roles.

Inflammatory memory is an ancient characteristic of innate immune cells. Vertebrate–invertebrate divergence preceded the appearance of adaptive immunity, and yet evidence in invertebrates as disparate as meal worm beetles and shrimp, *Drosophila* and lower metazoans all points towards inflammatory responses altered by prior exposure to infectious agents (reviewed in [1]).

Innate immune memory in mammals was first observed during the early 20th century. The bacillus Calmette–

Guerin (BCG) vaccine was found to save more children than died of tuberculosis, indicating an off-target effect [2]. This work has progressed over decades to illustrate innate memory in a range of experiments studying mammalian defence against secondary pathogens. Crucially, studies in severe combined immune deficiency (SCID) mice have shown the BCG off-target protection to be driven by innate – not adaptive – memory [3,4]. Such research has focused mainly on myeloid cells, with other haematopoietic cell types also attributed to innate memory in recent years (reviewed in [5] and [6]).

Despite the abundance of research on innate memory, stroma is still largely overlooked when considering repeat challenges in immune or inflammatory episodes. Indeed, the recent discovery of epithelial innate memory [7] prompted a *Nature* news and views article, which stated:

'This is the first identification of inflammatory memory in a non-immune cell' [8]. It is clear that stromal memory is not well-known or appreciated.

Given the recent resurgence of interest in stromal memory responses, and the latest observations showing that haematopoietic, epithelial and stromal cell types all alter their responses based on previous insults, we believe it is time to discuss the field of stromal innate memory. In this review, we will collate three decades of stromal memory research, discuss its strengths and unanswered questions and suggest key avenues of enquiry. Innate memory is proving ever more important to our understanding of inflammation biology, and it is time that stroma, the forgotten cells of the memory field, were remembered.

Because some of the important terms in this review may be subject to different interpretations, we will begin with some definitions. The 'stroma' comprises ubiquitous structural cells, providing extracellular matrix (ECM) and support functions in every organ. In this review we take 'stromal cells' to exclude cells of haematopoietic origin; for example, tissue-resident macrophages. Macrophages and other myeloid cells are discussed below merely by way of introduction to the concept of memory. To our knowledge, the vast majority of the research relevant to stromal memory has been performed on fibroblasts and endothelial cells. We therefore focus on these cells, but we consider it very likely that memory also exists in other stromal cell types. We define 'memory' as a state of altered responsiveness that does not depend upon the continued presence of the stimulus by which it is evoked. A key characteristic of this form of memory is that it outlasts the primary signalling response of the cell. In the experiments discussed below memory lasts from days to weeks, or even longer. There will clearly be some overlap between memory and differentiation, for example in a few experimental designs discussed below, where stromal cells are exposed to persistent inflammatory stimulation rather than being stimulated, rested, then stimulated again. As far as this review is concerned, we define 'inflammatory memory' as a change in the capacity of stromal cells to respond to inflammatory stimuli, as opposed to an inflammation-induced change in steady-state gene expression.

### Stroma in inflammation

During the last three decades, our understanding and appreciation of the roles played by stromal cells in healthy and pathological inflammation has increased exponentially. Two well-studied stromal cells [endothelial cells (EC) and fibroblasts] are described below.

EC line the blood and lymph vessels, and thus have an important role in inflammation. Both host and foreign cells must interact with EC in order to extravasate into tissue. Their release of chemokines recruits leucocytes to a site of infection or injury, and their expression of adhesion

molecules facilitates adhesion, rolling and diapedesis of circulating cells (reviewed in [9]).

