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Inflammation – a critical appreciation of the role of myeloid cells

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> "The receptor concept is to pharmacology as homeostasis is to physiology, or metabolism to biochemistry. They provide the basic framework, and are the 'Big Ideas' without which it is impossible to understand what the subjects are about."
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> H.P. Rang. The receptor concept: pharmacology's big idea. British Journal of Pharmacology (2008) 17: S9–S1

'What is inflammation's big idea?' In this brief overview of the role of myeloid cells in inflammation we will critically discuss what drives the initiation, amplification and resolution of inflammation in different anatomical sites in response to different pathological stimuli. It can be argued that we have a good understanding of the basic principles that underlie myeloid cell activation and the mobilization of innate immune cells to sites of injury and infection in acute inflammation. The challenge now for inflammation biologists is to understand how resolution of this normal physiological response goes wrong in hyper-acute and chronic inflammation. A better understanding of how inflammation is regulated will allow us to develop new anti-inflammatory drugs that will reduce the burden of inflammatory disease without compromising the patient's immune defenses against infectious disease. Ideally such drugs should encourage a return to homeostasis and enhance tissue repair processes.

Introduction – a historical and evolutionary synthesis

Multicellular organisms have had to develop a rapid response to infection and tissue injury. In all animals on our planet this involves mobilization of specialized cells to the focus of the infection or injury. This important insight was beautifully illustrated by Elie Metchnikoff whose detailed drawings of cells being recruited to the site of injury caused by a rose thorn in a starfish embryo gave us the first glimpse of cells he termed macrophages and neutrophils, which he termed 'microphages'. If not the first person to observe phagocytosis and leukocyte diapedesis, Metchnikoff was probably the first person to fully appreciate the role of these two important cellular processes in 'natural' or innate immunity (1).

An important question arises from Metchnikoff's observations in model organisms and histological images of human tissues infected by pyogenic bacteria - what are the locally

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produced molecular signals that mediate innate immune cell mobilization? *A priori*, we would expect these signals and the receptors that recognize them to have arisen early in evolution of multicellular organisms and for their function to be maintained by selection pressure exerted by everyday exposure to infectious disease and physical injury. A glimpse of how local signals to both pathogens and physical injury might have arisen comes from the Atlantic horseshoe crab, *Limulus polyphemus*. The horseshoe crab is often described as a 'living fossil' due to its near identical form to species present in the Triassic period 230 million years ago. The blood (or haemolymph) of *Limulus* species coagulates in response to intact bacteria and bacterial endotoxin effectively walling off invading pathogens. The *Limulus* protein that recognizes lipopolysaccharide (LPS) and Lipid A present in the outer membrane of Gram negative bacteria is a 132kDa protein called Factor C. LPS binding to the LPS / Lipid A recognition domain of Factor C activates a serine protease domain within the same protein. Activated Factor C activates haemolymph protein, Factor B, which initiates the clotting cascade to cause local haemolymph coagulation. This observation formed the basis of the Limulus amoebocyte lysate (LAL) assay for detecting low-level endotoxin contamination of tissue culture reagents, biologicals and medical devices (2). *Limulus* haemolymph also contains two conserved oligomeric serum proteins, the 'short' pentraxins C-reactive protein (CRP) and Serum Amyloid P component (SAP) (3). These two pentraxins, together with the evolutionarily conserved 'long' pentraxin PTX3, play important roles in mammalian host defense (4). A recent analysis of CRP knockout mice showed a marked sensitivity to *Streptococcus pneumoniae* infection in animals lacking endogenous CRP production that could be rescued by infusion of purified human plasma CRP or generation of anti- *Strep Pneumoniae* antibodies (5). These experiments strongly suggest that CRP has evolved to protect neonatal mammals from specific virulent bacterial pathogens. Specific roles for SAP in mammalian host defence have been harder to identify (possibly due to functional redundancy) but are likely to centre around recognition of bacterial peptidoglycan, damaged host membranes and complement activation, reviewed in (6). PTX3 was originally described as a non-redundant mammalian pattern recognition receptor essential for defence against the fungal pathogen *Aspergillus fumigatus* and later recognised to bind the bacterial pathogens *Pseudomonas aeruginosa* and uropathic *E. coli* as well as influenza virus (7, 8).

Mammalian hepatocytes synthesize a range of other host defense proteins as part of the acute phase response, most notably ~30 proteins of the complement cascade. Complement is another evolutionary ancient defense against pathogens that shares with the coagulation cascade local activation and local amplification via serine protease cleavage of inactive enzymes (zymogens). It is clear that the complement system has evolved to be much more than a simple plasma pathogen recognition system that can kill microbes through deposition of a membrane attack complex (C5b, C6–9) (9). Proteolytic cleavage of the plasma protein C3 leads to deposition of the C3b protein fragment on target cells greatly enhancing phagocytosis by professional phagocytes of the innate immune system (neutrophils and macrophages). Cleavage of the C5 complement protein by the C3 convertase complex generates a high local concentration of C5a, a potent chemoattractant for innate immune cells via the G protein coupled receptor (GPCR) C5aR1 (10).

The unrelenting evolutionary pressure exerted by the twin drivers of infectious disease and tissue injury means that any germline encoded signaling molecule or cellular response system that enhances tissue defense can be rapidly fixed, duplicated and mutated within eukaryote genomes. Multiple examples are provided from comparative genomics. One striking example is the *Drosophila* dorsoventral regulatory gene network, spätzle/Toll/cactus, which has been 're-engineered' and 're-purposed' during vertebrate evolution to give the cytokine activated NF-κB signaling pathway (11). Gene duplication has generated a family of Toll-like receptors (TLRs), which act as cellular pattern recognition receptors (PRRs) for highly conserved molecules on microbial pathogens termed pathogen associated molecular patterns (PAMPs) by Charles Janeway and Ruslan Medzithov (12, 13).

Another striking example of duplication and diversification of immune defense genes comes from consideration of chemokines and their receptors. Comparative genomics reveals that the chemokine-chemokine receptor system has proven a useful module for directing cell-type specific chemotaxis and activation. In addition to mediating T cell chemotaxis the CXCR4 - CXCL12 interaction is used to keep haematopoietic stem cells (HSCs) within a specific bone marrow niche (14). Indeed, a small molecule CXCR4 antagonist (AMD3100) has found clinical application in mobilizing donor HSCs from bone marrow to peripheral blood for more efficient and less painful harvesting. The chemokine-chemokine receptor system has been exploited by the adaptive immune system for dendritic cell migration to lymph nodes, homeostatic leukocyte trafficking, lymphocyte homing to different tissues (e.g. the gut) and recruitment of specific lymphocytes subsets to sites of inflammation (15). The original description of the chemokine system as a inflammatory cell recruitment system, and the impressive results obtained using chemokine receptor gene knockout animals in models of chronic inflammation e.g. Boring et al 1998 (16), suggested that small molecule drugs that inhibit chemokine receptor signaling would make potent, cell type-specific anti-inflammatory drugs. To date this initial optimism has not (yet) been converted into clinically useful drugs (17, 18).

No critical discussion of the role of myeloid cells in inflammation would be complete without consideration of inflammasome activation. The seminal contribution of Jurg Tschopp to immunology and inflammation biology was the recognition that the secretion of active Interleukin-1 (and Interleukin-18) is critically dependent on the formation of a large (>=700kD), cytoplasmic, multi-subunit caspase-activating complex (19). Since Tschopp's first description of this macromolecular structure it has been shown that inflammasome activation can be triggered by a wide range of bacterial, viral, fungal and even helminth PAMPs as well as by a range of host damage associated molecules. Inflammasome activation leads to high local concentrations of IL-1 and inflammatory cell death by pyroptosis and pyronecrosis. Recent experiments in murine models have revealed tantalizing glimpses of a link between inflammasome activation and the inflammatory component of metabolic diseases such as obesity and Type 2 diabetes.

