GLAXOSMITHKLINE / MRS PAPER

The inflammatory macrophage: a story of Jekyll and Hyde*

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ABSTRACT

Recent investigations have highlighted new roles for the macrophage (Mφ) in the biology of inflammation. Selective depletion of Mφs from inflamed sites has confirmed their predominant role in immune-mediated damage. The components of this injury have been dissected. Mφs mediate death of stromal, parenchymal and other immune cells by engaging the death programme, resulting in apoptosis. In addition, Mφs induce destruction of matrix and extracellular structures both directly and indirectly by inducing stromal cells to release matrix metalloproteinases. However, there is another side to the inflammatory Mφ. Evidence is provided that Mφs at the same sites possess the ability to aid cell proliferation, secrete and stabilize new matrix components and induce resident cells to secrete matrix components themselves. Mφ phagocytosis of apoptotic cells brings about a change from the cell-killing matrix-degrading cell to the matrix-generating cell-proliferating tissue-healing cell. Just as both Mφ types are necessary at the inflamed site, the right balance of these two populations is required for healing and resolution. Evidence of excessive inflammation as a manifestation of impaired phagocytosis of apoptotic cells emphasizes that defects in the transition from one Mφ type to another may account for the uncontrolled excessive inflammation seen in disease. Recent insights into the mechanisms by which apoptotic cells signal the change of function to the Mφ offer the prospect of novel targets for manipulation of Mφs in the inflamed tissue.

INTRODUCTION

Inflammation is characterized by the presence of the inflammatory macrophage (Mφ), be it simple wounded skin or complex autoimmune inflammation, such as Type I diabetes mellitus. It is also characterized by the influx of other inflammatory cells, such as neutrophils and lymphocytes. The former die mainly by apoptosis at the inflamed site, whereas the latter both proliferate and die at the inflamed site. Parenchymal and stromal cells start to proliferate and die by apoptosis or sometimes necrosis. Stromal or mesenchymal cells become migratory and deposit excessive pathological matrix. Normal tissue structure and architecture is often destroyed, leading to organ dysfunction. Yet, amongst all this chaotic activity, there is evidence of co-ordination, since apparently severe...
inflammation can resolve, leaving histologically and functionally normal structures. So why do some types of inflammation resolve, some ‘burn-out’ with persistent scarring, whereas others are characterized by persistent inflammatory cell infiltrate?

Recently, it has become apparent that the Mφ is a major player in the inflammatory response. Furthermore, evidence has accumulated that there is more than one type of inflammatory Mφ; indeed, there may be different populations of Mφ sub-serving different functions simultaneously. Newly described roles for the inflammatory Mφ are often diverse, sometimes contradictory, and in conflict with other roles. Initially, the inflammatory Mφ was shown, like the neutrophil, to produce cytotoxic mediators injurious to eukaryotic cells as well as prokaryotic cells. In addition to the capacity of Mφs to phagocytose debris, it was shown that Mφs phagocytose cells dying by apoptosis [1]. Mφs have also been shown to produce a myriad of cytokines, small, often soluble, peptide molecules that are the effectors of Mφ function.

In the present paper, evidence is provided that a key role for the inflammatory Mφ is killing by apoptosis and clearance of both infiltrating leukocytes and proliferating resident stromal and parenchymal cells. Furthermore, ingestion by Mφs of the cells they have killed leads to dampening down of the pro-inflammatory activities of the Mφs, altering the Mφ to a cell that has the capacity to initiate tissue healing and repair.