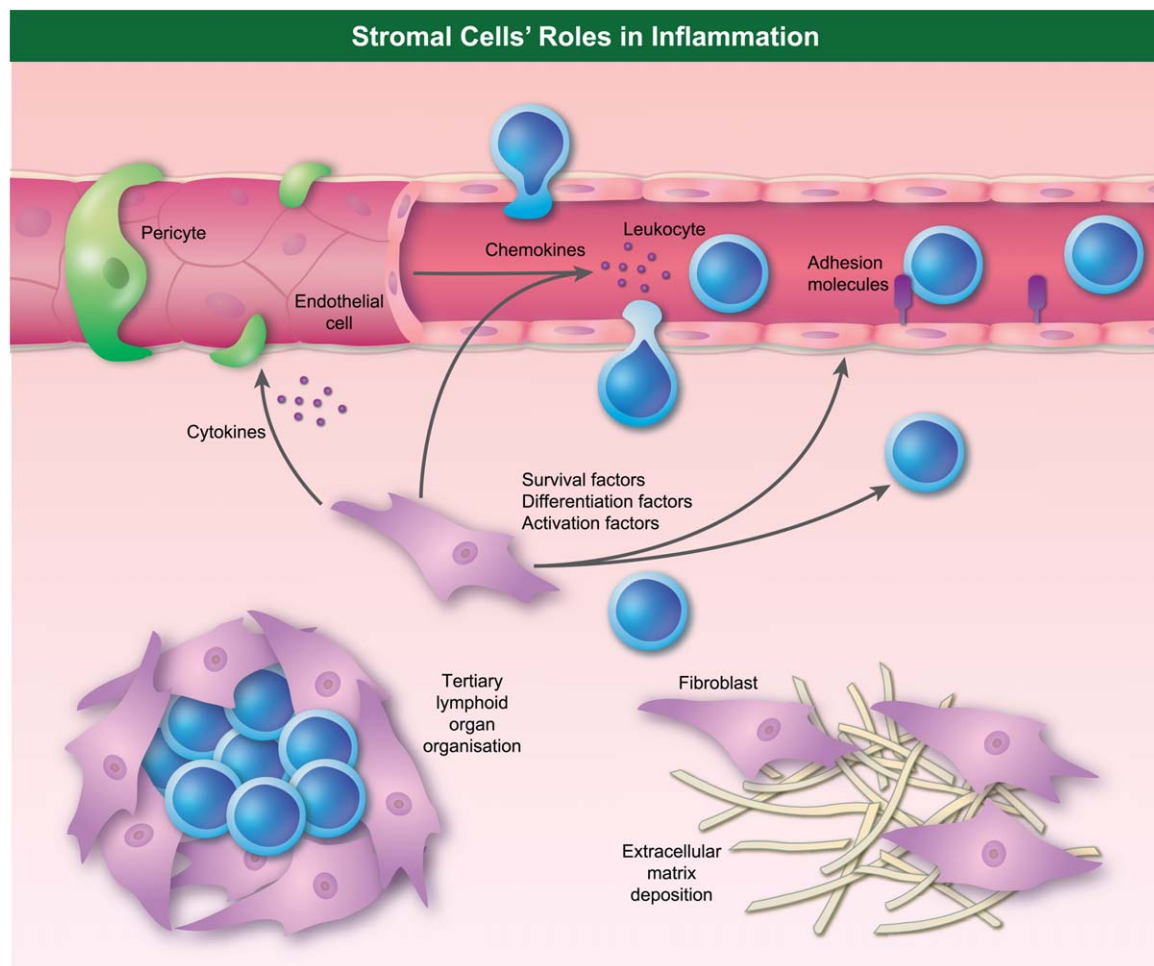
Fibroblasts are a major source of ECM and play organ-specific roles, such as lubricin production in the joint and lymphocyte support in lymphoid organs. They act as immune sentinels, with some capacity for phagocytosis [10] and antigen presentation [11]. They also release chemokines to recruit leucocytes and cytokines to modulate the nearby EC [12]. The fibroblast influence on EC adhesion molecule expression has profound effects on leucocyte extravasation. In healthy inflammation fibroblasts limit extravasation through EC, while fibroblasts from inflamed joints promote increase leucocyte extravasation [13,14].

Once leucocytes extravasate into tissue, fibroblasts influence their behaviour via the regulation of survival, activation, differentiation and retention [15,16]. It is therefore not surprising that pathologically altered fibroblasts are integral to several chronic inflammatory diseases. The roles of stromal cells in inflammation are illustrated briefly in Fig. 1. Due to the number of roles played, understanding how these cells alter their behaviour after an initial inflammatory episode could be crucial to our understanding of human pathology.

### Myeloid innate memory

Monocyte/macrophages are innate cells of the myeloid lineage, which act as immune sentinels, phagocytes of pathogens and cellular debris and as professional antigen presenters. In this sense, they are often recognized as the link between innate and adaptive immunity. This is also true in a temporal sense, as many studies have found that monocytes infiltrate tissue after neutrophils but before lymphocytes [17,18]. Others also found that the phagocytosis of apoptotic neutrophils by macrophages (known as efferocytosis) is a key point in progressing from the acute to the adaptive phase of inflammation (reviewed by [19]).

As an intermediary between the two arms of our immune system it is perhaps not surprising that, of all innate cells, macrophages should have memory. This may take either positive or negative forms, in that the response to a second challenge may be either enhanced or diminished by memory of a prior challenge. Both short- and long-term forms of myeloid cell memory have been described. Long-lived myeloid cell memory that underpins the beneficial off-target effects of vaccines such as BCG has been coined 'trained immunity' by Netea and colleagues [1]. This phenomenon is exhibited only after the cells have been educated by an initial infection, lasts for several weeks or even months after the initial infection, and induces an augmented inflammatory response to second infection. The long-term alteration is driven by epigenetic modifications, which are described in more detail later. Trained immunity has been studied in a wide range of primary and secondary challenges, and particular attention has been paid to the BCG vaccine and fungal



**Fig. 1.** Roles of stromal cells in inflammation. Illustration of the functions stromal cells use to influence the fate of an inflammatory episode. Secreted and presented mediators are illustrated, as are as key organizational roles.

products ( $\beta$  glucan) or organisms (*Candida albicans*) as providing wide-scale protection against a range of secondary agents. This protection takes the form of lower mortality rates and better pathogen clearance [4].

The other form of memory is a transient reduction of the inflammatory response. In 1947 bacterial substances were found to have diminishing pyrogenic effects in rabbits receiving successive doses [20]. In 1965, this was furthered by research showing that rabbits could be saved from death by a lethal dose of liposaccharide (LPS) if they had previously received a sublethal dose [21]. This phenomenon was termed 'endotoxin tolerance', and was attributed to macrophages some time later. While tolerance implies no effect of endotoxin on the macrophage, this is an oversimplification. It is true that successive doses of endotoxin abrogate the expression of proinflammatory mediators but, contrastingly, increase expression of genes related to phagocytosis [22]. This results in a phenotype which avoids tissue damage while still facilitating removal of infectious agents. It is therefore important to distinguish it from

'immune paralysis': the state of disorganized immune response seen after trauma or burns, and a contributing factor to mortality in sepsis patients [23]. Endotoxin tolerance is therefore a sophisticated state of altered responsiveness, rather than simply an 'off switch' for macrophages.