Finally, it is important to remember that the prototypic acute inflammatory response can be triggered by non-microbial stimuli. A good example of such 'sterile inflammation' is provided by administration of substances such as monosodium urate (MSU) crystals and calcium pyrophosphate (CPP) crystals, the pathogenic drivers of gout and pseudo-gout

respectively. Sensing of these and other crystalline insults is absolutely dependent upon the presence of a functional NALP3 gene and subsequent caspase activation, showing the central role of inflammasome activation and IL-1β in this neutrophil-dominated response to tissue injury (20). Necrotic cell death releases specific intracellular molecules that can induce activation of innate immune cells *in vitro* and a prototypic acute inflammatory response *in vivo* (21). Following the intellectual lead provided by Charles Janeway, signaling molecules released by necrotic tissue have been termed Damage Associated Molecular Patterns (DAMPs) by some, 'Alarmins' by others (22). Such molecules include extracellular ATP, mitochondrial DNA, uric acid and chromatin associated proteins, including the chromatin high mobility group-1 protein HMG-1. In a recent study Kataoka et al (23) directly compared the role of multiple signaling pathways in two mouse models of leukocyte recruitment in response to intraperitoneal injection of necrotic cells or induction of hepatocyte necrosis with paracetamol. The authors showed that neutrophil mobilization at early time points was significantly decreased in the absence of Complement C3, natural antibodies and the protease-activated receptor PAR2. Local depletion of ATP or deletion of the P2X7 receptor gene had no effect on leukocyte mobilization in these two models of necrotic cell induced inflammation, in contrast to findings in cultured cells or other models of sterile injury.

Another potential 'danger signal' in the context of tissue injury is damage and modification of the extracellular matrix (ECM) (24). Hyaluronan (HA) is an abundant nonsulphated glycosaminoglycan component of the ECM found in many tissues. Multiple biological functions have been ascribed to HA and pro-inflammatory functions have been assigned to low molecular weight forms of HA (LMW HA) generated by the action of a range of hyauronidase enzymes. An interesting pre-clinical study by Huang et al (25) demonstrated that commercially available LMW HA and hyaluronidase enzyme preparations are contaminated with endotoxin and other proteins. This contamination of key reagents had lead to the erroneous conclusion that LMW HA is a ligand of the TLR2 and TLR4 receptors leading to pro-inflammatory cytokine production. By using an endotoxin-free pure preparation of the human hyaluronidase enzyme PH20 (rHuPH20) in an LPS dorsal air pouch model of acute inflammation Huang et al. demonstrated a marked anti –inflammatory effect of hyaluronidase characterised by no change in pro-inflammatory cytokine production but marked reduction of neutrophils. The pro-inflammatory role of ECM damage merits further study in the context of both acute and chronic inflammation as well as in the context of tissue repair and fibrosis.

Experimental Models of Inflammation

Animal models of inflammation are always open to criticism of how well they mimic clinical events in human disease. For instance mouse models of atherosclerosis do not display the classical clinical sequelae of human atherosclerosis, i.e. myocardial infarction and ischaemic stroke. Another obvious concern is that drugs that work well in pre-clinical models of human disease do not always translate into successful clinical trials. An early example was the success of anti-TNF therapy in murine and primate models of endotoxic shock and the subsequent failure of anti-TNF monoclonal antibodies to impact on morbidity and mortality in human septic shock and sepsis in randomized clinical trials (RCTs). Despite

these obvious limitations, our current knowledge of inflammatory mediators, inflammatory cell biology and the resolution of inflammation owes much to a wide range of well characterized pre-clinical models some of which are outlined below.

Animal models of acute inflammation

Peritonitis is the inflammation of the peritoneum, a thin membrane lining the abdominal cavity. Experimentally it can be triggered by infectious stimuli, which if not treated immediately can spread to the blood and lead to septic shock. Peritonitis can also be induced by injection of sterile inflammagens or implantation of necrotic cells or tissues. Rodent models of peritonitis have provided insight into the generation of local mediators that aid the resolution and return to tissue homeostasis, such as Annexin-A1, lipoxins, resolvins, protectins and maresins. A variety of inflammatory stimuli have been used to induce peritonitis including zymosan, IL-1β and Brewers thioglycollate. Zymosan induced peritonitis is a simple and reproducible model of self-resolving inflammation. It has become a ‘go to’ model to study not only the kinetics of leukocyte recruitment and pro-inflammatory mediator production, but also pro-resolving actions on processes such as macrophage efferocytosis. Zymosan is a yeast cell wall extract of *Saccharomyces cerevisiae* and is recognised by TLR2 and Dectin-1. Intraperioneal injection with low dose zymosan (0.1–1mg/ml) leads to an initial wave of PMN recruitment into the peritoneal cavity which peaks between 6–8 hours followed by a second wave of mononuclear cells (>16 hours) during the resolution phase(26).

Carageenan (CG) induced paw oedema is a well-established model of acute inflammation used to test the effects of a variety of anti-inflammatory drugs/compounds and to understand the role of mediators during an acute inflammatory response. CG is a gelling agent consisting of sulphated galactans and three main forms have been identified; iodo-CG, kappa-CG and lambda CG. The lambda species is most widely used in mice and rats and is given as sub-plantar injection in one paw. A similar volume of saline is injected into the contralateral paw as a control and oedema is usually measured by plethysmometry (27). Upon sub-plantar injection with 1–3% CG, a biphasic inflammatory response ensues with pain, increased vascular permeability, oedema and PMN influx observed as a result of the release of range of mediators being generated locally including substance P, histamine, bradykinin, prostaglandins, complement and reactive oxygen species. Peak oedema levels in the first phase of the response is observed between 4–6 hours followed by a second more intense phase developing between 48–72 hours. A study carried out by D’Agostino et al, (2007) examined the role of peroxisome proliferator-activated receptor (PPAR)-α agonistsin modulating CG induced paw oedema in mice. The authors found that intracerebroventricular administration of an endogenous PPAR-α agonist, palmitoylethanolamide (PEA) 30 minutes before CG administration reduced oedema formation. This reduction was linked to a decrease in COX-2, iNOS synthase expression and IκB degradation. Mice lacking PPARα showed no reduction in oedema formation following pre-treatment with PEA. This study elegantly demonstrated for the first time that activation of PPAR-α in the CNS could control peripheral inflammation (28).

The air-pouch model is another simple model widely used to study inflammation in vivo. Two subcutaneous injections of air (day 0 and 3) into the dorsal intrascapular region leads to the formation of a discrete pouch. Injecting inflammatory stimuli such as zymosan, MSU or cytokines into the air-pouch results in rapid influx of PMNs and the local generation of mediators, including IL-8 and C5a. Depending upon the dose and the type of inflammagen used a second wave of mononuclear cells can follow. The air-pouch offers many advantages over the peritonitis model including ease of multiple dosing and application of compounds with poor solubility profiles. It also offers users the ability to recover relatively clean exudate samples, which can be used to measure mediators with low levels of abundance(29).

Animal models of chronic inflammation

The collagen induced arthritis model (CIA) has been an invaluable tool in furthering our understanding of some of the key mechanisms involved in the pathogenesis of human rheumatoid arthritis (RA). CIA is an inflammatory polyarthritis that shares many of the clinical and histological manifestations associated with human RA, i.e. disease being centered on the joints and the destruction of cartilage and bone. CIA is induced following sensitization with heterologous type II collagen (CII) in complete Freund's adjuvant (CFA) followed by a second immunization (day 21) with CII in incomplete Freund's adjuvant (IFA). This results in the initiation of cell mediated and humoral adaptive immune responses, which are characterized by increased levels of anti-CII IgG, complement fixation in joints, leukocyte infiltration and activation into the joint space and synovium, which leads to the generation of pro-inflammatory mediators such as TNFα and IL-17/23 that continue to drive the disease. A study carried out by Notley et al in 2008 utilised the CIA model to assess the effects of TNF blockade on IL-17 production. They found that mice treated with TNFR-Fc fusion protein or anti-TNF monoclonal antibody had reduced arthritis severity and showed reduced accumulation of Th1 and Th17 cells in the joint, but expanded Th1 and Th17 cell numbers in lymph nodes. Collectively, these findings suggested two opposing roles for TNF blockade in CIA, first blocking the accumulation of Th1 and Th17 cells in joints and secondly sequestration of pathogenic T cell numbers in peripheral lymphoid organs(30).

