MODELING THERAPEUTIC INTERVENTION IN SEPSIS

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ABSTRACT

Inflammation is the physiological response to infection and tissue injury. It is characterized by release of proteins called cytokines and other mediators produced by cells of the immune system. Sepsis, the systemic inflammatory response to infection, is a public health issue because of its high incidence, mortality and economical cost. Experimental paradigms and therapeutic strategies designed to harness systemic inflammation are still based on a reductionist framework based on linear dynamics. Recently, a neural pathway that modulates pro-inflammatory cytokine production was identified. This pathway originates in the central nervous system and reaches immune cells in the spleen via the vagus and the splenic nerves and attenuates cytokine production. Electrical stimulation of the vagus nerve has been shown to attenuate circulating levels of TNF and to reduce organ damage, thus improving survival in experimental models of sepsis. Here, we built on a previously published mathematical model of systemic inflammation to qualitatively explore the therapeutic potential of vagus nerve stimulation, a novel approach to reduce inflammatory mediators in sepsis and other inflammatory diseases.

INTRODUCTION

Inflammation is the physiological response to infection and tissue injury. It is characterized by release of proteins called cytokines and other mediators produced by cells of the immune system. From a teleological standpoint, inflammation serves to limit the extent of pathogen invasion and to promote tissue repair. Typically, local regulatory mechanisms adjust the magnitude of the inflammatory response such that the injurious condition is resolved and homeostasis is maintained. Ocassionaly, for reasons that are not completely understood, cytokine release in not restrained and what started originally as a local inflammatory process becomes a systemic inflammatory response, a pathologic condition associated with further tissue injury that leads to organ failure and death (1). This sequence of molecular and cellular events underlies the clinical signs of systemic inflammation and is common to several diseases with different triggering events like infection, severe trauma or hemorrhage, among others (2).

Sepsis, the systemic inflammatory response to infection, is a public health issue because of its high incidence, mortality and economical cost. It is estimated that severe sepsis, defined as sepsis plus hypotension, has an estimated incidence of 751,000 cases per year in the United States with annual costs of $16.7 billion USD and a mortality of 28.6% (3). Despite several years of research and clinical

trials testing promising drugs, sepsis is considered to be an intractable disease. Research in sepsis focuses primarily on identifying single cytokines or groups of cytokines whose neutralization aims to counterbalance the inflammatory status of septic patients. As new molecular targets raise and fall, endocrine and neural regulatory mechanisms, unrecognized until recently, have led to regard sepsis as a multi-systemic disease. However, experimental paradigms and therapeutic strategies designed to harness systemic inflammation are still based on a reductionist framework with linear dynamics.

More recently, the pathophysiologic process of sepsis has been formulated in terms of complexity theory. Bodily organization and function are conceived as an emergent property of a non-linear, non-equilibrium system organized as a scale-free network (4). Loss of homeostasis in sepsis is perceived as the progression from a stable behavioral pattern or attractor to another characterized by organ dysfunction as a consequence of reduced connectedness between the system's elements (5-7). From this standpoint treatment should aim to restore communication within the elements of the system. While this paradigm better describes an adapting living system, several fundamental questions remain to be addressed. How can an attractor be defined in sepsis? How do initial conditions affect the dynamics of the system? Can the change in dynamics induced by treatment by monitored? If so, is it possible to know when and with which magnitude to intervene? Here, we built on a previously published mathematical model of systemic inflammation (8) to explore the therapeutic potential of vagus nerve stimulation, a novel approach to reduce inflammatory mediators in sepsis and other inflammatory diseases.

MOLECULAR EVENTS IN SEPSIS

Macrophages are among the first immune cells to respond to pathogens. Tissue-residing macrophages encounter bacteria and release cytokines upon activation of specialized surface receptors. One such cytokine, tumor necrosis factor (TNF), acting on macrophages in an autocrine and paracrine way induces the release of other cytokines like interleukin-8 (IL-8), a key molecule in attracting neutrophils to the site of infection. TNF itself contributes to migration of neutrophils from the blood to the infected tissue by inducing the expression of adhesion molecules on the surface of endothelial cells in postcapillary venules. Recognition of adhesion molecules by ligands expressed on neutrophils facilitates their capture and migration into the tissue, a process known as leukocyte trafficking (9). Toxic compounds like reactive oxygen species or proteolytic enzymes released by activated neutrophils kill pathogens but induce tissue damage as well. Because the overall effect of cytokines like TNF and IL-8 is activation of immune cells and their recruitment to the site of infection (manifested as redness and swelling, pain and warmth) such mediators are referred to as pro-inflammatory.