### Fibroblast inflammatory memory

The study of fibroblast memory goes back to 1992 [24]. Early papers arose from discoveries pertaining to modulation of interleukin (IL)-8 expression by interferon (IFN). After the discovery that IFN- $\beta$  or - $\gamma$  would reduce tumour necrosis factor (TNF)- $\alpha$ -induced IL-8 expression [25], a series of publications examined the effect of IFN on endogenous or exogenous induction of IL-8, including the consequences of challenging fibroblasts with one stimulus and then another [24,26–28]. Thus, the field was born.

The earlier years of the new millennium saw no new publications on the subject, and it appeared forgotten. In the interim, appreciation of fibroblast involvement in chronic

inflammatory diseases was continuing to grow. With the greater understanding of stromal epigenetic alterations resulting in long-term increases or decreases of chromatin accessibility, authors began to suggest that a 'fibroblast inflammatory memory' may be involved in the perpetuation [29,30] or re-ignition [31], of inflammatory conditions.

At the same time, the primary publications on fibroblast memory were re-emerging. In 2009 gingival fibroblasts were shown to maintain their inflammatory response to re-challenge with LPS, whereas macrophages became refractory [32]. In 2011, however, gingival fibroblasts were shown to be capable of tolerance, but in a gene-specific fashion [33]. A recent spate of publications [34–38] has advanced our understanding further. Sohn *et al.* displayed inflammatory memory of T cell chemoattractants in fibroblast-like synoviocytes (FLS) derived from patients with rheumatoid arthritis (RA) [39]. Our own work built upon these findings by displaying positive memory to be shared by FLS from both inflamed and non-inflamed joints [37]. While both papers showed gene-specificity of memory in response to endogenous stimuli, the latter also displayed tropism, showing that FLS had memory while dermal fibroblasts did not. These findings suggest that inflammatory memory can be a property of the site of origin of fibroblasts rather than disease state.

Recently, Klein *et al.* also demonstrated the tropism of fibroblast memory and its gene-specificity [38]. Repeat challenges with LPS induced a refractory state seen only in fibroblasts of certain sites, and only in genes linked to the anti-viral response. Dakin *et al.* showed that tendon fibroblasts from chronic tendinopathy patients mounted stronger inflammatory responses *in vitro* than those of healthy tendons, thus demonstrating the longevity of innate immune memory in fibroblasts [35,36]. They also discovered that while intracellular signalling diminished over time after removal of stimulus, surface markers of fibroblast activation such as podoplanin (PDPN) and vascular adhesion molecule-1 (VCAM-1) were maintained at high levels. This suggests that memory in stromal cells does not necessarily manifest as continued inflammatory secretion, but rather as an altered state of readiness with increased activation receptors [36] and altered chromatin accessibility [38,39]. The altered expression of some fibroblast cell surface markers at sites of chronic inflammation could be interpreted as a differentiation phenomenon. Alternatively, it could reflect expansion of a fibroblast subpopulation that responds differently to inflammatory challenge. These interpretations cannot be resolved without deeper understanding of the molecular mechanisms involved.

### Endothelial cell inflammatory memory

Research into the concept of endothelial cell memory is newer than that of fibroblast memory, but a range of challenges have been examined. This field dates at least to 2002

[40], and publications are increasing in frequency. As seen in fibroblast memory, some authors have not found an altered response to second challenge [41], while others have shown both positive and negative memory responses indicative of a complex interplay of stimuli and cellular pathways. The examination of cross-stimulation (initial and secondary stimuli inducing different pathways) has led to the conclusion that some mechanisms of innate memory are shared with the myeloid lineage [42], but not all observations match, suggesting cell- and stimulus-specific pathways.

The anatomical site of origin and inflammatory stimulus have been shown to dictate EC memory responses, and the vessel architecture has also been demonstrated as important. Shear stress (pressure exerted by blood flow on the vessel walls) is higher in straight vessel sections than arches or junctions. Work from the Evans laboratory has shown that EC under low shear stress have constitutively active c-Jun N-terminal kinase (JNK) [43], which is suppressed by dual specificity phosphatase-1 (DUSP-1) in areas of high shear stress [44]. This high level of JNK activity correlates with VCAM-1 expression and thus the ability to facilitate leucocyte adhesion. The authors described JNK as 'priming' EC for inflammation, and pointed out that primed areas were atheroprone, while areas with high shear stress and suppressed JNK activity were atheroresistant.

Studies have confirmed the relevance of EC memory in both infectious and non-infectious inflammatory contexts. Prior infection by *Schistosoma mansoni* increases leucocyte adherence through the endothelial layer [45]. Others have shown that EC exposed to circulating contributors to atherosclerosis have augmented inflammatory responses to subsequent challenges by exogenous or endogenous stimuli [40,46]. In the examples above, altered EC responses were driven by persistent exposure to inflammatory stimuli. Whether such changes should be considered as memory or differentiation is arguably a question of semantics.

In 2008, Zemani *et al.* found that EC progenitors performed better as therapeutic cells in models of ischaemic injury if they were primed *ex vivo* with C-X-C motif chemokine ligand 12 (CXCL12) before donation [47]. Similarly, in 2016 Stark *et al.* found the endotoxin tolerance seen in EC could be manipulated. An initial challenge with LPS induced an inflammatory response, which was followed by non-responsiveness to subsequent LPS challenges. If, however, monophosphoryl lipid A (MPLA) was used as the initial stimulus, the non-responsiveness to LPS was achieved without an initial inflammatory event [48]. This suggests that EC memory may be amenable to therapeutic manipulation. Thus, there is an obvious need to understand endothelial memory at basic science and clinical levels.