In recent years, inducing rheumatoid arthritis in mice using serum transfer has become a more widely used model of RA, in part because the CIA model only works well in selected inbred mouse strains (a common feature of several mouse models of inflammation). The K/BxN serum transfer arthritis model was first described in the mid-90s as an inadvertent byproduct of crossing KRN T-cell receptor transgenic and nonobese diabetic (NOD) mice that lead to the development of spontaneous arthritis (31). Transfer of pathogenic anti-glucose-6-phosphate isomerase (GPI) antibodies from these mice to normal recipients resulted in the development of a severe arthritis as a result of alternative complement pathway activation and continual recruitment of neutrophil and mast cells into joints. A study from the laboratory of Mathis and Benoist employed this model to delineate the role of chemokines in leukocyte recruitment during the initiation of auto-antibody mediated arthritis. The authors induced arthritis by transferring serum into several chemokine and chemokine receptor deficient mice (CCR1–7, CCR9, CXCR2, CXCR3, CXCR5, CX3CR1, CCL2, or CCL3) and found that only the absence of CXCR2, a classical neutrophil

chemokine receptor, was critical for the development of autoantibody-mediated joint inflammation and arthritisin C57/Bl6 mice (32).

The ability of ApoE$^{-/-}$ mice on a C57BL6/J background to develop atherosclerotic lesions was first reported in 1992 and a similar phenotype in Ldlr$^{-/-}$ mice fed a high fat-high cholesterol diet was reported a few years later. These two mouse strains have been used extensively to study the cell and molecular biology of atherosclerosis *in vivo* (33). Mouse models of atherosclerosis involve many of the key features of human atherosclerosis including trapping of apoB-containing lipoproteins in the sub-endothelial space of major arteries, monocyte recruitment and macrophage differentiation.

Acute Inflammation is a Process

Generally ascribed to the Roman physician Celsus, the cardinal signs of acute inflammation in response to infection or tissue injury have been recognized for 2,000 years i.e. redness (rubor), heat (calor), pain (dolor) and swelling (oedema). Acute inflammation, like grieving, is a process (Billy Crystal, Robert de Niro, Analyze This, movie 1999 http://www.imdb.com/title/tt0122933/). The coordinated recruitment of plasma proteins, lipid mediators and myeloid cells in the inflammatory exudate can be defined by careful examination of sterile inflammation in the experimental model systems outlined above and can be organized under three headings.

Initiation

The earliest event in acute inflammation is sensing of pathogens and tissue damage, typically by tissue resident macrophages and mast cells. The most important role of these sentinel cells, which are relatively few in number, is to generate signaling molecules that lead to *local* endothelial cell activation in post capillary venules and autocrine and paracrine activation of macrophage effector functions within tissues.

Amplification

Locally generated signaling molecules cause important changes in the properties of nearby endothelium, including the release of the contents of Wiebel-Palade bodies, endothelial cell contraction, up-regulation of cell adhesion molecules (e.g. ICAM-1) and synthesis and presentation of chemokines. Acting in concert, all these changes in the endothelium allow for the local elaboration of an inflammatory exudate of plasma proteins and myeloid cells, especially neutrophils. Changes in the properties of endothelial cells cause the observed clinical signs of inflammation – redness and heat via increased local blood flow, oedema through elevated oncotic pressure caused by elevated albumin in tissues and localized pain through the action of locally produced mediators including bradykinin and Prostaglandin E_2.

It is the recruitment of plasma proteins and their subsequent proteolytic cleavage at sites of tissue damage or suspected pathogen invasion that leads to the massive *amplification* phase of inflammation that is essential for the localized recruitment of myeloid cells from the blood and their subsequent activation. Local activation of serine protease cascades in response to DAMPs and PAMPs (using the same molecular mechanism adopted by the

horseshoe crab 220 million years ago) generates large quantities of potent protein and peptide mediators such as complement C3a, C5a and bradykinin.

Resolution

This is probably the least well-understood aspect of the acute inflammatory process. Termination of further leukocyte recruitment requires catabolism of inflammatory mediators, neutrophil apoptosis, macrophage efferocytosis of apoptotic cells, lymphatic drainage and the initiation of tissue repair processes. Described by many as an ‘active’ rather than a ‘passive’ process, definitive experiments addressing the cell biology of inflammation resolution using *in vivo* model systems are at a premium. An under-researched area of inflammation biology is the role of the lymphatic system in clearance of the inflammatory exudate during the resolution phase. One important anatomical arena where future research seems merited is the infarcted myocardium following recent studies showing changes to the cardiac lymphatic system following injury(34).

Animal models of sterile peritonitis provide some idea of the potential timescale of the key events in acute inflammation. In the widely used mouse zymosan peritonitis model resident F4/80^{hi} macrophages leave the serosal cavity to local lymph nodes within 60 minutes of inflammagen injection and neutrophil recruitment peaks at around 4 hours. PMN recruitment is followed by a wave of inflammatory Ly6C^{hi} monocytes, which peaks between 16 and 24 hours. In this model neutrophil and monocyte numbers in the cavity return to baseline within 96 hours but these timings and the absolute number of myeloid cells depends on the dose and nature of the inflammagen. A recent paper from the group of Derek Gilroy extended analysis of the classic zymosan peritonitis model out past this 96-hour window and showed that a true return to tissue homeostasis following clearance of zymosan particles took more than three weeks (35). After disappearance of PMNs from the peritoneum, Newson et al observed changes in monocyte derived macrophage subsets and a significant increase in the number of B and T lymphocytes in the peritoneum. The recruitment and retention of this collection of lymphoid and myeloid cells long after the disappearance of the inciting sterile stimulus is intriguing. It will be important to see exactly how the mixture of cell types present post-inflammation contribute to tissue repair and defence against infectious disease. An important myeloid cell type that we will not consider in this brief review is the dendritic cell. An important question for immunologists and pathologists is, ‘Do dendritic cells play a unique role in the initiation, amplification or resolution of inflammation that cannot be provided monocyte derived macrophages recruited to the site of inflammation?’ Perhaps the unique, non-redundant role of DCs in inflamed tissues is to engage the anti-inflammatory/ pro-repair arm of the adaptive immune response, most notably T_{reg} cells and perhaps some lesser-studied B-lymphocyte subsets such as B1 and B_{reg} cells. Published studies on the role of DCs and B cell subsets in animal models of atherosclerosis may lead the way in this regard.

A typical time course of an acute inflammatory response is shown in Figure 1 (after Christopher Buckley, University of Birmingham, U.K.). Representing the intensity of the inflammatory response on the Y-axis (measured as leukocyte recruitment, leukocyte activation or local inflammatory cytokine concentrations) the initiation, amplification and

resolution phases of a stereotypic, ‘healthy’ acute inflammatory response can be clearly demarcated. Failure to clear the initial inflammatory insult or failure of inflammation resolution leads to chronic inflammation, represented by the horizontal arrow. Hyper-acute inflammation is represented by a continuing escalation of leukocyte recruitment, leukocyte activation (locally and systemically) and unrestrained inflammatory cytokine production.

