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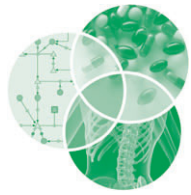

The macrophage and its role in inflammation and tissue repair: mathematical and systems biology approaches

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Macrophages are central to the inflammatory response and its ability to resolve effectively. They are complex cells that adopt a range of subtypes depending on the tissue type and stimulus that they find themselves under. This flexibility allows them to play multiple, sometimes opposing, roles in inflammation and tissue repair. Their central role in the inflammatory process is reflected in macrophage dysfunction being implicated in chronic inflammation and poorly healing wounds. In this study, we discuss recent attempts to model mathematically and computationally the macrophage and how it partakes in the complex processes of inflammation and tissue repair. There are increasing data describing the variety of macrophage phenotypes and their underlying transcriptional programs. Dynamic mathematical and computational models are an ideal way to test biological hypotheses against experimental data and could aid in understanding this multi-functional cell and its potential role as an attractive therapeutic target for inflammatory conditions and tissue repair. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

We start with a necessarily simplified account of a macrophage's central role in inflammation and tissue repair in order to set the stage for our subsequent description of relevant mathematical modeling.

Inflammation is the body's normal response to tissue injury and infection with the purpose of repairing any damage and returning tissue to a healthy state. It is a highly regulated process with both pro- and anti-inflammatory components that work together to ensure a quick resolution and restoration of tissue structure. In contrast, disruption of this process can lead to chronic inflammation. This self-perpetuating condition, which is now recognized to underlie a wide range of diseases, including rheumatoid arthritis, inflammatory bowel disease, asthma, atherosclerosis, neurodegenerative diseases, and cancer,[1] can disrupt the transition to repair processes and the restoration of normal tissue architecture.[2]

Macrophages are highly versatile large white blood cells that play a central role in all stages of the inflammatory response[3] (see Figure 1). They derive from blood monocytes and reside in all tissues, where they act as sentinels responding to damage by activating.[4] Once activated, macrophages are efficient phagocytes; this is the process by which they remove unwanted material including apoptotic cells (those that have died naturally). They secrete a wide array of cytokines and chemokines that direct the inflammatory response and aid in tissue repair.[3,4] Macrophages acquire distinct functional phenotypes directed by tissue type and environmental cues[4,5] (see

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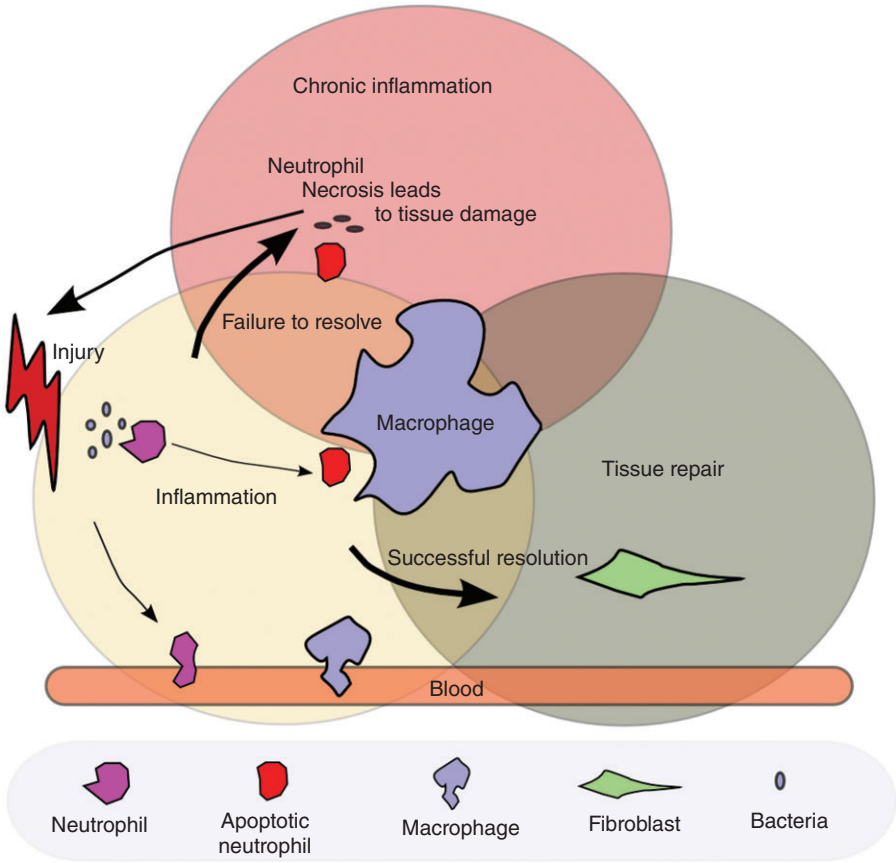