### Mechanisms underpinning innate memory

The mechanisms behind innate memory have been investigated intensively in the myeloid lineage. The effects of Toll-

like receptor (TLR)-4 signalling in particular have received most attention, and have been reviewed comprehensively [49]. For this review, it suffices to say that TLR-4 signals through the myeloid-differentiation primary response protein 88 (MyD88) and Toll/IL-1R (TIR)-domain-containing adapter-inducing IFN- $\beta$  (TRIF) pathways. MyD88 induces proinflammatory cytokine expression through pathways such as that of nuclear factor kappa B (NF- $\kappa$ B). TRIF induces the interferon response and thus the production of IFN response factors (IRF) and anti-viral effectors. These pathways are well established and reviewed by [50].

### Negative feedback signalling

The proinflammatory TLR-4 response is, as with all healthy inflammatory episodes, curtailed by in built feedback loops [51,52]. Both the MyD88 and TRIF pathways induce the expression of negative regulators. These may inhibit TLR-4 signalling at levels distal, intermediate or proximal to gene expression. IL-1 receptor-associated kinase (IRAK) M and A20, for example, inhibit, respectively, the release of IRAKs and TNF receptor-associated factor (TRAF)6 from MyD88, and the actions of TRAF6 and TRIF [53,54]. Conversely, SH-2 containing inositol 5' polyphosphatase (SHIP) 1 inhibits inhibitor of  $\kappa$ B kinase (IKK) activity downstream of MyD88 and IRF3 downstream of TRIF [55]. Finally, the non-canonical NF- $\kappa$ B p50 homodimer can inhibit canonical NF- $\kappa$ B binding to promoters, presumably by steric hindrance [56].

Two studies of fibroblasts are useful to note here. The first is that of Lee *et al.* [57], who showed that rheumatoid arthritis RA-derived FLS maintained an inflammatory response to TNF- $\alpha$  up to at least 4 days after initial challenge. The authors also displayed FLS expression of several of the negative regulators to be negligible compared to expression in macrophages. The second is that of Ara *et al.* [32], who found that neither *Escherichia coli* LPS nor *Porphyromonas gingivalis* LPS (TLR-4 and TLR-2 agonists, respectively) could induce tolerance in gingival fibroblasts. They also showed that human gingival fibroblasts lack expression of classic negative regulators. During myeloid endotoxin tolerance, negative regulators are up-regulated, leading to disrupted signalling through both pathways. While the above fibroblast studies showed a deficiency in negative regulators, neither showed explicitly that this was the reason for propagation of the inflammatory response. Future studies are necessary to link these observations with alterations in memory responses, as has been confirmed in myeloid cells [58].

### IFNs

IFNs are induced in response to infectious agents, and also in response to tumour cells and proinflammatory cytokines. Their role in innate memory appears multi-faceted. IFN- $\gamma$ , for example, can rescue endotoxin tolerant

macrophages from their refractory state by opening condensed chromatin [59]. Conversely, IFN- $\beta$  can induce tolerance of a subset of genes in fibroblasts [24,33]. Even in studies of the same cell type (i.e. fibroblasts), IFNs were shown to have both inhibiting [24,26,28] and augmenting [26,28] roles. This disparity was not due to the IFN, but rather to the timing of its administration. Sohn and colleagues found that IFN- $\alpha$ , - $\beta$  and - $\gamma$  could all induce augmented responses from TNF- $\alpha$ -primed FLS [34]. This not only confirms that different IFNs may have redundant roles in innate immune memory, but also provides evidence that they may act as the initial or secondary challenge.

Koch *et al.* developed a theory based on cross-priming EC with TLR agonists [42]. They suggested that initial stimulation induced an increase in IRF-7 accumulation, facilitating a greater response to subsequent challenges. Such a theory may be supported by the finding that FLS have a TNF- $\alpha$ -induced IFN- $\beta$  autocrine feedback loop which increases chemokine release [60]. If both mechanisms were conserved across FLS and EC, one could envisage the initial stimulation providing a large IRF pool, leading to a greater cellular response to autocrine IFN feedback in the second response. This model relies upon uniting mechanisms from different cell types. Even among fibroblasts of different origins this may be inappropriate, as synovial fibroblasts release IFN- $\beta$  [60], while those from the gingiva do not express it [33]. The effects of tropism, and of different experimental designs with different cell types, stimuli and readouts, means that three decades of research have yet to provide a clear model for the role of interferons in stromal memory.

### NF- $\kappa$ B

The NF- $\kappa$ B pathway is well known for its involvement in the expression of proinflammatory genes. In macrophage endotoxin tolerance, the canonical NF- $\kappa$ B pathway is refractory. Its inhibition is induced by the increase in inhibitory p50 homodimers at the loci of proinflammatory genes [56]. There is also increased repression of the IKK complex. Due to this, the abundance of I $\kappa$ -B $\alpha$  is higher in tolerized than naive cells [61], and it has also been reported to replenish its cytoplasmic pool faster in the second challenge compared to the first [58,59].