A couple of important discussion points arise from consideration of this simplistic representation of the ‘classical’ acute inflammatory response. The first is whether there is ever a complete absence of inflammation in host tissues i.e. should the Y-axis be set to zero at t=0? A paper published in 2010 by Jeffrey Weiser’s laboratory showed that that low-levels of systemic peptidoglycan derived from the gut microbiota primes the host innate immune system via the Nod1 receptor leading to more efficient neutrophil killing of S*treptococcus pneumonia* and *Stahylococcus aureus* (36). A second important discussion point arising from consideration of Figure 1 is ‘What regulates the magnitude of the acute inflammation response?’ and more specifically ‘Does the magnitude of the acute inflammation to the same inciting stimulus differ between tissues in the same individual and between individuals in the same population?’ A brief consideration of the mechanisms that might drive hyper-acute inflammation as presented in Figure 1 is also warranted. One simple explanation for the continuing amplification of acute inflammatory amplification seen in say, septic shock, could simply be the continuing proliferation of the initial bacterial infection that acted as the stimulus for the initiation of inflammation at t=0. Circumstantial evidence supporting such an explanation comes from a retrospective analysis of mortality data for patients admitted to hospital with suspected sepsis. In a cohort of 17,990 patients given antibiotics upon admission to 165 intensive care units with severe sepsis or septic shock the probability of in-hospital mortality increased steadily with time to antibiotic administration (37). An alternate explanation for hyper-acute inflammation could be failure of endogenous pathways that act to limit myeloid cell responses to PAMPs, a phenomenon that has been termed endotoxin tolerance (38) or the more recently appreciation of altered myeloid cell metabolism induced by sepsis (39).

In Figure 2 we represent this question in a simple diagram. We propose that the *magnitude* of the host response to an inflammatory insult in any given anatomical location is the net result of the action of local pro-inflammatory mediators (upward arrows) and the opposing action of endogenous anti-inflammatory mediators (downward arrows). We have termed our model the ‘Inflammatory Set point Hypothesis’ and it stands apart from a previous theoretical consideration of the acute inflammatory response, which placed more weight on the *kinetics* of inflammation resolution by deriving a ‘Resolution Interval’ for comparing the effects of different therapeutic interventions in experimental animal models, typically zymosan peritonitis (40). The inflammatory set point model as presented in Figure 2 immediately suggests that pharmacological interventions that lower the activity of specific pro-inflammatory mediators (e.g. anti TNF antibodies or chemokine receptor antagonists) will reduce the maximal intensity of the acute inflammatory response. Another approach to reduce the peak level of inflammation would be to augment the activity of relevant endogenous anti-inflammatory mediators. This therapeutic rationale could be particularly efficacious in treatment of human diseases where the magnitude of the initial inflammatory

response overwhelms the inflammation resolution machinery i.e. chronic ('non-resolving') inflammation and hyper-acute inflammation (see Figure 1).

Consideration of experimental evidence for the importance of the downward arrows in Figure 2 comes from the enhanced inflammatory response seen in mice carrying gene deletions for the anti-inflammatory mediator Annexin A1 and its receptor *Fpr2* (41). Finding evidence in support of the model presented in Figure 2 using human rather than murine models will be challenging but use of the beetle blister model could be instructive. Derek Gilroy and colleagues performed an interesting experiment where they gave human volunteers aspirin (75mg, oral once a day for 10 days) or placebo and then induced used a fixed dose of beetle cantharidin toxin to induce an acute inflammatory response in the skin characterized by dermal oedema and localized leukocyte recruitment. Volunteers taking low dose aspirin showed no change in blister oedema volume at 24 hours compared to volunteers taking placebo but did show reduced neutrophil and monocyte numbers in the inflammatory exudate (42). In terms of our set point model of Figure 2 the effect of low dose aspirin is likely two-fold, reducing pro-inflammatory drive by reducing local PGE_2 production and simultaneously enhancing endogenous anti-inflammatory 15-epi-lipoxin A_4 production.

Monocytes and Inflammation

Macrophages have been described as the "mature forms of circulating monocytes that have left the blood and taken up residence in the tissues" (The Immune System, Peter Parham, 3rd edition, Garland Science, NY and London, 2009). We now know that the origin of tissue macrophages is not exclusively from the circulating pool of monocytes. Under homeostatic conditions, many tissue resident macrophage populations are probably of embryonic origin and capable of self-renewal (reviewed in Sieweke and Allen (43). Most relevant to this chapter, however, is that an inflammatory stimulus will significantly increase the contribution of circulating monocytes to the tissue macrophage pool. In mice, the pool of circulating monocytes has been broadly divided into $Ly6C^{hi}$and $Ly6C^{lo}$ subsets, which are also referred to as classical and non-classical monocytes, respectively reviewed in (44). In humans, the corresponding subsets are $CD14^+$ $CD16^-$ and $CD14^{lo}$ $CD16^+$, but the proportions of classical:non-classical monocytes varies by species (1:1 in mice and 9:1 in humans). Non-classical monocytes are thought to be derived from classical monocytes, but there is some evidence also for independent origin (45).

The functions of circulating monocyte subsets in inflammation have been extensively studied, particularly in mice. CCR2-mediated mobilization and chemotaxis are major drivers of classical monocyte recruitment from bone marrow to blood, and from blood to inflammatory sites. This has been borne out in multiple studies of $CCR2^{-/-}$ mice. CCR2 deficient mice have fewer monocyte/macrophages in models of both acute e.g., thioglycollate-induced peritonitis (46) and chronic inflammation e.g., atherosclerosis (16). Likely this reflects both the impaired release of classical monocytes from the bone marrow as well as the reduced chemokine mediated recruitment of circulating monocytes to the site of inflammation.

Though non-classical monocytes are low expressors of CCR2, in contrast to the classical cells they express high levels of CX3CR1. This chemokine receptor is thought to contribute not only to the migratory behaviour of the cells, but also to enhance the survival of both the non-classical monocytes in the blood and the tissue macrophages derived from them (47). Like classical monocytes, non-classical cells are recruited to sites of inflammation, but less abundantly so in both acute and chronic models (48). Also in contrast to the classical subset, they perform “patrolling” functions in the vasculature and can be recruited to non-inflamed tissues (49). Another contrast between the two subsets is the polarization of the tissue macrophages derived from each type. It is thought to be towards the activated M1 state for those of classical, and towards the anti-inflammatory, tissue repair M2 state for those of non-classical origin (50). There are a number of exceptions, however, to this “rule” (51), suggesting that the phenotype of the macrophages derived from each subset is likely to be context dependent.

Though the bone marrow is typically the major source of circulating monocytes, in certain circumstances, there can be acute and substantial contributions from extra-medullary sources. One example is reported by Swirski, Nahrendorf, and their colleagues in a series of elegant papers reviewed in (52). In a mouse myocardial infarction (MI) model, in which the myocardium experiences ischemia-reperfusion damage, they observed, not surprisingly, that the inflammatory process followed that in wound healing; namely that there was biphasic entry of monocytes into the injured tissue, the first wave being classical ($Ly6C^{hi}$) cells responding to a burst of locally produced CCL2, the ligand of CCR2. These cells became activated, M1-like macrophages. The two surprises were that the second wave also consisted of $Ly6C^{hi}$ cells. These converted to $Ly6C^{lo}$ cells in the injured tissue, where they became tissue-repair, M2-like macrophages (53) in a variation to the “rule” that tissue M2 macrophages are derived from circulating non-classical monocytes. The other surprise was that the source of the circulating monocytes was not the bone marrow directly, but rather the spleen, where monocyte progenitors (HSPCs) that travelled from bone marrow to specialized niches were stimulated to proliferate and to give rise to monocytes that entered the circulation and were recruited to the injured tissue (54). These observations leave open the question of whether drugs that inhibit CCR2 activity will ever find therapeutic utility post-MI patients. The Swirski-Nahrendorf results raise the possibility that blocking $CCR2^{+}$ cell recruitment post MI will interfere with tissue healing and remodeling ultimately resulting in reduced cardiac output(55).