FIGURE 1 | Macrophage role in inflammation and tissue repair. Upon stimulus monocytes and resident macrophages activate. They remove tissue debris and produce inflammatory signals that promote the inflammatory response. Macrophages produce a wide array of cytokines, chemokines, and growth factors that promote inflammation, its regulation, and the successful restoration of tissue. They also participate in the regulation of inflammation by removing apoptotic neutrophils, an important process in turning the inflammatory process to one of tissue replacement and remodeling, apoptotic neutrophils that are not removed can undergo necrosis, spilling their toxic content and perpetuating the inflammatory response.

Figure 2). This enables the macrophage to play multiple roles in the inflammatory response and the repair of tissue, both encouraging and discouraging these processes. This heterogeneity in function is thought to be pivotal to the successful resolution of inflammation and the restoration of healthy tissue.[3] While inflammation has long been studied, there is still much controversy surrounding macrophages, their heterogeneity, what they express when, how they respond to different injuries and infections, as well as their response in different tissue types.[3,4]

The employment of high-throughput techniques is resulting in a rapidly expanding body of biological data. The macrophage is no exception to this assault, with a wealth of data being produced that describes the macrophage's function, morphology, gene, protein, and transcription factor expression in response to a wide array of stimuli.[7–9] There is growing interest in understanding these data at a systems level and this is highlighted by the publication of a website[10,11] that aims to collate and present macrophage data. This has led to some novel and visually appealing attempts to define the components of the networks that underpin macrophage heterogeneity and assemble them in a way that is not only visually appealing and but easy to interrogate.[12–14]

The processes involved in inflammation and the repair of tissue act over a wide range of temporal and spatial scales—from subcellular, through to cellular and macroscopic—while the timescales may vary from seconds (or less) for signal transduction pathways to months or even years in the case of chronic inflammation. Understanding this system-level behavior is a daunting task. Mathematical and computational approaches can aid in this not only by animating current static knowledge of protein level interactions into dynamic temporal models that allow perturbations and their effects to be explored but

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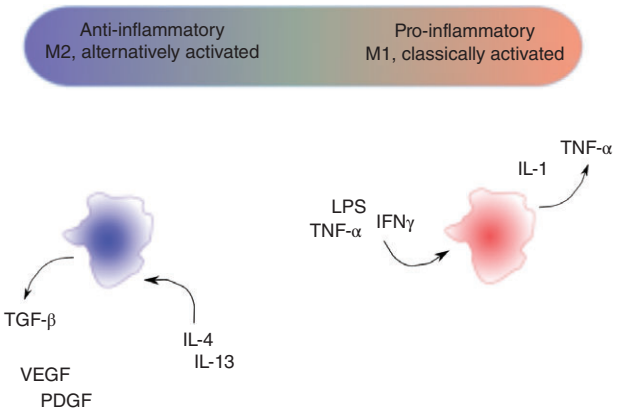

FIGURE 2 | Macrophages play a key role in inflammation and tissue repair tailoring their behavior to signals from their environment. Traditionally, two main macrophage phenotypes have been identified: M1 (classically activated) that are pro-inflammatory in nature, promoting the killing of pathogens and driving the inflammatory response and M2 (alternatively activated) that are thought to upregulate anti-inflammatory cytokine secretion and activate downstream tissue repair. The understanding of macrophage heterogeneity is changing rapidly, driven by gene expression data that displays no single marker of phenotype; it is now thought that macrophages display shades of activation, suggesting there may be a continuum of phenotypes. While the M1/M2 paradigm can provide a simplified description that can be used to aid in deciphering the role of macrophages in health and disease classification of macrophage heterogeneity is only likely to expand. Source: Mosser and Edwards.[6]

also by the development of comprehensive multi-scale models that link subcellular events through to macrophage function and its role in inflammation and repair. In this study, we review some of the current mathematical and computational models that focus on macrophages and their interactions in these processes. In doing this, we highlight how mathematical modeling and computational techniques provide useful insights into the key role of macrophages in the inflammatory process.