Studies have shown that repeated TLR-4 engagement leads to repression of the NF- $\kappa$ B pathway in endothelial cells [42,46]. This included decreased activation of the IKK complex and concomitant reduction in NF- $\kappa$ B-dependent cytokines. In direct contradiction, Wang *et al.* found that the second challenge with TLR-4 agonist increased EC cytokine production, and this correlated with increased NF- $\kappa$ B pathway activity [41]. Both studies used human umbilical vein ECs and *E. coli* LPS for their experiments, so the explanation for the disparity is unclear. What is clear, however, is that the action of the NF- $\kappa$ B pathway during

the memory response correlated with the increase or decrease of inflammatory mediators elicited by the second challenge. There is also evidence for NF- $\kappa$ B-dependent altered responses in fibroblast memory. In fibroblasts stimulated with TNF- $\alpha$  for 24 h then rested for 24 h, both NF- $\kappa$ B activity and IL-6 expression subsided to basal levels during the rest period. Restimulation of the rested cells caused more prolonged NF- $\kappa$ B activation, and increased expression of IL-6 that was dependent upon NF- $\kappa$ B. At least in this case, the inflammatory memory of fibroblasts is not simply persistence of cell activation, but reflects priming of the NF- $\kappa$ B signalling pathway for an augmented response [37]. The Kalliolias group showed that TNF- $\alpha$ -treated RA FLS continued to express inflammatory mediators for up to 4 days in a manner dependent upon sustained NF- $\kappa$ B activity [56]. They showed later that NF- $\kappa$ B was also required for priming of these TNF- $\alpha$ -exposed cells to respond more strongly to IFN stimulation [34].

### Chromatin access and epigenetics

Epigenetic modification is generally regarded as key to the innate memory seen in macrophages. Trained immunity induces an augmented response to second challenge weeks or even months after the initial infection. Changes on such a time-scale are likely to be epigenetic and, indeed, evidence abounds for such a theory. Permissive modifications have been described by several groups [4,62].

Endotoxin tolerance is a memory response of a much more short-term nature. While previous examples show evidence for upstream mediators, the study of epigenetics has transferred well to the tolerance field. Examples of this include the length of endotoxin tolerance in macrophages, which has been shown to be from 24–48 to 5 days in duration [22,63]. Medzhitov and colleagues showed that macrophage endotoxin tolerance was induced by gene-specific transcriptional repression, occurring at the level of chromatin condensation [22]. This is in accordance with findings of other groups [59,64].

The chromatin landscape in stromal memory is receiving increasing attention. When EC were primed with TLR-3 agonist, the CXCL10 induction by subsequent TLR-4 agonist was augmented and this was reduced by inhibiting histone deacetylases [42]. This suggests that the induction of chromatin opening is part of the augmented stromal memory response. Studies in fibroblasts have also suggested epigenetic changes [29].

More explicit evidence came from Sohn *et al.*, who showed that chronic exposure of RA FLS to TNF- $\alpha$  led to decreased histone H4 levels, increased H4 acetylation and increased NF- $\kappa$ B and PolII occupancy at the promoter of genes which could be augmented subsequently upon rechallenge. Blocking TNF- $\alpha$  with infliximab for 24 h before the rechallenge did not reduce the augmented response, suggesting that a permissive state had already

been established. This may correlate well with the latent enhancers found to have prolonged transcription factor occupancy in trained macrophages [65]. The recent publication by Klein *et al.* also showed the chromatin landscape to determine which genes became tolerant in macrophages and synovial fibroblasts derived from different anatomical sites [38]. Genes that could not be tolerized had similar epigenetic markers in the first and second responses to LPS. Those that were refractory in the second challenge displayed decreases in histone markers associated with accessibility, thus showing that epigenetic silencing contributes to the control of which genes display innate immune memory profiles.

A generic example of the signalling events known to occur in stromal cells undergoing tolerance or augmented responses is illustrated in Fig. 2.

### Role of stromal memory in infection and chronic inflammatory disease

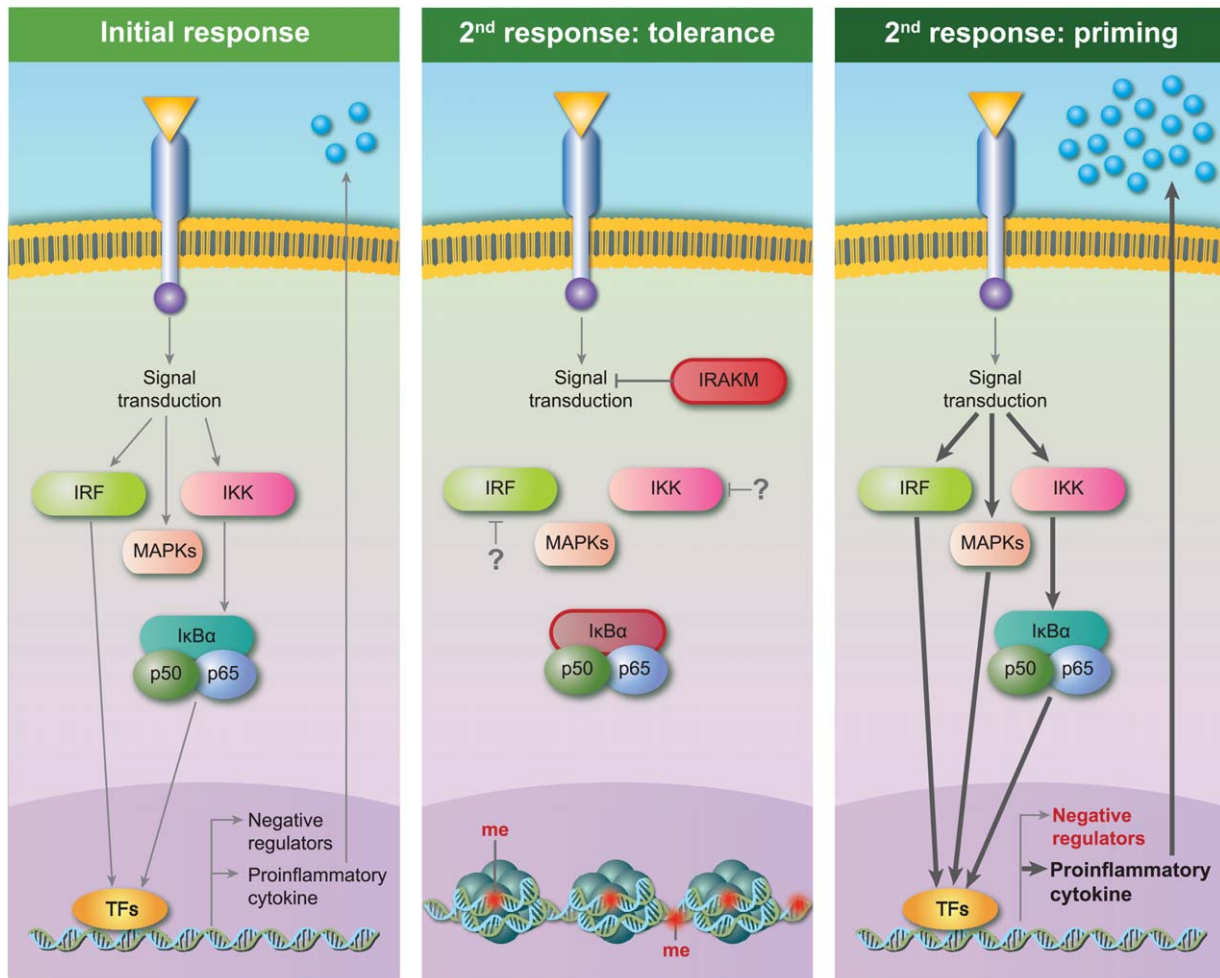
We are well aware of the deleterious nature of flawed adaptive immunology. The number and variety of autoantibodies contributing to a large range of autoimmune diseases displays the danger of what is ostensibly beneficial in infection.