Inflammatory Mediators

Our brief consideration of acute inflammation and its resolution has highlighted the importance of sequential mobilization and differentiation of innate immune cells. Could sequential recruitment and differentiation of leukocyte subsets fit the bill for Henry Dale’s ‘Big Idea’ for inflammation biology? If so, it is clear that we will need to understand how different classes of inflammatory mediators are generated, how they change leukocyte migration and activation and ultimately how these mediators work together to effect tissue repair programmes.

Different classes of mediators (briefly)

1. Vasoactive amines - Activation of mast cells leads to rapid degranulation and the release of their potent pro-inflammatory granule contents e.g. vasoactive amines histamine and serotonin. These mediators act via specific GPCRs that can lead to vasodilatation and very rapid changes in cellular behaviour, e.g. endothelial cell contraction. Systemic mast cell degranulation can be fatal, for instance binding of food allergens to specific IgEs bound to mast cells via Fc_e receptors.

2. Cytokines and chemokines Cytokine genes are transcribed within minutes of exposure of macrophages to DAMPs and PAMPs and these cytokines can act in an autocrine and paracrine manner to further amplify the transcription of pro-inflammatory cytokines. Typically cytokine production by macrophages is measured in response to a single PAMP using primary cells (often bone marrow derived macrophages) cultured *ex vivo*. The advent of single cell transcriptomics will allow us to follow changes in the transcription pattern of the whole genome in sentinel cells responding to pathogens in infected tissues *in vivo*. To take full advantage of this surge in information we will need to stop thinking about cytokines in isolation and start thinking in terms of cytokine networks.

3. Lipid mediators- In macrophages TLR signaling increases prostaglandin synthesis by activating cytosolic phospholipase A_2 (cPLA2). $cPLA_2$ releases arachadonic acid from membrane phospholipids and up-regulates cyclooxygenase-2 and microsomal prostaglandin E synthase-1 (mPGES-1) expression. These changes in intracellular lipid pools and lipid metabolizing enzymes set the scene for generation of multiple pro-inflammatory prostaglandins and leukotrienes. Later in the acute inflammatory response there is a marked ‘eicosanoid class switching’ which is marked by a switch to production of anti-inflammatory, pro-resolution lipid mediators such as Lipoxin A_4 (56, 57).

4. Gases as mediators - Since the Nobel Prize winning discovery of nitric oxide NO as a signaling molecule we now better appreciate two other gaseous signaling molecules that can act as anti-inflammatory mediators, carbon monoxide CO and hydrogen sulfide H_2S (58). Vascular endothelial cell production of NO from L-arginine is catalyzed by endothelial nitric oxide synthase (eNOS, NOS3) and this almost ephemeral, very short-lived signaling molecule strongly influences vascular tone via cGMP signaling. CO is generated by heme catabolism by the enzyme heme oxidase-1 (HO-1) and CO exerts its anti-inflammatory effects via the MAPK pathway (59). The use of multiple H_2S donors and selective inhibitors of H_2S synthesis has helped to define the cellular actions of this gaseous signaling molecule. The therapeutic effects of H_2S releasing drugs seen in animal models of inflammation has lead to H_2S releasing drugs been taken forward into clinical trials (60).

A Brief Note on Inflammasomes and Autoinflammation

As alluded to above, the field of inflammation biology owes much to the seminal papers of Jürg Tschopp (1951–2011) who first identified the cytoplasmic molecular machinery for secretion of active Interleukin 1β, a structure that he termed the inflammasome (61). The majority of inflammasomes are formed with one or two Nod-like receptor proteins (NLRs). Other non-NLR proteins including absent in melanoma 2 (AIM2) and pyrin can also form inflammasomes, as reviewed in (62). The N-terminal pyrin domain (PYD) within NLRs associates with apoptosis-speck like protein containing CARD (ASC) and this permits the recruitment of pro-caspase 1 to the inflammasome. The NLRP3 inflammasome is the most extensively studied inflammasome to date. NLRP3 activation can occur is response to a wide range of stimuli including infectious agents including intracellular bacterial products e.g. *Shigella* shiga toxin, extracellular ATP, monosodium urate or cholesterol crystals as well as changes in osmolarity or pH (63, 64). MCC950 is a highly selective inhibitor of NLRP3 that blocks canonical (ATP, monosodium urate) and non-canonical (cytosolic LPS) NLRP3 inflammasome activation at nanomolar concentration. Administration of MCC950 to mice with EAE, a murine pre-clinical model of human multiple sclerosis, has been shown to improve clinical symptoms and attenuate IL-1β production (65). The ability to pharmacologically inhibit NLRP3 inflammasome activation in pre-clinical models will greatly aid investigation of the role of this signaling complex in the pathogenesis of inflammation and could be the starting point for development of novel small molecule anti-inflammatory drugs.

Over the past 25 years rheumatologists have come to recognize that autoinflammation as a distinct disease pathology from autoimmunity. Tumour Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS) is a rare, genetic disease that causes recurrent episodes of fever that are associated with chills and muscle pain. In 1999 Kastner et al showed that TRAPS did not involve T or B lymphocyte activation but rather inappropriate innate immune activation caused by germline mutations in the 55kDa TNF receptor 1 (66). Kastner et al coined the term ‘autoinflammation’ for the observed defects in the regulation of systemic inflammation. Molecular analysis of other rare genetic diseases characterized by systemic inflammation with no obvious infectious disease or autoimmune component identified a group of rare monogenic diseases with defects in IL-1β production and the NALP3 inflammasome including Cryopyrin-Associated Periodic Syndromes (CAPS), Muckle Wells syndrome and familial cold autoinflammatory syndromes (67). Detailed analysis of other rare monogenic disorders continues to provide further examples of how loss of endogenous regulatory pathways can lead to inappropriate inflammatory and innate immune responses (68).

Hyper-acute Inflammation – Bacterial Septic Shock and Viral Cytokine Storm

Local activation of macrophages, mast cells and endothelial cells is essential to mobilize an acute inflammatory response to sites of pathogen invasion and tissue injury. However, systemic activation of these cells by bacterial PAMPs can lead to the life-threatening septic

shock. An excessive systemic host reaction to viral pathogens such as influenza, frequently termed a ‘cytokine storm’, can also have life-threatening consequences, often in young people with a robust immune system (shown diagrammatically in Figure 1). The clinical sequelae of septic shock and cytokine storm show the importance of regulating the magnitude of the initial inflammatory response (Figure 2). The recent appreciation of the need to distinguish sepsis from septic shock serves only to emphasise the importance of return to tissue homeostasis following the initial triggering of a hyper-acute inflammatory response (69). There is a substantial unmet clinical need to develop better treatments for septic shock and sepsis but a better understanding of the disease process is being held back by the lack of good experimental models and the continuing failure to translate basic science findings into effective new treatments (70). One fruitful area for future research might be to identify and augment endogenous pathways that can rapidly decrease the maximal host inflammatory response without ‘paralysing’ the innate immune system altogether.

Chronic Inflammation– Pathogenesis and Current Treatments

In an excellent 2010 review article Carl Nathan and Aihhao Ding wrote, “The problem with inflammation is not how often it starts, but how often it fails to subside. Non-resolving inflammation is not a primary cause of atherosclerosis, obesity, cancer, chronic obstructive pulmonary disease, asthma, inflammatory bowel disease, multiple sclerosis, or rheumatoid arthritis, but it contributes significantly to their pathogenesis.” (24). In their review the authors lay out multiple overlapping and competing models for how chronic inflammation can arise *in vivo*.