**Mφ Activation States**

Monocytes are the circulating precursors of tissue Mφs. They lack phagocytic capacity and their cell surface receptor/ligand complement renders them relatively inert whilst in the blood compartment. In vivo, they rapidly cross the endothelial barrier using selectins, integrins and other receptors of the immunoglobulin superfamily to initially role on the endothelium, then become tethered, and finally diapedesed [2,3]. At the inflamed site, activated endothelial, epithelial and mesenchymal cells of the tissue release chemokines and cytokines, which signal to the naïve monocyte. Binding of immune complexes or phagocytosis of opsonized particles and debris triggers activation through receptor signalling. Activating cues are also received from lymphocytes and disturbed matrix [4,5]. Products of invading organisms, as might be found in wounded tissue, also potently stimulate activation through specific receptor-ligand interactions. Once the monocyte leaves the circulation, it differentiates into the Mφ (defined by its capacity to phagocytose). Naïve Mφs entering the inflamed site develop one of at least several phenotypic states depending on the array of information sensed at the cell surface by such receptor binding. Collectively these phenotypic states are known as classical activation (Table 1). Many factors can push the Mφ toward the classical activation phenotype. Ligation of chemokine receptors [such as those for Mφ chemotactant protein-1 (MCP-1)], exposure to pathological collagen deposition and hypoxia can all activate the Mφ [4–7].

There are, however, important differences in the phenotype of Mφs activated by different stimuli. For example, binding of the Th1 lymphokine interferon (IFN)-γ has been shown [8] to augment the activation of Mφs in response to bacterial products, such as lipopolysaccharide (LPS), pro-inflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-12, or activated T-lymphocytes through CD40 ligand. The absolute amount of cytokines and toxic nitrogen radicals released by Mφs that have been exposed to IFN-γ and one of those activating stimuli is many fold higher than Mφs that have been exposed to activating stimuli without IFN-γ. Mφs can be activated by immune complexes signalling through the Fc-γ receptors (Fc-γRs), but, instead of NO production, toxic oxygen radicals predominate. Instead of high IL-12 release (known to be necessary in the development of cell-mediated immune responses) like when Mφs are treated with LPS, Fc-γR1 activation results in the release of low levels of this cytokine [9,10]. Stimulation of Mφs with IFN-γ also up-regulates expression of those receptors necessary for effective presentation of antigens to the adaptive immune system. Thus differences in the pattern of cytokine release by Mφs may translate into differences in function, rendering the term ‘classical activation’ of Mφs an oversimplification.

Not all cytokines, however, cause Mφs to become classically activated. Exposure of naïve Mφs to Th2 lymphokines, such as IL-4, or the cytokine transforming growth factor (TGF)-β, prevents the Mφ inflammatory phenotype, even when such Mφs are challenged with pro-

**Table 1** Stimuli for classically and alternatively activated Mφs

<table>
<thead>
<tr>
<th>Mφ activation</th>
<th>Stimulus</th>
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<tbody>
<tr>
<td>Classical</td>
<td>IFN-γ plus pro-inflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td>Bacterial lipoproteins (TLRs)</td>
</tr>
<tr>
<td></td>
<td>Bacterial DNA (TLRs)</td>
</tr>
<tr>
<td></td>
<td>Parasitic proteins/carbohydrates (TLRs)</td>
</tr>
<tr>
<td></td>
<td>Opsonized particles (FcR, CR)</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
</tr>
<tr>
<td></td>
<td>Abnormal matrix</td>
</tr>
<tr>
<td>Alternative</td>
<td>IL-4</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
</tr>
<tr>
<td></td>
<td>IL-13</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
</tr>
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<td></td>
<td>Glucocorticoids</td>
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inflammatory cytokines. These cytokines cause deactivation.

In fact, several other cytokines (IL-10 and IL-13) and glucocorticoids have the same effect. The term deactivation is inadequate, however, since Mφs exposed to these ‘non-inflammatory’ cytokines do not remain naïve or inert; instead, they show enhanced capacity for antigen presentation and enhanced phagocytosis of debris and particles, though not pathogens. The ‘alternatively activated’ Mφ generates anti-inflammatory cytokines, suppresses synthesis of pro-inflammatory cytokines and is resistant to re-activation [8,11,12].

**MR HYDE: INDUCTION OF TISSUE INJURY**

Tissue injury is a rather vague term without precise definition. It leads to organ dysfunction and damage of cellular structures, but might also imply the induction of cellular death. It indicates damage to the tissue architecture and therefore not only cells, but the extracellular matrix.