MATHEMATICAL MODELS

Various systems biology and mathematical techniques have been used to construct models that describe the inflammation response and its subsequent reparatory processes. Many of these models either focus on, or include, macrophages. Some models focus on a particular part of the inflammatory process, such as the ability of the inflammatory response to resolve[15] or the effect of inflammatory cell motility on the outcome of the response;[16–18] others capture specific macrophage functions such as their ability to remove debris;[19] while yet others investigate specific diseases that are inflammatory in nature and as such include populations of macrophages.[20] Rather than attempting a comprehensive review of all models that include macrophages in the context of the inflammatory response or tissue repair, we present an illustrative review of the breadth and depth of models that incorporate the macrophage in this context. A summary of the models and the macrophage behavior that they capture is given in Table 1 and the key macrophage parameters utilized by, or estimated in, these models are listed in Table 2.

Acute Inflammation and Tissue Repair

Recruitment of macrophages, and/or other leukocytes, into tissue is a key step in the inflammatory process. This occurs through a highly regulated process that allows leukocytes to permeate from blood vessels, although gaps between endothelial cells that line them, into tissue.[15] Once in tissue, cells are chemoattracted to the site of injury by the release of small molecules from damaged tissue and/or pathogens. Early attempts at modeling the inflammatory process focussed on a generic leukocyte's localization in tissue to a bacterial infection.[16–18] These simple models, with two or three variables representing bacteria, leukocytes, and chemoattractants (that attract leukocytes to the inflammatory stimulus), have been used to investigate how variation in key parameters, such as leukocyte capacity for motility or for phagocytosis of bacteria, affect susceptibility to bacterial infection.

Penner et al.[20] extend a classic mathematical model of cell motility in response to a chemical signal (the Keller–Segel model[30]) to include an anti-inflammatory mediator. Interpreting the cell as a macrophage, which responds by moving up the concentration gradient of the chemical signal (the pro-inflammatory mediator), a newly introduced anti-inflammatory mediator, along with the original chemical signal, is produced by the macrophages. The production of the anti-inflammatory mediator follows a slower timescale than that of the pro-inflammatory mediator and thereby allows the existence of self-supporting traveling waves that generate propagating patterns reminiscent of the rapidly evolving bands seen in Erythemic gyratum reopen (also known as Gammel's disease), a rare rash often associated with malignancy.[31]

In contrast to the above, Vodovotz and coworkers[26,32] neglect cell movement and chemoattractant diffusion. They develop simplified models of the inflammatory response that focus on generic leukocytes and their ability not only to dampen the

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TABLE 1 | A Summary of the Mathematical Models Reviewed and the Macrophage Functions That They Capture

Model	Model Type	Macrophage Function	Stimulus	Wider Context
15	PDE (3)	Generic leukocyte that moves by chemotaxis and removes pathogens	Pathogen	Leukocyte motility
16	ODE (2)	Generic leukocyte that removes pathogens	Pathogen	Leukocyte and pathogen interactions
17	PDE (2)	Generic leukocyte that moves by chemotaxis and removes pathogens	Pathogen	Leukocyte motility
20	ODE (3)	A combined generic leukocyte and pro-inflammatory mediator produces a second pro-inflammatory mediator at a slow rate. The leukocyte removes pathogens	Pathogen	Sepsis
21–23	ODE (2-4)	Generic leukocyte that is activated in response to pathogens and tissue damage. The leukocyte removes pathogens, induces tissue damage and produces an anti-inflammatory mediator that inhibits tissue damage	Pathogen	Sepsis, response to endotoxin and anthrax inhalation
25	ODE (8)	An extension of 19 to include the inflammatory mediators IL-1, TNF and IL-10	Pathogen (endotoxin)	Fitted to experimental data describing the inflammatory response of rats to endotoxin administration
19	PDE (3)	A single population of macrophages that move by chemotaxis and release pro- and anti-inflammatory mediators	Pathogen	Rash formation
28	ODE	A single population of macrophages that remove pathogens and release generic pro- and anti-inflammatory mediators	Pathogen	Respiratory system
14	ODE (4-5)	A single population of macrophages that remove apoptotic neutrophils. The removal of apoptotic cells stimulates macrophages to release a generic anti-inflammatory mediator	Tissue damage	Generic tissue damage
32	PDE (7)	Macrophages proliferate and produce growth factors	Secretion of PDGF	Ischemic chronic wounds
38,39	ODE (3-7)	Two distinct populations of macrophages (inflammatory and repair) that migrate and proliferate	TGF-β and balance of inflammatory and repair macrophages	Diabetes. Investigates the effect of two commercially available skin substitutes on wound repair
41	ODE (5)	A single population of macrophages that produce two mediators (VEGF and TFF-β). The mediators regulate the growth of the lymphatic system	Latent TGF-β	Lymphatic system
44,46	ODE (1-7)	Population of macrophages that engulf and digest apoptotic cells. Macrophage populations are	Supply of apoptotic cells to the experiment	Model output compared to *in vitro* experimental data macrophage (from normal and diabetic