- **a.** Failure to clear a pathogen that was the original trigger for inciting acute inflammation. Classic examples would be failure to clear mycobacterial infection or chronic virus persistence in hepatitis.
- **b.** Response to continuing tissue injury, for instance necrotic cell damage and the release of DAMPs caused by ischaemia reperfusion injury.
- **c.** Continuing presence of antigen, e.g. antigens recognized by autoantibodies in rheumatoid arthritis.
- **d.** Non-resolving inflammation, for instance the failure to clear macrophages and macrophage derived foam cells from atherosclerotic lesions in major arteries leading to the build up of stable and unstable atherosclerotic plaques, a form of chronic inflammation that persists for decades.

Consideration of the multiplicity of pathogenic mechanisms in human diseases caused by chronic inflammation reminds one of the opening lines of Leo Tolstoy’s novel *Anna Karenina* ‘All happy families resemble one another, each unhappy family is unhappy in its own way’. All successfully resolved bouts of inflammation resemble one another in showing a return to ‘happy’ tissue architecture and essentially normal tissue function. In contrast, each chronic inflammatory disease shows ‘unhappy’ tissue architecture caused by multiple defects in the return to homeostasis.

Current treatments for chronic inflammation include steroids, non steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti rheumatic drugs (DMARDs) and 'biologicals'; i.e. recombinant monoclonal antibodies or decoy receptors that block inflammatory cytokine function. An important mode of action shared by these drugs is that they reduce inflammatory leukocyte recruitment and dampen down adaptive immune responses. Although current anti-inflammatory drugs have different targets and widely different modes of action, they all share a common side effect, namely a potentially life-threatening reduction in the host immune response to infectious disease. In a recent review Ira Tabas and Chris Glass give a good discussion of the challenges of developing new anti-inflammatory drugs and how we might achieve selective dampening of myeloid cell recruitment without compromise of 'first responders' to infectious disease and wound repair (71). One promising approach might be to target pro-resolving agents specifically to sites of chronic inflammation, a proof of concept of this approach using nanoparticles was recently published by Tabas and co-workers(72).

Unanswered Questions and Future Directions in Inflammation Biology

Following our brief overview of the role of myeloid cells in inflammation we identify seven areas where further research is needed and offer seven questions for those working in the field.

(1) Macrophage differentiation and plasticity in vivo

For an excellent historical overview of the macrophage M0, M1, M2 concept and a critical assessment of the current literature the authors thoroughly recommend a recent review by Fernando Martinez and Siamon Gordon (73). It is tempting to think about macrophage subsets in the same way we think about T cell subsets in disease. However, advances in flow cytometry, including single cell detection of cytokine production, have thrown up many more T cell classifications than the simplistic Th1, Th2, Th17 classifications that we previously used to explain the role of T cells in disease pathogenesis.

Key questions for the field include 'Do M1 macrophages, defined by surface expression of a handful of different markers really do something *substantially* different from 'common or garden' F4/80^{hi} tissue resident macrophages in the context of an ongoing inflammatory response?' Similarly, 'Do M2 (alternatively activated) macrophages, defined in vitro by a cluster of markers, really enhance tissue repair in the context of inflammation resolution or wound repair? Put another way; 'How plastic is macrophage differentiation *within tissues*?

The issue of macrophage plasticity *in vivo* has been placed centre stage by two important papers published in 2014 (74, 75). Lavin et al. elegantly demonstrated the plasticity of tissue resident macrophage differentiation by taking F4/80^{hi} peritoneal macrophages from CD45.1 donor mice and instilling them into the lungs of CD45.2 recipient mice (without irradiation). In parallel they transferred F4/80^{hi} alveolar macrophages from CD45.1 donor mice and injected them into the peritoneum of CD45.2 recipient mice. CD45.1 donor cells were recovered from their new tissue microenvironments three weeks later and their transcriptomes and chromatin marks were analysed and compared to those of resident peritoneal and alveolar macrophages in the same tissue. Strikingly Lavin et al showed a

complete re-programming of chromatin marks and gene expression patterns in donor macrophages so as to match the macrophages already resident in the recipient tissue. Combined with the intellectual framework provided by Chris Glass and co-workers from their studies of enhancer transcripts and transcription factor binding in myeloid cells we now have a much better understanding of how macrophage subset gene expression patterns are established and re-programmed(76).

Building on these seminal studies, we need develop robust methodologies for switching specific macrophage M1 and M2 functions on or off *in situ*. Such an experimental approach will allow us to move from observing changes in gene expression to actually changing macrophage cellular behaviours within a site of chronic inflammation. The application of optogenetic technologies, until now largely confined to the CNS, might be one way to achieve this ambitious goal.

> In the future could changing macrophage behaviour in chronic inflammation be used as a novel therapeutic modality, e.g. enhancing macrophage emigration from atherosclerotic plaques, or changing the behaviour of tumour associated macrophages?

(2) Functional assays for pro-resolution macrophages

Over the last 30 years intense study of the molecular biology of inflammatory mediators, be they pro- or anti- inflammatory, has occurred at the expense of studying the cell biology of tissue repair and healing. Inflammation research would benefit greatly from developing useful *ex vivo* and *in vivo* models of tissue repair and fibrosis. Ideally such models should incorporate features of (patho)physiological ECM and tissue architecture, e.g. 3D cultures rather 2D tissue cultures on plastic. In recent papers Dalli and Serhan have shown that sulfido-conjugates of the pro-resolution lipid mediator maresin hasten the resolution of acute inflammatory responses. To test the ability of these mediators to promote tissue regeneration they have turned to tissue regeneration models using a Planaria flatworm model (77, 78). Tissue repair in mammals exhibits significant differences to the tissue regeneration seen in flatworms and urodeles following amputation. This important caveat emphasizes the need for developing more model systems to study the important process of tissue repair and healing.

> Will a better understanding of successful tissue repair processes in mammals allow us to develop treatments to prevent the irreversible effects of chronic inflammation - tissue destruction and fibrosis?

(3) Time of day and time of life – circadian rhythms and Inflamm-aging

Nearly all aspects of mammalian physiology have been shown to be strongly influenced by the time of day. A series of experiments from the laboratory of Ajay Chawla elegantly demonstrated that even something as critical to the host as response to *Listeria monocytogenes* infection is susceptible to circadian rhythm. Having demonstrated a 2–3 fold circadian oscillation in Ly6C^{hi} monocyte numbers in blood and spleen Nguyen et al. infected two groups of C57BL/6J mice being kept on a 12 hour light / dark cycle with the same intraperitoneal dose of *L. monocytogenes*8 hours apart in terms of their Zeitgeber time

(ZT). Mice infected at ZT8 had reduced bacterial counts in blood and tissues 2 days post infection compared to mice infected at ZT0 and this was correlated with improved recruitment of $Ly6C^{hi}$ monocytes to the site of infection. Mice with myeloid-specific deletion of the clock gene Bmal1 showed no rhythmic alterations in $Ly6C^{hi}$ monocyte numbers in blood, spleen or bone marrow and none of the circadian variation in chemokine gene expression seen in wild-type animals (79). The impact of circadian oscillation on innate immune cell numbers and myeloid cell gene expression patterns may explain the link between shift work, altered sleep patterns and increased risk of obesity, diabetes, certain cancers and cardiovascular disease (80).

Aging populations, including our own, are characterized by chronic, low-grade inflammation often accompanied by elevated cortisol levels. This phenomenon merits further investigation because with few exceptions all age related diseases have a strong inflammatory component. The term ‘Inflamm-aging’ was coined by Franchesi and colleagues in 2000 and has become somewhat of a ‘poster child’ for systems biology ever since (81). In Figure 3 we have tried to represent key features of inflamm-aging in an idealized time course of inflammatory responses to a single inflammatory stimulus. The first point to note is that the inflammatory response in older subjects starts from an elevated baseline at t=0 but an important and key question is whether older experimental subjects show an elevated maximal response to the same inflammatory stimulus and whether the resolution of inflammation occurs more slowly in comparison with younger test subjects. In contrast to studying how the inflammatory response varies in inbred mouse strains over a 24-hour period, experimental investigation of how inflammatory responses are altered in older cohorts of experimental animals will be challenging but could bring novel mechanistic insights. Studies on exactly how inflammatory responses in humans change with age will be purely comparative (for instance GWAS of people living to 100 years of age), but studying the efficacy of immune responses to say influenza vaccines in different human cohorts might be a promising place to start teasing apart changes in the immune response with age.