The ability of Mφs to induce tissue injury is well established. In models of inflammation, such as severe glomerulonephritis in the kidney (induced by deposition of antibody on the basement membrane of highly specialized glomerular capillaries), depletion of Mφs in the early stages of disease brought about amelioration [13]. Kupffer cell depletion in three models of liver injury using liposomal clodronate attenuated disease in all cases [14]. In the T-cell-dependent autoimmune model of multiple sclerosis, experimental allergic encephalomyelitis (‘EAE’), depletion of Mφs prevented relapse completely. T-cells were still present, indicating that infiltrating Mφs were crucial effectors of tissue injury [15]. The Mφ as the effector of tissue injury is supported further from depletion studies in allograft and tumour rejection [16,17]. Interestingly, however, Mφ depletion during an animal model of autoimmune diabetes not only blocked disease, but prevented the maturation of effector cytotoxic T-cells, due to an absence of the Mφ-derived cytokine IL-12 [18] (Figure 1).

More than 30 years ago it was reported that cells can die by a controlled programme of death called apoptosis [19], unlike the chaotic destruction of necrosis. The principal differences between apoptosis and necrosis are both functional and morphological. Functionally, apoptotic cells maintain integrity of the plasma membrane and specifically disable many intracellular signalling and homoeostatic systems. Furthermore, DNA is specifically cleaved to render it unusable. These processes might be seen as non-inflammatory in that toxic intracellular vesicles are not released and DNA is safely disposed of [20].

Dramatic increases in apoptosis of cells at the inflamed site have been described [1,19,21,22]. Some of these apoptotic cells are neutrophils, inflammatory cells that are pre-programmed to die as soon as they exit the bone marrow, where they are produced in large numbers. Many are cells derived from the inflamed tissue itself, including resident stromal and parenchymal cells [23–29]. In the models of severe inflammation described above, there is evidence of dramatic increases in apoptosis [14,26,30–33]. What is the significance of these apoptotic cells? If the loss of resident cells outstrips the generation of new cells by proliferation, the outcome is irrevocable loss of tissue, hypocellularity and failure of organ

![Figure 1](image)

**Figure 1** Diverse and disparate functions of the Mφ.

Monocytes can differentiate into classically or alternatively activated Mφs. Their respective functions are shown. Overlapping functions are also indicated.
Table 2 Induction of resident cell apoptosis by Mφs in inflammation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Disease</th>
<th>Cell-type</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>GN, UUO</td>
<td>Mesangial, tubular</td>
<td>Duffield et al. [36], Tesch et al. [34], and Lange-Sperandio et al. [50]</td>
</tr>
<tr>
<td>Liver</td>
<td>Cirrhosis, hepatic failure</td>
<td>Stellate, hepatocyte</td>
<td>Schumann et al. [14], and J. S. Duffield and J. P. Iredale (unpublished work)</td>
</tr>
<tr>
<td>Vasculature</td>
<td>Atherosclerosis</td>
<td>Smooth muscle</td>
<td>Boyle et al. [40]</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>Fibroblasts</td>
<td>Polunovsky et al. [83]</td>
</tr>
<tr>
<td>Skin</td>
<td>Wounding</td>
<td>Fibroblasts, neutrophils</td>
<td>Desmouliere et al. [24], and Mezaros et al. [35,84]</td>
</tr>
<tr>
<td>Pancreatic islets</td>
<td>Diabetes</td>
<td>β cells</td>
<td>Jun et al. [18], Suk et al. [85], and Thomas and Kay [86]</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>EAE</td>
<td>T-cells</td>
<td>Pender and Rist [87]</td>
</tr>
<tr>
<td>Immune cell interactions</td>
<td></td>
<td>Lymphocytes</td>
<td>Saio et al. [88], and Brown and Savill [41]</td>
</tr>
</tbody>
</table>
to apoptotic death by the Mφ release of the soluble form of Fas ligand [40,41].