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TABLE 1 | Continued

Model	Model Type	Macrophage Function	Stimulus	Wider Context
		distinguished by the number of apoptotic cells that they have engulfed but not digested (up to a maximum of 7)		prone mice) engulfment and digestion of apoptotic cells
45	ODE (variable)	Resting macrophages activated by contact with apoptotic cells. Apoptotic cells undergo necrosis that stimulates macrophages to secrete harmful cytokines	Arrival of apoptotic cells	Diabetes
18	ODE (variable)	Populations of macrophages that engulf (phagocytose) quartz crystals. Populations are distinguished by the number of silica particles that they have engulfed	Quartz crystals	Alveolar macrophages and silicosis
52,53	ODE (4-6)	Interactions of the cytokines TNF-α, IL-1, and IL-10 with monocytes	Monocyte exposure to TNF-α or lipopolysaccharide	Septic shock and rheumatoid arthritis
54	ODE (2)	Interactions of pro- and anti-inflammatory cytokines, produced by macrophages, or other cells of the synovial lining of joints	Anti-inflammatory cytokine	Rheumatoid arthritis
56	ODE (10)	Subcellular interactions and enzyme production in two populations of macrophages; a classically activated (M1) phenotype and an alternatively activated (M2) phenotype	Either the enzyme NOS-2 [for activation of classical (M1) phenotype] or arginase-1 [for activation of the alternative (M2) phenotype]	Model output compared to experimental data describing NO and urea production (following stimulation by IFN-y and a combination of IL-4 and cAMP) in populations of RAW 264.7 macrophages
57,58	ODE (19)	Macrophage subcellular protein interactions that lead to calcium flux	Ligand C5a	Model output is compared to time-course data describing ligand induced calcium flux (through a G-protein coupled receptor) in macrophage RAW 246.7 cell populations

The type of model is indicated (ODE, ordinary differential equations; PDE, partial differential equations) alongside the number of equations incorporated into the model (bracketed). Key macrophage functions that are incorporated into the models and the form of model stimulus are described alongside the wider context that drove model development (such as disease scenarios and experimental data).

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TABLE 2 | Some Key Parameter Values, Relevant to Macrophages, Which Are Utilized or Estimated by the Referenced Mathematical Models

Parameter Description	Notes	Value	Source
Macrophage density	In tissue (mice)	5×10^5 cells mL^{-1}	21
	In inflamed tissue (mice)	1×10^7 cells mL^{-1}	
	Density in skin	1875 cells mm^{-3}	
Rates of influx or movement	Influx into tissue (not inflamed)	5×104 cells mL^{-1} day^{-1}	21, based on 22
	Random motility (generic leukocyte)	10^{-9}–10^{-2} cm^2 $seconds^{-1}$	18 based on data from 23,24
	Chemotaxis (generic leukocyte)	18 and 60 cm^2 $seconds^{-1}$	
Rates that macrophages die or leave tissue	Leave tissue	0.1 day^{-1} [this value agrees with [2] who state that macrophages are resistant to apoptosis and leave tissue after several weeks (via the lymphatics)]	15,21
		0.2 day^{-1}	25
	Die	1.2 day^{-1}	17
		2.88 day^{-1} (these two values are for generic leukocytes. As other leukocytes die quicker than macrophages, *in situ* values are expected to be greater	26
Rate of phagocytosis	Rate of removal of bacteria	100 bacteria $cell^{-1}$ h^{-1} (reduced by a factor of 20 to simulate defective phagocytosis)	17
	Rate of engulfment (*in vitro*) of apoptotic cells by murine peritoneal macrophages	12×10^{-7} mL $cell^{-1}$ h^{-1} (reduced to 4×10^{-7} in macrophages from diabetic prone mice)	27,28
	Rate of digestion (*in vitro*) of apoptotic cells by murine peritoneal macrophages	1 h^{-1}	27,28
Rates that macrophages produce cytokines	TGF-β	0.07 pg $cells^{-1}$ day^{-1}	15,29
	PDGF	0.015 pg $cells^{-1}$ day^{-1}	25
	VEGF	0.0019 pg $cell^{-1}$ day^{-1}	