> Future clinical and pre-clinical studies should take more account of the age of participants and the time of day when studies are performed. For instance does the efficacy of anti-inflammatory drugs vary with the time of delivery and/or age of the patient? Another important challenge will be to decide whether anti-inflammatory / pro-resolution agents should be given in extended versus short release formulation. The optimal drug formulation may well depend upon the ‘target’ and the specific disease.

(4) Micro RNAs – Nature’s own cytokine network regulators?

Micro RNAs (miRNAs) are estimated to modulate the expression of ~30% of all protein encoding genes in the mammalian genome so it is not surprising that multiple miRNAs have been shown to modulate myeloid cell inflammatory responses in a number of different settings. Silencing miRNA expression *in vivo* through the use of chemically modified oligonucleotides called ‘antagomirs’ has emerged as a new therapeutic modality for the development of novel anti-inflammatory drugs. The path to randomized clinical trials faces obvious problems, not least the mode of delivery and targeting antagomirs or miRNAs to the site of inflammation. However, the recent FDA approval of KYNAMRO, mipomersen

sodium of a closely related therapeutic class of molecules- anti-sense oligonucleotides (ASOs)- gives rise to optimism for this approach. In a recent report Wang et al compared the efficacy of systemic delivery of an anti-miR21 compound versus the same reagent delivered via a drug eluting stent in an experimental animal model of in stent restenosis. The authors showed that an anti-miR21 oligonucleotide could prevent in stent restenosis in a balloon injured human internal mammary artery transplanted into nude rats equally well with either delivery method, but an anti-miR21 coated stent gave rise to fewer side effects(82).

Will a better understanding of the roles miRNA splay in modulating inflammatory responses lead to the development of new drugs for regulating cytokine networks?

(5) Metabolic inflammation and obesity -mediators and microbiota

Increasing levels of obesity in the developed world have led to an alarming increase in the incidence of type 2 diabetes and cardiovascular disease. These conditions are associated with increased systemic inflammation and leukocyte infiltration into white adipose tissue (WAT). Healthy WAT in lean subjects is characterized by a population of M2 macrophages with the type 2 cytokines IL-4 and IL-13 for M2 polarisation being provided by eosinophils, which are present in reduced numbers in obese WAT. Furthermore, pro-inflammatory monocytes recruited into WAT differentiate into M1 macrophages that contribute to insulin resistance and dyslipidemia (83). It is not yet clear whether targeting leukocyte recruitment to and leukocyte activation within adipose tissue will be a viable strategy to prevent the conversion of obesity and metabolic syndrome into type 2 diabetes.

Obesity is one of the more obvious manifestations of a “Western-Lifestyle” but some immunologists have postulated a link between the increased incidence of allergies, asthma and autoimmunity with changes in diet and host microbiota generated metabolites (84). Studies in pre-clinical models of inflammation have revealed the importance of a series of metabolite receptors including GPR41, GPR43, GPR109 and GPR120 expressed by innate immune cells that can have potent anti-inflammatory actions. One of the first papers to reveal a link between diet, the gut micobiota, short chain fatty acid (SCFAs) and inflammation was provided by the laboratory of Charles Mackay in 2009. Maslowski et al showed that Gpr43$^{-/-}$ mice had excessive inflammation in models of colitis, arthritis and asthma. Germ free mice showed a similar exacerbation of inflammatory responses consistent with bacterial fermentation of complex carbohydrate generating SCFAs for stimulation of GPR43’s anti-inflammatory actions (85).

In the future will we be able to exploit our increasing knowledge of crosstalk between the microbiota, microbial metabolites and host metabolite receptors to modulate host innate and adaptive immune responses for therapeutic benefit ?

(6) Towards a more molecular definition of inflammation

In all pathology textbooks inflammation is defined by timing, being either short-term (acute) or long-standing (chronic). An alternative classification of inflammation could be envisaged that takes account of the inciting stimulus, e.g. metabolic inflammation, or the inflammatory cytokines that drive a specific chronic inflammatory disease process e.g. TNF versus IL-6 driven disease (86). Rapid advances in combined liquid chromatography mass spectroscopy

(LC-MS) techniques for inflammatory exudates may soon allow us to draw up alternative classifications based on tissue responses to infectious diseases. A striking example is provided by two recent papers that followed the time course of lipid mediator appearance in the lungs of humans hospitalized with influenza infection and mice infected with the same titer of different strains of influenza that differed in their virulence. Morita at al and Tam et al. undertook a detailed analysis of host lipid mediators and their regulation during the course of influenza infection and correlated these changes with viral replication, host immune responses including transcriptomics and measurement of cytokine and chemokine profiles. One important result from these two extensive lipidomics studies in clinical cohorts and pre-clinical infection models was the identification of the endogenous lipid mediator protect in D1 as a potent inhibitor of influenza infection. In the longer term, advances in LC-MS technology and our ability to interpret large data sets may allow us to better assess the severity of respiratory distress and virus pathology through analysis of nasal swabs of patients hospitalized with influenza (87, 88).

Ultimately, will better identification of the molecules and receptors that drive acute and chronic inflammation lead to better treatments and better patient outcomes?

(7) Future anti-inflammatory drug targets and clinical trials

In twenty years time when the current frontline biologics *du jour* such as anti TNFα, anti IL-1β, anti IL-6, anti IL-17 etc. have gone generic, we may well view these pioneering monoclonal antibodies and decoy receptors as excellent ‘test reagents’ used to identify the specific cytokine networks or specific cell types that cause chronic inflammatory responses. Currently a sub-group of RA and MS patients seem to respond better to B-lymphocyte depleting antibodies, such as the anti-CD20 monoclonal Rituximab, than they do to anti-TNFα biologics. In the future we would like to identify patients whose disease will respond better to anti B-lymphocyte therapy as soon as possible to avoid ‘hit or miss’ dosing with powerful and expensive drugs. Careful analysis of current clinical outcomes combined with biomarker analysis and pharmacogenomics studies will have the twin benefit of improved outcomes for patients and new insights in the pathogenesis of chronic inflammatory disease in human cohorts(86).

Rheumatologists have long sought after biomarkers, haplotypes, environmental factors and genetic markers that can identify patients at increased risk of developing rheumatoid arthritis. Recently biomarker panels have been expanded to include serum titers of anti-citrullinated peptide antibodies (ACPA). Early reports have given rise to the idea that this class of auto-antibodies may be driving chronic inflammation in tissues other than the joints, most notably within the lungs of patients before they present with joint inflammation and are diagnosed with RA (86). If ACPA screening could identify people who will go on to develop debilitating RA up to a decade later, what will we do with this knowledge? Should we treat this pre-RA lung inflammation aggressively with systemic anti-cytokine biologics or should we use inhaled glucocorticoids? Alternatively, should we call back people with high ACPA titers for radiological assessment every year and initiate aggressive anti-inflammatory treatment as soon as we see any sign of joint disease? An analogy can perhaps be drawn with the link between elevated plasma LDL, accelerated atherosclerosis and the increased

risk of cardiovascular disease where primary prevention emphasizes lifestyle changes and prescription of statins.

In the future will we start prescribing anti -inflammatory drugs for people with pre-diabetes, pre-RA or even pre-dementia?