The magnitude of the inflammatory response is normally modulated by regulatory mechanisms which inhibit the release of pro-inflammatory cytokines or neutralize

their binding to effector cells. For example, IL-10 inhibits TNF production while interleukin-1 receptor antagonist (IL-1ra) prevents binding of the inflammatory mediator IL-1 to effector cells. Other mediators like resolvins and lipoxins dampen migration of neutrophils and participate in the tissue repair process (10). It is important to note that the previous description is merely mechanistic and reflects the reductionist approach that permeates the field. The complex and dynamic interplay of immune cells and the pro and anti-inflammatory mediators that they release can also be regarded as a distributed informational network organized in top-down and bottom-up fashion (11).

Occasionally, because pathogen load is overwhelming or because regulatory mechanisms fail to restrain the pro-inflammatory response, co-lateral tissue damage induced by neutrophils and other immune cells is magnified. When this occurs, death cells release proteins that are normally contained within (12). One such protein, HMGB1, further induces the release of TNF and other pro-inflammatory mediators from macrophages, perpetuating the TNF-tissue damage-TNF cycle which eventually leads to organ failure. HMGB1 is released much after the initial TNF response, and its neutralization with antibodies improves survival in models of sepsis. For this reason, it is considered as a late mediator in sepsis (13). These essential features of the inflammatory response to infection are summarized in figure 1A.

The inflammatory response is also regulated by input from the nervous system. Glucocorticoids and epinephrine released by the hypothalamic-pituitary-adrenal (HPA) axis attenuate inflammation by blocking the activation of macrophages, reducing secretion of TNF, and hindering leukocyte trafficking. Glucocorticoids and epinephrine, as well as cytokines, are humoral mediators that act as hormones by spreading through the systemic circulation and acting on target cells located far from their site of release. In contrast, neural mediators such as norepinephrine and acetylcholine, and other neurotransmitters released by nerve endings, act immediately upon cells located in the immediate vicinity of the nerve terminal from where they are released. Therefore, neural modulators of inflammation are cell and organ specific, and fast acting. Recently, a neural pathway that modulates pro-inflammatory cytokine production was identified (14). This pathway originates in the central nervous system and reaches immune cells in the spleen via the vagus and the splenic nerves and attenuates TNF production (15) (figures 1B and 2). Electrical stimulation of the vagus nerve has been shown to attenuate circulating levels of TNF and to reduce organ damage, thus improving survival in experimental models of sepsis (16).

An important consideration with the use of therapeutic strategies against sepsis whose aim is to attenuate pro-inflammatory cytokine synthesis or block their effect has to do with dose and timing. If the anti-inflammatory effect is of enough magnitude it can cause suppression of the immune response and favor secondary infections or exacerbation of the preexisting one. Even if the anti-inflammatory effect is of adequate magnitude, its duration and timing could be

inappropriate and it is possible to observe immune suppression or paradoxically, enhanced inflammation. In order to study the effectiveness of VNS in sepsis we used a previously published mathematical model of acute inflammation (8). In particular, we were interested in qualitatively exploring clinical outcomes as a consequence of suppressing TNF during the systemic inflammatory response to infection.

RESULTS AND DISCUSSION

Figure 1A shows a case of infection that is successfully kept in check. The pathogen induces release of pro-inflammatory mediators (e.g. TNF) whose coordinated action eliminate the infection. Activation of the inflammatory response also elicits migration of neutrophils into the infected tissue which participate in the killing of the pathogen by release of non-selective enzymes and other molecular species which also induce tissue damage. This scenario could be interpreted as a small infection which is successfully eradicated with some to none tissue damage and where homeostasis is eventually is maintained. There is no need to intervene therapeutically.