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inflammatory response through the removal of pathogens, but to promote it through damage to tissue. Model development was motivated by a desire to gain insight into the mechanisms that underlie sepsis, a whole body response to a pathogen with an underlying inflammatory component that has a high rate of mortality and relatively few effective therapies.[33] A four variable model[26] includes an anti-inflammatory mediator released by the phagocytes and allows the investigation of therapeutic interventions that highlight the dependence of a healthy outcome on the timing of such interventions. These models have been modified and extended to aid in investigating host pathogen interactions and the timing of possible treatment strategies that occur in response to administration of anthrax and the bacterial byproduct endotoxin.[34–36]

In another homogeneous model that explicitly identifies the macrophage as the sole leukocyte to participate in the acute inflammatory response to a bacterial infection, Herald et al.[37] develop a model that incorporates bacteria, macrophages and both pro- and ant-inflammatory mediators. Bacteria promote the inflammatory response through the production of pro-inflammatory mediators and macrophages control the response through their removal of bacteria and the production of anti-inflammatory mediators that dampen the pro-inflammatory signal. The authors discuss the sensitivity of the outcome (whether the bacterial infection is removed or sustained) in terms of key parameters such as the intensity of macrophage recruitment. They find that the success or failure to remove the bacterial infection can be therapeutically manipulated, an example being the application of anti-inflammatory mediators that while able to reduce the intensity of a severe bacterial infection are insufficient to eliminate it.

Apoptosis of inflammatory cells, such as neutrophils, followed by their timely removal by macrophages is thought to be a key event in the successful resolution of inflammation. Dunster et al.[15] develop a spatially averaged model of acute inflammation in response to tissue injury that, in contrast to the previously described models, distinguishes between distinct populations of neutrophils and macrophages. The model captures the key interactions between these inflammatory cells and generic pro- and anti-inflammatory mediators. Utilizing bifurcation theory they investigate how variation in the system parameters affects the ability of the inflammatory process to resolve or progress to a self-perpetuating state. They find that therapeutic manipulation of the rate of macrophage phagocytosis of neutrophils can aid in pushing the inflammatory response to a healthy outcome but predict that this success is critically dependent on the rate of neutrophil apoptosis. This leads to the prediction that an effective treatment protocol would take a dual approach, targeting macrophage phagocytosis alongside neutrophil apoptosis.

The above models focus on the acute inflammatory response but macrophages also influence the repair and replacement of tissue through the production of a range of growth factors. Vascular endothelial growth factor (VEGF) attracts endothelial cells and promotes the formation of new blood capillaries (angiogenesis) that are vital to increasing blood flow to an area of damage.[38] Angiogenesis has been well studied both experimentally and mathematically, this interest being stimulated by angiogenesis being not only crucial to the ability of a wound to heal but to tumor growth. Many models of angiogensis, in the context of wound repair, do not explicitly account for macrophages but focus on fibroblasts, endothelial cells, capillary tips and sprouts, and growth factors such as VEGF.[39,40] An exception to this is a model of dermal wound healing that not only incorporates the above but also macrophages that excrete the growth factors that attract fibroblasts and promote angiogenesis.[41] Macrophages are assumed to die by apoptosis, at a rate that is enhanced under ischemic conditions where there is a lack of blood flow into the wound. Simulations demonstrate how this lack of flow limits macrophage recruitment to the wound site and impairs wound closure. A review of the extensive mathematical research into angiogenesis is given by Scianna et al.[42]

Macrophages are a prominent source of TGF-β^3 which, among other functions, stimulates collagen production and the replacement of tissue through its influence on fibroblasts. Theoretical models of tissue repair tend to focus on the easily observable reparatory processes that occur when skin is damaged (wound healing). In a wound, damage results in inflammation and the formation of a blood clot. The upper layer of the clot is replaced by the migration of epidermal cells while the lower layer (often termed granulation tissue) provides a platform for fibroblast migration and the formation of new blood capillaries. Fibroblasts generate collagen fibrils, which are then remodeled in an ongoing process with the type and alignment of the collagen fibrils thought to influence the degree of scarring and hence the quality of the resulting tissue. TGF-β is thought to influence this process and has been proposed as a novel anti-scarring therapy.[43] Models of wound healing exist for all facets of this process and a review is given by Sherratt and Dallon.[44]