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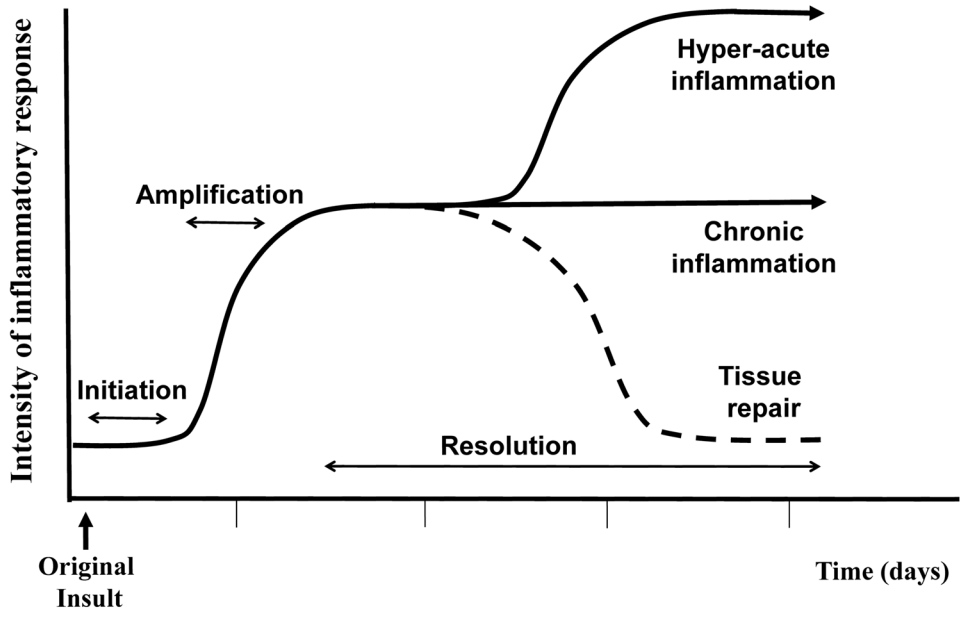

Figure 1. Time course of a typical acute inflammatory response

A schematic representation of the ideal outcome of an acute inflammatory response, i.e. resolution, is shown as a dashed line. Two potential outcomes leading to significant clinical sequelae are shown, hyper-acute inflammation e.g. septic shock and non-resolving, chronic inflammation e.g. rheumatoid arthritis.

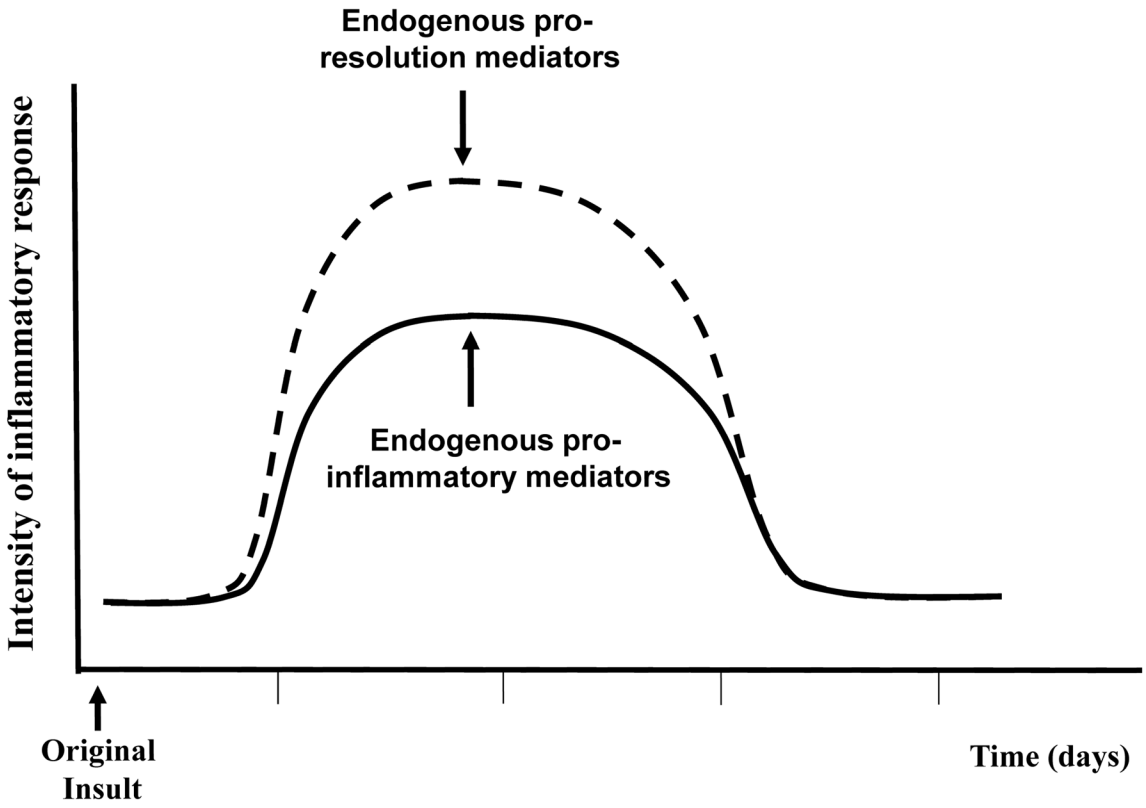

Figure 2. The inflammatory set point hypothesis

This schematic representation highlights the balance between locally produced pro-inflammatory and endogenous anti-inflammatory / pro-resolution mediators in determining the magnitude of the inflammatory response in response to a given stimulus.

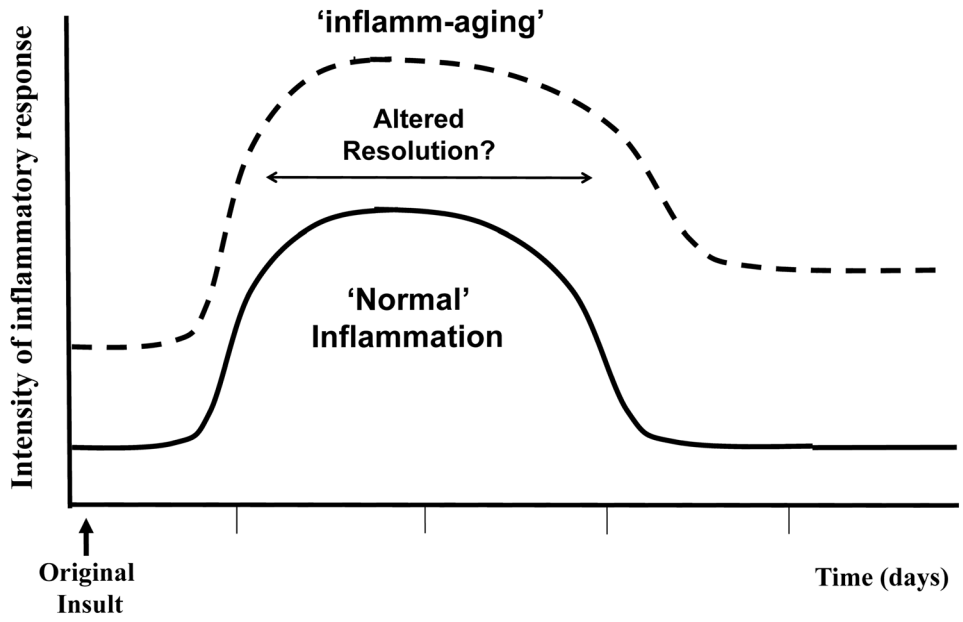

Figure 3. Inflamm-aging, do inflammatory response change with age?

This schematic representation compares the 'normal' inflammatory response (solid line) with the inflammatory response seen in aged populations (dashed line). Aged populations show increased basal levels of systemic inflammation and may show differences in the magnitude of the response to inflammatory stimuli and/or altered resolution.