Tissue injury, however, is not only cell death. The matrix supporting the cellular architecture is progressively destroyed, resulting in collapse and distortion of normal structures. Many matrix components are crucial to normal tissue function and homeostasis. One example of a specialized extracellular protein matrix is the glomerular basement membrane in the kidney that acts as a molecular sieve. During disease, this sieve malfunctions, resulting in leakage of plasma proteins and erythrocytes into the urine.

The classically activated inflammatory Mφ plays a crucial role in bringing about matrix destruction through the production of matrix metalloproteinases (MMPs) both directly and indirectly. For example, classically activated Mφs up-regulate the activity of MMPs with gelatinase, caseinase and elastinase activity; in particular MMP-9, MMP-2 (gelatinases), MMP-12 (metalloelastase) [42] and MMP-7 (matrilysin) [43]. Mφs are also known to release collagensases (MMP-1 and MMP-13) in particular circumstances [44]. In co-culture assays, Mφs can induce myofibroblasts to release collagenases. Thus particulate-activated Mφs induce lung fibroblasts in the rat to generate collagenase-3 (MMP-13) activity, whereas fibroblasts alone did not produce this collagen-degrading enzyme [45]. In studies of kidney disease, inflammatory Mφs of the glomeruli induced myofibroblast mesangial cells to produce stromelysin (MMP-3) [46]. Thus the classically activated Mφ may bring about degradation of normal and abnormal matrix.

There is evidence that matrix destruction is not a linear unidirectional process, rather a dynamic turnover. During the early phases of normal wound healing, both Mφs and myofibroblasts express tissue inhibitors of metalloproteinases (TIMPs). These natural inhibitors of the MMPs bind specifically and block MMP activity. TIMP-1 and -3 are up-regulated early in the injury response [47]. In other inflammatory models, such as ureteric obstruction, Mφs early in disease express MMP-1 and -2, but simultaneously express TIMP-1 and -2 [48].

How exactly Mφ-directed matrix degradation and its inhibition balance out has not been elucidated. However, conditioned medium from quiescent rat alveolar Mφs does not have overall proteolytic activity in enzymography assays, whereas medium from classically activated Mφs does. Furthermore, in whole tissue assays (i.e. those including the resident and inflammatory cells), expression of TIMP-1 predominates over MMPs in liver fibrosis during the early stages of disease, implying that overall proteolytic activity of tissue Mφs is inhibited, though the matrix may be turned over. Later, however, during the resolution phase of liver fibrosis, expression of the collagenase MMP1/13 predominates [25], suggesting that during this later phase Mφ function switches and overall proteolytic activity predominates.

Thus the classically activated Mφ, analogous to Mr Hyde, kills inflammatory, stromal and parenchymal cells by apoptosis. In addition, this cell-type has overall matrix proteolytic activity, releasing many enzymes that break down extracellular structures. Why should such a Mφ exist? It is likely that, since the inflammatory process functions to keep invading organisms from entering the host as well as bring about repair, a cell-killing Mφ that is able to migrate easily through matrix structures is essential for the first phase of wound healing; that is containment and clearance of debris. It is also clear that if proliferation of resident cells were not kept in check the consequences might be even greater damage to the tissue.

**DR JEKYLL: EXTRACELLULAR MATRIX REMODELLING AND RESOLUTION OF INFLAMMATION**

Just as Mφ-depletion studies have shown a clear role for Mφs in killing cells and damaging extracellular structures, in other, often milder, resolving models of inflammation, Mφ-depletion has had different results.