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Wounds That Will Not Heal

The success of the acute inflammatory response to resolve is crucial for the transition to the following phases of tissue replacement but many factors can interfere with this process. This is particularly apparent in patients with diabetes who often suffer from the debilitating condition of wounds that are either slow, or fail, to heal. Diabetic wounds are characterized by an ongoing inflammatory state to which macrophages with compromised function are thought to contribute.[45,46]

Waugh and Sherratt[47] present a homogeneous model that focuses on two distinct populations of macrophages (with either an 'inflammatory' or a 'repair' phenotype) and the mediator TGF-β. They find that the balance between the populations of macrophages of an inflammatory and repair phenotype vary under diabetic (compared to normal) conditions, affecting the subsequent healing process. This work was subsequently extended[25] to include macrophage production of platelet-derived growth factor (PDGF), the subsequent attraction of fibroblasts and their synthesis of collagen and hyaluronan. These additions allowed investigations into the effect of applying two commercially engineered skin substitutes (Apligraf™ and Dermagraft™) to diabetic wounds. The model showed close agreement to data from clinical trials and demonstrated that hyaluranon is the key component in these treatments. It promotes a shift in macrophage phenotype that drives wound healing.

The restoration of blood and lymphatic capillaries are essential for a wound to heal but, biologically, the formation and repair of lymphatic capillaries (lymphogenisis) has been studied much less than that of blood vessels.[48] This is reflected in the few mathematical models that capture the formation of lymph vessels. An exception to this is a simple homogeneous model of five equations that accounts for the formation of lymphatic capillaries following a wound to the skin.[29] Like the above models,[25,47] the focus of this work is on how the healing process is altered under diabetic conditions. The model includes a single population of macrophages that are attracted by, and produce, TGF-β. Subsequent macrophage production of VEGF stimulates the arrival of lymphatic capillary cells that cluster to form capillaries. The model's parameter values are inferred from data. Results indicate that possible treatment strategies for situations where lymphogenesis is impeded, such as under diabetic conditions, are lowering macrophage production of TGF-β and increasing basal lymphatic endothelial cell growth rate.

Additional models exist that investigate macrophage function under diabetic conditions but they are reviewed in the next section where we focus on models that capture a macrophage's ability to engulf particles and apoptotic cells.

Ability of Macrophages to Remove Cells and Debris

The capacity of macrophages to clear debris or cells that have undergone programmed cell death (apoptosis), through a process of attraction, engulfment and digestion (phagocytosis), is central to inflammation. Phagocytosis of apoptotic cells has two benefits: it prevents the apoptotic cell from undergoing secondary necrosis, a process where cells lose membrane integrity and spill their toxic contents, subsequently perpetuating the inflammatory process; and it can also contribute to a shift in macrophage phenotype, from one of a pro-inflammatory to an anti-inflammatory phenotype.[49] Very little is known about the details of phagocytosis of apoptotic cells,[50] but some mathematical models have been formulated to incorporate phagocytosis and investigate mechanisms that can explain experimental data.

A quantitative comparison of rates of engulfment and digestion of apoptotic cells by macrophages from nonobese diabetic-prone (NOD) and normal (BALB/c) mice is given in papers by Marée et al.[21,27,28] The first study[27] compared time course *in vitro* macrophage feeding experimental data to a basic mathematical model of macrophage clearance dynamics. The authors included and subsequently quantified uptake and digestion of apoptotic cells by macrophages from both mouse strains. This work highlighted the importance of observing both the engulfment-only and the digestion-only phases of phagocytosis in order to identify possible therapeutic targets. The subsequent collection of new experimental data describing these phases enabled identification of an acceleration in rates of engulfment after ingestion of the first apoptotic cell in both macrophage strains (though this was identified to be slower in diabetes-prone mice), and quantification in engulfment rates that are 5.5 times faster in normal than diabetes-prone mice. This led to the suggestion that targeting macrophage defects in engulfment could be an effective therapeutic strategy for type 1 diabetes.