Endotoxin tolerance reduces the inflammatory response of myeloid cells to infectious triggers and thus preserves the host's life. Very low doses of LPS, however, do not induce tolerance, but rather a chronically inflamed state [66]. In this sense, it is possible that low-dose stimulants actually prime the host to augmented second responses. The augmented second response to infection is well reported *in vivo* and in macrophages [5]. The 'red alert' of innate immune cells may induce heightened responses to infectious triggers, but also similarly to endogenous factors.

The concept of endogenous factors priming the response to damage-associated molecular patterns (DAMPs) is established in the stromal memory field. Homocysteine (HCy) is a circulating amino acid used as a clinical marker for atherosclerosis risk. It has been shown to synergize with TNF- $\alpha$  to induce stronger activation of EC [40]. The authors hypothesized that HCy may prime EC for augmented responses to damage. While not tested explicitly in the publication, this was proved by another group, who showed that concentrations of HCy only slightly higher than normal physiological range could prime aortic EC to an augmented inflammatory response to LPS or thrombin [an exogenous pathogen-associated molecular pattern (PAMP) and an endogenous DAMP, respectively] [48].

The role of EC as inflammatory contributors to atherosclerosis is well established [44,67], and the role of myeloid trained immunity in the disease is also gaining attention (reviewed in [68,69]. These publications and others [34,37,40,48,70] acknowledge the dangers of innate





**Fig. 2.** Mechanistic responses to first and second response to stimulation. Simplified stromal cell response to initial response (left), and abrogated (centre) or augmented (right) memory response to second challenge.

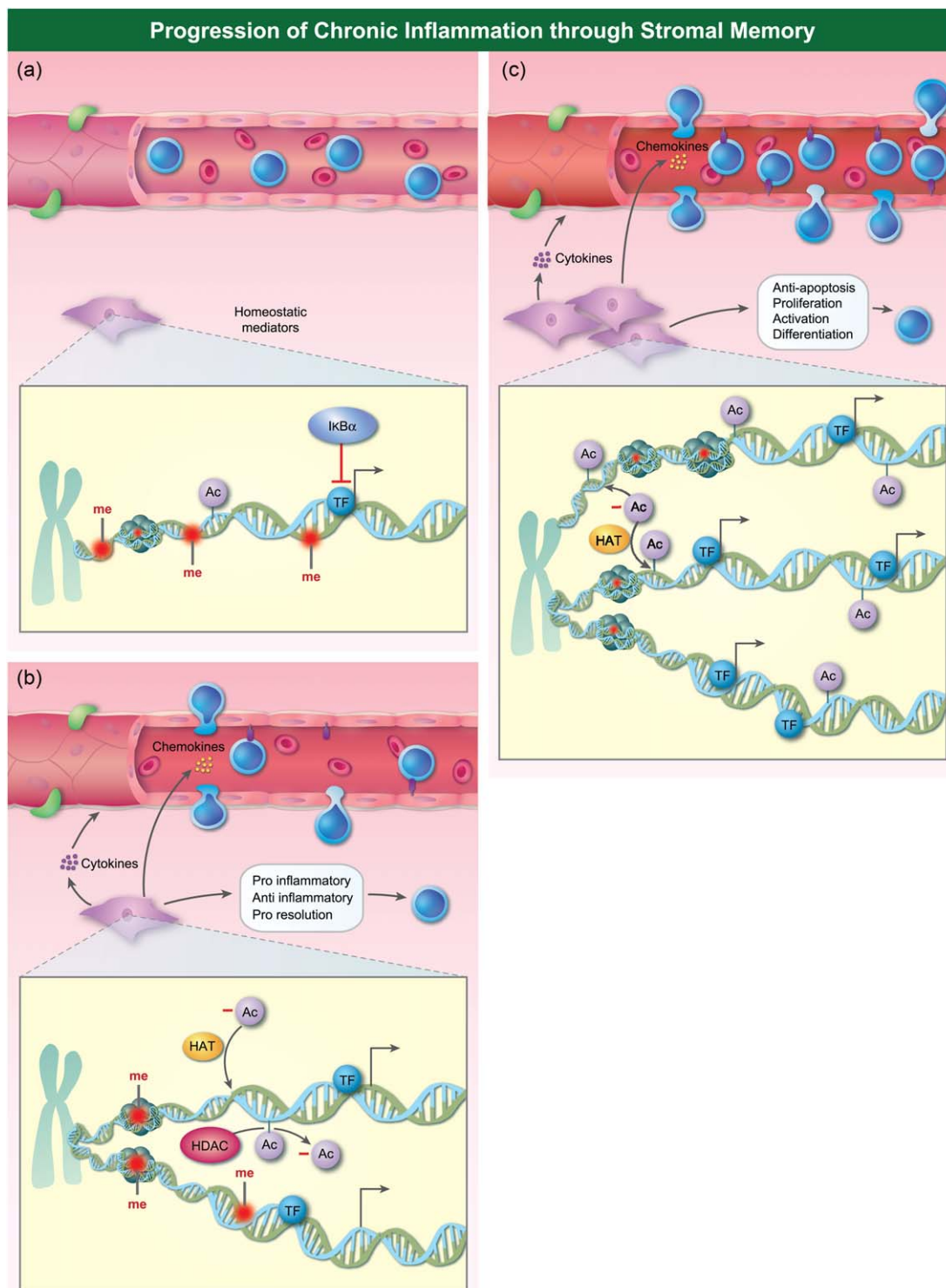
memory facilitating over-aggressive inflammatory responses based on priming by endogenous mediators. DAMPs and other host products inducing this high alert state may lead to chronic inflammatory conditions because they will tend to favour sustained inflammatory responses rather than resolution of inflammation. In some of these examples, whether augmented inflammatory responses are dependent upon continued presence of the 'priming' agent remains to be tested formally. The effects of stromal memory responses to endogenous ligands is shown in Fig. 3.