In the healing wound model, Leibovich and Ross [49] depleted Mφs using anti-Mφ serum and demonstrated decreased matrix production and fibrosis, indicating that Mφs were responsible for laying down matrix. In recent studies by Lange-Sperandio et al. [50], Mφs were unable to transmigrate and enter the injured renal interstitium following ureteric obstruction in mice lacking selectin, cell-surface molecules important in diapedesis. These selectin-deficient animals showed a much diminished laying down of scar tissue as well as a reduction in tubular cell apoptosis [50]. These observations have been corroborated in our own department. De Heer et al. [51], investigating the self-resolving model of rat Thy1.1 glomerulonephritis, found that in some rodent strains there were many influxing Mφs, whereas in others far fewer. The presence of Mφs correlated with fewer mesangial (myofibroblast) cells, but more matrix deposition. However, those strains with more Mφs showed resolution of disease, in contrast with those with fewer Mφs, which had persistent hypercellularity. The latter did not recover and went on to have permanent renal scarring. This interpretation of Mφ function was supported by Mφ-depletion studies using clodronate in the same disease model [52]. Thus such studies indicate that Mφs, or at least a population of Mφs at the inflamed area, are responsible for generating matrix components. This is in direct contrast with studies by Tipping et al. [13] who depleted Mφs in another model of kidney inflammation, progressive nephrotoxic nephritis. In these studies, depletion of Mφs attenuated inflammation and prevented proteinuria [13,53].

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Direct evidence for Mϕ-mediated matrix deposition has been derived from in vitro studies: when Mϕs were alternatively activated by IL-4 or the cytokine TGF-β, followed by co-culture with myofibroblasts, the latter increased fibronectin production, proliferated more and produced more collagen I [43,54]. IL-4-activated Mϕs themselves produced more fibronectin and other matrix proteins, such as TGF-β-inducible gene H3 (‘βIG-H3’) [55]. Furthermore, Mϕs in healing wounds highly expressed tissue transglutaminase [56], which cross-links matrix proteins, such as fibronectin, collagen, fibrinogen, laminin and osteopontin (OPN), rendering the polymeric structures resistant to breakdown by proteases. In rodent injury models of the myocardium, skin and other tissues (by freezing), a population of inflowing Mϕs abundantly produced the matrix protein OPN [57]. Its production by Mϕs is limited to the early phase of the injury response. OPN is not only involved in normal fibrillogenesis of collagen, but also Mϕ recruitment, maintaining Mϕ activation and down-regulating enzymes which breakdown matrix, namely MMPs. In support of a role for TGF-β in signalling to myofibroblasts to generate new matrix, two recent studies of wound healing in knockout animals lacking either TGF-β or smad-3 (one of the components involved in TGF-β receptor signalling) indicated Mϕ-derived TGF-β as necessary for matrix deposition in the normal healing process [58,59].

The in vitro studies of Song et al. [43] and Pierce et al. [54] indicated that the alternatively activated Mϕ can induce myofibroblast-like stromal cells to proliferate. Corroborative evidence for this comes from several studies in different organs (Table 3). Analysis of low-grade inflammation in the kidney emphasizes the point: the presence of Mϕs in the tissue correlated with mesangial (myofibroblast) proliferation [60]. Song et al. [43] and others [54,61,62] were able to confirm that Mϕ-derived platelet-derived growth factor (‘PDGF’) and insulin-like growth factor (‘IGF’) were important pro-survival and pro-proliferative factors for myofibroblasts.

There is also data to suggest that Mϕs are important in angiogenesis, since they generate a multitude of angiogenic soluble cytokines, including vascular endothelial growth factor (VEGF) [63], basic fibroblast growth factor (‘bFGF’), insulin-like growth factor-1, TGF-α and TNF-α [64,65]. The importance of angiogenesis in normal healing of the injured tissue has recently been emphasized in the rat Thy1.1 model of nephritis that normally resolves. Blockade of the potent angiogenic cytokine VEGF was associated with the failure of healing and progression to scarring ensued instead [66]. Although not exclusively produced by Mϕs, they represent a potent source of this cytokine.

Collectively, these studies point to a different population of Mϕs in those models of inflammation where healing is occurring, and indicate a role for Mϕs in healing and repair. Thus the alternatively activated Mϕ is analogous to the good Dr Jekyll.