Tran et al.[19] focus on the capacity of macrophages to remove debris, in the form of quartz crystals, rather than apoptotic cells. Their interest in silicosis, a disease caused by the inhalation of silica particles, led them to develop an ordinary differential equation model that captures macrophage sequential

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engulfment of quartz particles. The model describes the detrimental effects that ingestion of quartz has on macrophages; when overloaded with quartz particles macrophages have reduced mobility, emit distress signals in the form of pro-inflammatory mediators and die prematurely. This prevents macrophages from safely transporting their load to the mucociliary escalator, the main clearance pathway of the lung. The model was parameterized from literature and showed good agreement to data from rats exposed to increasing concentrations of quartz particles.

While not explicitly modeling macrophage engulfment, Richards and Endres[51] derive a model of engulfment that, while compared to experimental data in the form of time lapsed movies of neutrophils engulfing IgG-coated polystyrene beads, could equally well be adapted to that of macrophage engulfment. Neutrophil and macrophage phagocytosis involves recognition of surface bound antibodies on the target, this leads to a change in shape and extension of pseudopodia that wrap around the target before engulfment.[52] A series of increasingly complicated partial differential equation models of receptor driven membrane engulfment of beads is developed that investigate receptor drift and/or diffusion in the membrane. The ability of the model to accurately describe the experimental data is dependent on the inclusion of receptors movement within the cell membrane being an active process that is driven by positive feedbacks through subcellular signaling mechanisms. The model predicts that engulfment occurs in two distinct stages: the first stage is dependent on receptors diffusing within the cell membrane but the second stage involves their active movement and occurs quickly.

Effect and Expression of Cytokines and Chemokines

Sites of tissue trauma are characterized by high levels of molecules such as damage-associated molecular patterns (DAMPS) released from injured cells. Binding of these molecules to macrophages initiates signal transduction cascades resulting in activation and the release of a wide array of mediators with cytotoxic, pro- and anti-inflammatory and fibrogenic activity which aid in promoting inflammation and healing.[53] Some of the key cytokines such as tumor necrosis factor α (TNF-α) are thought to be viable anti-inflammatory targets for a number of diseases, indeed anti-TNF-α drugs are already licensed for the treatment of inflammatory diseases such as rheumatoid arthritis.[54,55]

Seymour and coworkers extend a previous mathematical model[56] that explores the relationship between a population of monocytes stimulated, by TNF-α or lipopolysaccharide (LPS) to produce the pro-inflammatory cytokine IL-1 and the anti-inflammatory IL-10, where IL-10 has a deactivatory effect on IL-1. The extended study[57] explores the effects of therapeutically blocking TNF-α, which is an effective target for patients with rheumatoid arthritis (RA). TNF-α plays a key factor in the pathology of Systemic Inflammatory Response Syndrome (SIRS) but anti-TNF-α therapy has not shown the same benefits to these patients as to those with RA. Utilizing the known pharmokinetic properties of three soluble TNF-α inhibitors (TNFR2, Etanercept and Infliximab) the authors were able to demonstrate the effectiveness of the inhibitors in controlling concentrations of free TNF-α under the conditions of RA. They proposed, that in contrast, under the condition of SIRS, therapeutically sequestered TNF-α can act as a slow reservoir sabotaging the therapeutic strategy.

Also focusing on the cytokine networks that underlie rheumatoid arthritis Baker et al.[58] considered the interactions between generic pro- and anti-inflammatory cytokines produced by inflammatory cells including macrophages; their simple model of two variables displays a range of outcomes, including oscillatory behavior that is reminiscent of a remitting-relapsing disease state. They demonstrate anti-inflammatory responses to anti-cytokine therapy, finding that dose-regime (as well as dose-level) is important for a successful outcome.

Subcellular Events

The understanding of the signal transduction networks that underlie a macrophage's ability to extract information from its environment and mount functional responses, while now better understood,[59] can also be aided further by the construction of mathematical models and their careful comparison to experimental data. Whilst there is much work to be done in this area we next note a few examples that are specific, or relevant, to macrophages.

Childs et al.[60] incorporate key subcellular events that are thought to be central to the expression of both a classical (pro-inflammatory) and alternative (anti-inflammatory) phenotype into a mathematical model comprising ten ordinary differential equations. They utilize the model to investigate whether an individual macrophage can change phenotype. Model simulations are compared to experimental data describing stimulation of murine

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macrophages. The cells were stimulated *in vitro*, with the cytokines IFN-γ or IL-4 to induce classical or alternative activation respectively, using the production of Nitric Oxide and urea as evidence of the respective phenotype. They predict that macrophages can switch from the alternative (anti-inflammatory) phenotype to the classical (pro-inflammatory) one but are unable to reverse that switch.