Figure 1B can be considered as a case of severe infection. Although the pathogen is successfully eliminated there is a long-lasting inflammatory response characterized by persisting and elevated levels of TNF and significant tissue damage. Under these conditions, organ failure occurs and death eventually ensues. This outcome represents a significant fraction of the patients with sepsis. Most of the proposed therapies, initially devised to target persistently high pro-inflammatory mediators in order to block their effect and minimize tissue damage, showed little or no success (17). In part, this is because these therapies were directed against single cytokines. Figure 1C is a composite of figures 1A and 1B shown in three-dimensional space.

Electrical stimulation of vagus nerve attenuates TNF production of spleen macrophages and improves survival in experimental models of sepsis (18). In order to test its efficacy, we simulated vagus nerve stimulation by reducing TNF by a predetermined percentage when TNF reached a preset value. In the model, the immediate suppression of TNF reflects the fast delivery and organ specificity of neural anti-inflammatory mediators. This feature permits finer control of timing and dosage as opposed to humoral mediators or soluble therapeutic drugs whose delivery is slower and more difficult to control due to issues of distribution and metabolism. Figure 4A shows the case of persistent tissue damage described above with (blue line) and without (red line) vagus nerve stimulation. Under these particular conditions of vagus nerve stimulation, attenuation of TNF at the precise moment was able to restore the system to healthy initial conditions: low TNF, low tissue damage, and low pathogen load. In contrast, when vagus nerve stimulation was used in a case of low pathogen challenge, one that required no therapeutic intervention for the system to return to basal conditions, a

state of intermittent TNF and tissue damage with fluctuating pathogen load developed (Figure 4B).

Finally, to simulate the effect of immuno-stimulating strategies like the use gamma interferon (a pro-inflammatory cytokine) in the setting of a small infection with local inflammation, we increased the magnitude of TNF production induced by the pathogen. In this setting (figure 4C), enhancement of the inflammatory response led to a more efficient infection clearance with less tissue damage (red line).

The different scenarios presented are based on a simplistic model with certain assumptions. One critical parameter not considered, unknown at present time, is the duration of the TNF-suppressive effect by vagus nerve stimulation. This parameter can be determined by experimentation and it could be implemented into the model to have a more accurate picture of the intervention. More detailed models would also consider the effect of neurotransmitter on neurotransmitter receptor expression by spleen macrophages, and would address the system's refractoriness to the effect of vagus nerve stimulation. Similarly, the dynamical interaction of pro- and anti-inflammatory cytokines produced my macrophages could be included in the model. Such considerations have already been modeled for cytokine production by monocytes (19)

Rheumatoid arthritis is a chronic inflammatory disease characterized by joint inflammation driven by local TNF production (20). The relative success of anti-TNF therapies in patients with rheumatoid arthritis contrasts with their failure when used to block TNF in sepsis (17,21) . Recently, the effect of three anti-TNF therapies was explored in a mathematical model of rheumatoid arthritis (22). Results obtained from said model offer an explanation to the discrepancy in outcomes observed in both diseases. While in rheumatoid arthritis TNF is produced locally and its effect is confined to the joints, in sepsis its effect is systemic. Neutralization of TNF in a discrete anatomical location is achieved in rheumatoid arthritis while in sepsis neutralization is difficult to attain because multiple sites of inflammation most likely undergo out-of-phase inflammatory responses (22). More detailed models aimed specifically at characterizing therapeutic interventions in sepsis could provide further insight into the complex dynamics of this illness, and offer a more solid ground to try new therapeutic possibilities.

The use of mathematical models and complex theory to study diseases like sepsis can provide useful information to further our basic understanding of the sepsis process. However, is it feasible to put into clinical practice notions derived from non-linear dynamics like attractors, basins of attraction and bifurcations? Obtaining time series of variables such as cytokine concentrations or pathogen load is impractical due to technical limitations. However, analyses of time series like heart rate are already proven accurate to predict morbidity and mortality in sepsis and other inflammatory diseases (23,24). Perhaps someday technological

advances will allow accurate and fast data collection from patients and its analysis in real time, and offer the possibility of intervening at the precise time, location, and magnitude.