### MR HYDE CAN CHANGE INTO DR JEKYLL

Inflammatory and resident Mϕs are phagocytic and are able to phagocytose debris, invading organisms and opsonized particles. They have another role in the injured tissue, namely the clearance of apoptotic cells. As described earlier, inflammatory and resident cells die by apoptosis. Mϕs limit inflammation by rapidly clearing apoptotic cells, such as neutrophils, which, if left in the tissue, would undergo secondary necrosis, spilling pro-inflammatory proteases into the inflammatory milieu [1,20,67]. When Mϕs ingest opsonized particles or foreign agents, this triggers a pro-inflammatory response. Ingestion of apoptotic cells does not trigger activation. Indeed, recent evidence indicates that Mϕ ingestion of apoptotic cells is more than non-activating Mϕs, it is deactivating. Several in vitro studies have now demonstrated that when Mϕs ingest apoptotic cells, be they neutrophils, lymphocytes or stromal cells, such as mesangial cells, they become refractory to the production and release of pro-inflammatory mediators, including IL-1β, IL-6, IL-12, TNF-α, MCP-1 and Mϕ-inhibitory protein (‘MIP’)-1α [37,68–70]. The same Mϕs were not inert, but produced anti-inflammatory mediators, such as TGF-β, IL-10 (in some studies) and prostaglandin E2 (‘PGE2’).

In our own studies of models of inflammation in the kidney [36,37], resident myofibroblast mesangial cells were killed (by apoptosis) by inflammatory Mϕs. Those Mϕs phagocytosed the apoptotic mesangial cells and were inhibited from further killing (Figure 3). This process could be seen as analogous to a carnivore preying on an unsuspecting animal, killing it and devouring it, which leads to satiety. The carnivore no longer desires or

### Table 3 Induction of resident cell proliferation by Mϕs in inflammation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Disease</th>
<th>Cell-type</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Nephrosis</td>
<td>Mesangial, interstitial fibroblast</td>
<td>Diamond et al. [89], and Sharma et al. [90]</td>
</tr>
<tr>
<td>Liver</td>
<td>Partial hepatectomy</td>
<td>Hepatocyte</td>
<td>Rai et al. [91]</td>
</tr>
<tr>
<td>Vasculature</td>
<td>Atherosclerosis</td>
<td>Smooth muscle</td>
<td>Ross et al. [62]</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>Fibroblasts</td>
<td>Cao et al. [61], and Kodelja et al. [92]</td>
</tr>
<tr>
<td>Skin</td>
<td>Wounding</td>
<td>Fibroblasts</td>
<td>Hunt et al. [93]</td>
</tr>
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The inflammatory macrophage

Figure 3  Phagocytosis of apoptotic cells inhibits inflammatory Mφs from inducing death of myofibroblast mesangial cells

Mφs were differentiated from bone marrow using Mφ- colony-stimulating factor (‘M-CSF’). The purified Mφs were then cultured with mesangial cells (MC) in a 1:1.5 ratio. The co-culture was overlaid with pre-prepared apoptotic cells or similar sized latex beads. After incubation for 4 h, loose apoptotic cells were removed and the co-culture was activated with pro-inflammatory cytokines (IFN-γ and LPS). MC apoptosis was scored at 24 h and compared with MC apoptosis in MC cultures exposed to apoptotic cells and cytokines in the absence of Mφs. **P < 0.001 compared with ingestion of beads.

is able to kill. Thus it seems that the process of inducing apoptosis in resident cells with subsequent binding and phagocytosis of the dead cells ‘switches off’ the Mφ. The switching off of Mφ-mediated killing in this model of kidney inflammation coincided with down-regulation of TNF-α release and up-regulation of TGFβ, release [37]. Furthermore, the effect was long lasting; 48 h after ingestion of apoptotic cells, Mφs were incapable of responding to inflammatory stimuli (J. S. Duffield, unpublished work).

The immunosuppressive effect of apoptotic cells on Mφs has now been reported in animal models of multiple sclerosis [71], rheumatoid arthritis, [72], lung inflammation [73] and Trypanosome infection of the peritoneum [69], emphasizing its widespread implication in inflammation.