Research into fibroblast memory also suggests a pathological role. Our own research into non-inflamed and RA synovial fibroblasts suggests that the augmented proinflammatory response is inherent to the joint [37]. This may help to explain the capacity of antibodies against widely expressed antigens to drive joint inflammation. Recent research from Oxford University suggested that chronic tendinopathy was due to an initial (and supposedly resolved) insult, which led nonetheless to fibroblasts acquiring a fibrotic and hyper-responsive phenotype

[35,36]. As remarked above, the altered fibroblast phenotype may be explained in part by differentiation or expansion of a fibroblast subpopulation.

Two publications have shown healthy skin fibroblasts to be incapable of the augmented response [32,37]. In contrast, unpublished work from our department suggests skin fibroblasts from psoriatic plaques pathologically acquire augmented inflammatory responses to rechallenge (i.e. positive memory; manuscript in preparation). This concept of fibroblasts in a chronically inflamed setting acquiring inflammatory memory appears to be supported by fibroblasts from other tissues we have studied, and particularly gingival fibroblasts. These cells have inflammatory memory when isolated from periodontitis patients (manuscript in preparation), but not from healthy patients [32,33].

The concept of changes in fibroblasts contributing to chronic joint inflammation is well established [71,72]. The perturbation of surface markers leading to site-specific leucocyte recruitment (the stromal postcode) in chronic inflammation was reviewed more than 10 years ago [73].



**Fig. 3.** Progression of chronic inflammation through stromal memory. The increasingly inflamed stromal microenvironment during challenge with endogenous mediators is illustrated. (a) The stromal compartment at rest. (b) The inflammatory response of stromal cells leads to an inflamed microenvironment, but negative regulatory and pro-resolution pathways ensure timely resolution. (c) Continued challenges lead to increased endothelial cell adhesion molecule expression and chemokine expression. Fibroblasts also increase cytokine and chemokine release, inducing inflammatory responses from both endothelial cells and leukocytes. Magnified image illustrates the changes at the chromatin level.



The idea that fibroblast memory may be inherent in some sites, but gained aberrantly in others, is therefore not an unusual notion.

### The clinical significance of stromal memory

A host of publications have recognized the modulating influence of prior inflammatory events [68,74–77]. This will obviously due be partly to the adaptive immune response, but as this review has shown, the effect on other cell types, and so the tissue or even organism as a whole, are not to be dismissed. Didierlaurent and colleagues discussed the effects of repeat lung infection [74], while Dakin *et al.* hypothesized [31] and later confirmed [36] that an acute injury event may prepare tendons for chronic tendinopathy and fibrosis upon a second insult. Such concepts show a clear clinical relevance to repeat challenge of innate cells.

Vaccines have proved beyond doubt the clinical benefit of harnessing adaptive immunity. In the same sense, macrophage innate memory is host-protective in various infectious scenarios. Trained immunity protects the host by putting macrophages (and NK cells) on high alert for subsequent infections. Conversely, endotoxin tolerance reduces inflammatory secretions but maintains phagocytic function to defend the host from cytokine storm.

These two sides of innate memory present an attractive therapeutic opportunity. Mihai and van der Meer have expounded the theory of an ‘innate vaccine’ in order to harness trained immunity [5]. This has, in a way, already been invented in the form of the BCG vaccine mentioned earlier, and trials to explicitly test its validity as an infection non-specific vaccine have been conducted [78]. Importantly, the innate vaccine may also be used to defend the host against non-infectious conditions such as autoinflammatory and allergic diseases [79]. Harnessing the short-term memory of macrophages has yet to come to fruition, and the similar (although distinct) ‘immune paralysis’ of sepsis patients continues to play a deleterious role [80].