Subcellular elevations in calcium levels are thought to mediate many cellular responses, and the mechanisms underlying these events have long been of mathematical interest. The predictive power of these models is increasingly being enhanced by the ability of visualizing calcium ions within live cells. Maurya et al.[61,62] present a detailed model that, in a system of 61 coupled ordinary differential equations, captures the events downstream of the macrophage G-protein-coupled receptor, C5aR, through to calcium flux. Although model simulations compare well to data, describing calcium flux in populations of native and knock-down cell lines, a drawback of large models such as this is the number of parameters involved. This complexity can cause difficulties in parametrizing models and therefore gaining intuition from their results; these drawbacks may or may not offset the increased levels of biological knowledge that they encompass.

The NF-kB transcription factor is a key regulator of cellular responses to pro-inflammatory cytokines including IL-1 and TNF-α. Prolonged activation of NF-κB activity has been implicated in the development of chronic inflammatory diseases as well as cancer.[63] Mathematical models, tightly linked to experimental data, have been utilized to unravel the complex regulatory responses to different inflammatory stimuli. While the bulk of experimental data used to validate these models is derived from fibroblast cell lines, misregulation of NF-kB is a key component in macrophages' response to inflammatory signals,[64] which suggests that they should be of relevance in the current context also. A review of NF-κB signaling is given by Hoffman and coworkers who also produce models in this area.[65]

CONCLUSION

Macrophages are integral to the inflammatory response and tissue repair. They perform a wide array of functions that can promote and regulate the inflammatory response.

The above survey demonstrates that mathematical models exist that capture all of the key macrophage functions such as migration toward an inflammatory stimulus,[16–18,30] the secretion and adaption to both pro- and anti-inflammatory cytokines[36,38,39,59–61] and the removal of debris and apoptotic cells.[15]

Many of the above models focus on the inflammatory response in a generic context.[16–20,26,30,32,38,39] They have modeled the effects of macrophage behavior, type of inflammatory stimulus and the effects of generic mediators (often both pro- and anti-inflammatory) on the outcomes of the inflammatory response. These models are important as a first step in understanding how variation in individual components (such as could be achieved therapeutically) contributes to the ability of the inflammatory system to resolve or progress to self-perpetuating condition. Macrophage behavior changes according to the tissue type that the macrophage resides in and the disease conditions it is under. Some success has been made in adapting generic models of the inflammatory response to specific disease scenarios,[34–36] but this is hampered by availability of data and the ability to infer the large number of parameters incorporated in such models. Mathematical models have also been utilized to aid understanding of how macrophage functions alter under diabetic conditions by the development of models that capture macrophage removal of apoptotic cells.[28,51,52] The comparison of the models to *in vitro* experimental data led to model refinement and directed further experimentation. This allowed quantification of rates of phagocytosis and aided in the understanding of mechanisms that contribute to the reduced rate of removal of apoptotic cells seen in macrophages from diabetic prone mice.

Initial efforts have been made to address the complex issue of macrophage phenotype in mathematical models by the incorporation of adaption in macrophage expression of cytokines,[15] hypothesis surrounding the possible progression from a pro-inflammatory to an anti-inflammatory phenotype,[60] and the incorporation of these distinct phenotypes into a model that investigates how the balance between populations is altered in diabetic wounds.[25,47] The extension from a simple classification of macrophage phenotype to a wider spectrum of macrophages has yet to be addressed theoretically and offers a new and interesting challenge for models to contribute to understanding macrophage activation and the role it plays in the context of diseases.

Macrophage plasticity involves a complex gene expression program. The availability of high density experimental data linking subcellular events, such as gene and protein expression, through to macrophage

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phenotype is increasing. This represents an important step in understanding how macrophages respond to signals from their environment and curation of these data sets will make it easier to integrate this knowledge that spans from subcellular events through to cellular function into theoretical models. While demanding, the application of systems biology approaches provides a mechanism by which such large-scale data and mathematical models that capture biological knowledge and hypotheses that operate over the multiple time and space scales involved can be combined to elucidate macrophage biology.

The above survey suggests that, while much challenging work is still to be done, diverse models, from simple ODEs to sophisticated agent-based, stochastic and partial-differential-equation formulations will continue to have a useful role to play in understanding these complex cells that are so central to inflammatory diseases and tissue repair.

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