It is important to emphasise that this switch from classically to alternatively activated cell-type is not complete. The alternatively activated Mφ does not produce NO, but releases IL-10 in abundance. However, in our studies [37], ingestion of apoptotic cells neither down-regulated NO nor promoted IL-10 generation. Thus the Mφ that has ingested apoptotic cells exhibits many features of the alternatively activated Mφ, but it is not functionally identical.

In the investigation by Voll et al. [70] of human monocytes binding apoptotic lymphocytes, monocytes behaved similarly to Mφs in that binding of apoptotic cells inhibited the activation normally induced by LPS. The monocytes also released anti-inflammatory cytokines. Since monocytes have little capacity to phagocytose particles or cells, it may be that binding alone of apoptotic cells is sufficient to signal to the monocytes/Mφs to bring about the change in phenotype.

Figure 4  Some of the receptor–ligand interactions occurring when a Mφ binds an apoptotic cell

When an apoptotic cells engages with a Mφ several receptor–ligand interactions occur. New epitopes on the apoptotic cell surface, such as PS exposure, result in binding to the PSR. Intercellular adhesion molecule (‘ICAM’)-3 binds to CD14 and unknown ligands bind to the scavenger receptor CD36 and via a molecular bridge the integrin αvβ3 binds. CD31 expressed on live cells is repellant to CD31 on Mφs, but becomes adherent on the apoptotic cell. Homophilic engagement of CD31 on the Mφ can activate CD31 to bind, via SH2 domains, SHP-1 (SH2 domain-containing phosphatase), a phosphatase which counteracts the activation of cell signalling pathways.

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Blockade of Mφ receptors known to be involved in recognition and binding of newly exposed epitopes on the surface of apoptotic cells, namely the scavenger receptor CD36, the integrin $\alpha_\beta$ and the phosphatidylserine (PS) receptor (PSR), has been reported to inhibit the immunosuppressive effects of apoptotic cells on Mφs [69,70,73]. The ligands for CD36 and $\alpha_\beta$ remain unknown, but PSR recognizes the membrane phospholipid PS, which is exposed on the outer leaflet of the plasma membrane of apoptotic cells. Binding of agonistic antibodies to $\alpha_\beta$ or CD36 on the Mφ cell surface concurrently with the activating stimulus LPS has been reported to block the activation normally induced by LPS [69,70], thus mimicking the effect of apoptotic cells binding to the Mφ. Furthermore, intracellular signalling from CD47, also known as integrin-associated protein (‘IAP’), can block certain pro-inflammatory signalling pathways. This cofactor is closely associated with $\alpha_\beta$ and is necessary for its activation [74,75] (Figure 4). These observations open up the possibility that, by manipulating receptors on the Mφ that mediate apoptotic cell engulfment, it will be possible to manipulate the ongoing function or activity of a population of inflammatory Mφs. The population could be accelerated into the transition from killing matrix-degrading cells into reparative healing cells. Brown et al. [76] recently found that the cell-surface receptor CD31, located on both the Mφ and the apoptotic cell, bound tightly through homophilic interaction, thus mediating recognition. The same interaction between the same receptors on live cells was repulsive, that is promoted disengagement. Such a finding offers novel strategies for manipulating the function of the Mφ, as CD31 has properties of an inhibitory receptor: when ligated it becomes tyrosine phosphorylated at specific sites in the intracellular tail and this enables the binding of phosphatases, such as Src homology 2 (SH2) domain-containing phosphatase (‘SHP-1’). This phosphatase can block many activating signalling pathways, thereby inhibiting activation of the Mφ. Ongoing research points to CD31 and other inhibitory receptor family members as being responsible for mediating the immunosuppressive effects of apoptotic cells on the Mφ (Figure 4).