Although the field of stromal memory is still relatively understudied, clinical relevance has already been evidenced. As stated earlier, *ex-vivo* challenging of EC progenitors with CXCL12 induced greater homing to activated EC and greater tube formation [45]. The authors suggested that this priming dose would improve efficiency of cell therapy following ischaemic disease. In 2016, Stark *et al.* showed that *in-vitro* endotoxin tolerance could be achieved in EC by stimulating with MPLA rather than LPS [46]. This would render EC refractory to infectious triggers of inflammatory responses without the inflammation induced by the first LPS challenge. Such a mechanism would be of benefit in the setting of chronic inflammatory disease, wherein the EC themselves, or the underlying inflamed tissue, induce pathological recruitment to, and extravasation through, the endothelium. A possible example is periodontitis, in which microbiota and host immune responses

conspire to produce the most common chronic inflammatory disease in the world [81]. Zaric *et al.* showed that induction of tolerance could be achieved in gingival fibroblasts if IFN- $\beta$  was provided [33]. Such a reduction in inflammatory response may provide a break in the inflammatory cycle and facilitate the induction of resolution. While targeting circulating cells is always easier than a specific resident population, this latter option is progressing, with improved targeted drug delivery making the targeting of tissue-resident stromal cells a real possibility [82].

### Future questions

The increased interest in this field during the last few years has shed light upon several facets of stromal memory. It is, however, still a field in its infancy. The aspects we feel to be most pressing are as follows.

Although *in-vitro* challenge and rechallenge experiments can shed light upon mechanisms of stromal cell memory, they model the situation poorly *in vivo*. Ground-breaking experiments by Müller-Ladner and colleagues demonstrated that RA synovial fibroblasts could be maintained in tissue culture without losing their erosive properties in an *in-vivo* model of cartilage degradation [83]. It has been argued [70] that prolonged exposure of synovial fibroblasts to an inflammatory environment engenders lasting epigenetic changes, for example at the level of DNA methylation or micro-RNA expression, which strengthen responses to proinflammatory stimuli. However, the acquisition of the aggressive phenotype is not yet well understood. We need better integration between *in-vitro* approaches and elegant *in-vivo* experiments of this kind. This may require the use of irradiated chimeras, or even models deficient in immune cells.

The role played by metabolism is currently not known. The field of immunometabolism has expanded rapidly in recent years, and inflammatory triggers are known to alter metabolic processes in both haematopoietic and stromal cells [84]. Further, chronic inflammatory diseases are known to correlate with altered cellular metabolic profiles [85,86]. A precedent for the involvement of metabolism in innate memory already exists, as the Netea group have shown that monocyte training by prior challenge involved a shift towards glycolytic metabolism both *in vitro* and *in vivo* [62,87]. Given the link these papers made between innate memory, epigenetics and metabolism, the ability to understand and manipulate stromal memory could depend upon a greater appreciation of this axis.

As described earlier, there is disparity between *in-vitro* repeat stimulation and real-world inflammatory pressures. Therefore, although some features of inflammatory memory of stromal cells have been described, the adaptive value of the phenomenon remains unclear. Its gene-specific nature suggests that it may be a mechanism to fine-tune the recruitment of leucocytes during the evolution of an inflammatory episode, or to modify inflammation-induced

recruitment to sites that have previously experienced inflammation.

The contribution to chronic inflammatory disease is also yet to be defined clearly. Why is it that stromal memory differs according to site of origin [37,38,42,46]? Why may it also differ between cells from healthy and chronically inflamed tissue? Stromal positional identity is long established, and shows at both epigenetic [88,89] and functional levels [13,90,91]. If some sites (such as the joint) are more inclined to stromal memory, does this contribute to the likelihood of chronic inflammation in that site? Such knowledge could provide a powerful new prophylactic tool in those with susceptibility alleles.

Finally, if cells from chronically inflamed tissue have memory, is it part of the cause for inflammation or is it acquired in the course of inflammation? If the former, we have a new susceptibility marker which may be more tissue and disease-specific than most of the genome-wide association study (GWAS) hits (which are often shared among many diseases). If the latter, this provides a new therapeutic target which could be applied to any chronic inflammatory disease where memory is acquired. Such a therapeutic would have the dual benefit of targeting more than one disease, and of leaving the patient immunocompetent.

## Conclusions

Innate memory in humans was recognized nearly a century ago, and for years was seen as the prerogative of myeloid cells. Innate memory, whether in the guise of endotoxin tolerance or trained immunity, is host protective. The former protects from tissue damage and cytokine storm, while the latter provides a generic augmented response to future infection. Recently non-myeloid lineages were also recognized to exhibit memory, and stromal memory is starting to receive the attention it deserves.

The roles of stromal cells in inflammation are manifold, and crucial in inflammatory responses to both infection and injury. The field of stromal memory is still in its infancy, and yet already promises to reshape our understanding of stromal cells' roles in inflammatory episodes. Of the evidence presented herein, some suggest that stromal memory may be adaptive, and others deleterious. Some suggest a double-edged sword, protecting against infection at the risk of autoinflammatory conditions. The data are varied, but the consensus in the field is that this under-investigated mechanism will undoubtedly influence the nature, magnitude and duration of inflammatory episodes. Stromal memory, therefore, is well worth remembering.

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## Disclosure

None declared.

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