THE RIGHT AMOUNT OF MR HYDE AND DR JEKYLL

It can be seen that there is evidence of both types of Mφ in inflammation from the descriptions of Mr Hyde, the classically activated Mφ, and Dr Jekyll, the alternatively activated Mφ, in different inflammatory diseases. For example, in the resolving Thy1.1 nephritis, characterized by initial mesangiolytic injury, there are activated Mφs [77] and the induction of apoptosis [23]. There is also evidence of Mφ-mediated matrix accumulation following the initial mesangiolytic injury [52]. This pattern of cell death, matrix destruction followed by healing, is re-iterated elsewhere [24,25,50]. It suggests that the population of Mφs changes with time from one predominantly classically activated to one predominated by alternatively activated Mφs. Our studies [36,37] indicate that this transition from one Mφ population to another is brought about by the actions of the classically activated Mφ. Thus the very action of killing cells leads to phagocytosis, which leads to a transition from classically to alternatively activated.

In progressive inflammation, there is much more apoptosis of stromal and parenchymal cells than seen in resolving inflammation ([32,29], and G. L. Thomas and J. S. Duffield, unpublished work). It implies that there may be too many Mφs or too many activated Mφs at the inflamed site. On the other hand, too many of the alternatively activated Mφs also appears to be detrimental. For example, when TGF-$\beta$, a cytokine released by alternatively activated Mφs, is overexpressed in the lung, it promotes progressive uncontrolled fibrosis with a paucity of inflammatory cells. Glucocorticoids, often used as a treatment for severe inflammation, promote alternatively activated Mφs. When effective, these agents leave scarred organs. Moreover, alternatively activated Mφs are associated with protracted chronic inflammation that is seen in conditions such as psoriasis and rheumatoid arthritis [78,79]. Together, these observations beg the question: are there defects in the co-ordinated progression from a Mφ population predominated by classically activated Mφs to one predominated by alternatively activated Mφs that result in failed resolution of inflammation?

Evidence already exists that this is the case. Complement factor 1q (C1q), a soluble protein from the complement cascade and more recently recognized as a member of the collectin family of proteins, is able to bind apoptotic cells and promote phagocytic clearance. Inflammatory Mφs recognize C1q on apoptotic cells, which assists in mediating phagocytosis. The C1q knockout mouse exhibits failure to clear apoptotic cells in tissues in a C1q-dependent fashion. The animal develops excessive inflammation, glomerulonephritis and features of the autoimmune disease systemic lupus erythematosis (‘SLE’). Excessive apoptotic cells are seen in the organs. Furthermore, when nephritis is deliberately induced, the disease is much more dramatic in these animals [80–82], implying overactivation of inflammatory Mφs.

CONCLUSION

Inflammatory Mφs are a heterogeneous group of cells with diverse functions. Broadly, and simplistically, they fall into two groups: the ‘angry’ cell-killing matrix-destroying Mφ, analogous to Mr Hyde, and the matrix-
generating cell-nurturing tissue-regenerating Mφ, analogous to Dr Jekyll. As Mr Hyde turns into Dr Jekyll, there is now evidence that the classically activated Mφ can lose its cell-killing pro-inflammatory capacity and take on features of the alternatively activated anti-inflammatory Mφ (Figure 5).

It is clear that (i) both cell types are necessary in the inflamed tissue for normal damage limitation and healing, (ii) both cell-types are probably active concurrently, (iii) an excess of either is detrimental to resolution of inflammation, and (iv) co-ordinated changes in one overriding population to another are necessary for remodelling and resolution of the inflamed tissue. Central to this conversion is the killing and ingestion of apoptotic cells by classically activated Mφ. This process signals to the Mφ population to change from a killing to a reparative population.

Logically, defects in the process of cell killing, apoptotic cell clearance or intracellular pathways signalling to the Mφ upon engulfment of apoptotic cells will perturb this normal process, leading to excessive or inadequate activation of Mφs with the consequence of excessive or persistent chronic inflammation. Evidence already exists for such a defect in studies of the C1q knockout mouse. With the discovery of a role for members of the inhibitory receptor family in clearance of apoptotic cells by Mφ, current research is directed at defining the precise mechanisms by which apoptotic cell engulfment leads to the reprogramming of Mφ function. Understanding and manipulating defects in clearance and inhibitory signalling, such as C1q deficiency, may well lead to new therapeutic interventions in the future.

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