CONCLUSIONS

Sepsis, the systemic inflammatory response to infection, is a disease characterized by a complex interaction of a myriad of molecular mediators. Therapeutic strategies directed against single pro-inflammatory mediators have shown mixed results. In part, this is so because dampening the inflammatory response can often lead to enhanced immunosuppression. Attenuation of TNF production by vagus nerve stimulation is a promising strategy to harness systemic inflammation. In contrast to humoral mediators, neural mediators reach specific target cells in real time. This feature inherent to vagus nerve stimulation allows for intervening therapeutically with fine precision in time and location. We explored qualitatively the possible effects of suppressing TNF by vagus nerve stimulation by using a known model of systemic inflammation. As reported in experimental models of disease, stimulation of the vagus nerve in a scenario of severe infection resulted in successful control of inflammation and pathogen clearance if performed at the appropriate moment. However, if delivered in a case of low pathogen load, vagus nerve stimulation can exacerbate the inflammatory response. Therefore, attention should be paid to perform vagus nerve stimulation in a timely manner to avoid possible unwanted side effects.

METHODS

The model consists of three variables (pathogen, TNF and tissue damage) obeying predator-prey dynamics. Refer to (8) for the complete description of the model.

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k_p p(1-p) - k_{pm} mp$$

$$\frac{\mathrm{d}m}{\mathrm{d}t} = (k_{mp} p + l)m(1-m) - m$$

$$\frac{\mathrm{d}l}{\mathrm{d}t} = k_{lm} f(m) - k_l l$$

$$f(m) = 1 + \tanh\left(\frac{m-\theta}{w}\right)$$

FIGURE LEGENDS

Figure 1. (A) Simplified model of systemic inflammation. Infection (pathogen) triggers an immune response characterized by production of cytokines by cells of the immune system. This event can be summarized as macrophages releasing TNF, a pro-inflammatory cytokine. The immune response (TNF) keeps infection in check by killing the pathogen, and induces release of late mediators indiscriminately help in pathogen clearance and induce tissue damage. (B) When the vagus nerve is stimulated electrically, release of neurotransmitters in spleen act upon receptors expressed on macrophages to attenuate production of TNF.

Figure 2. Spleen sections 60 minutes after injection of endotoxin, a bacterial product that induces production of TNF by macrophages. Vagus nerve stimulation (B) or sham surgery (A) was performed prior to endotoxin administration. TNF is shown in red. (C) High magnification of nerve terminals (green) in close proximity to macrophages (blue) producing TNF (red). (A) and (B) 100x magnification. (C) 630x magnification.

Figure 3. (A) Example of inflammatory response with initial load pathogen load. The immune response contains the infection and homeostasis is restored. (B) High pathogen load. The immune system is able to clear infection but persistent high TNF levels and tissue damage occur. Under these circumstances organ failure develops and death eventually ensues. (C) Three-dimensional representation of (A) and (B)

Figure 4. (A) Initial conditions of high pathogen load with (red trajectory) or without (blue trajectory) vagus nerve stimulation. Under these conditions, timely suppression of TNF is abate persistent high TNF and tissue damage levels, and restore homeostasis. (B) Low pathogen load with (red trajectory) or without (blue trajectory) vagus nerve stimulation. In this scenario, vagus nerve stimulation elicits cycles of low-to-medium TNF and tissue damage with oscillating pathogen load. (C) Low pathogen load with (red) or without (blue) immuno-stimulating strategy.

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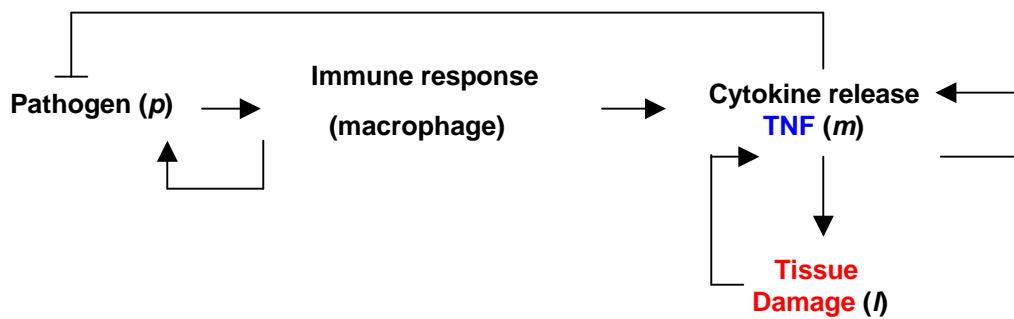
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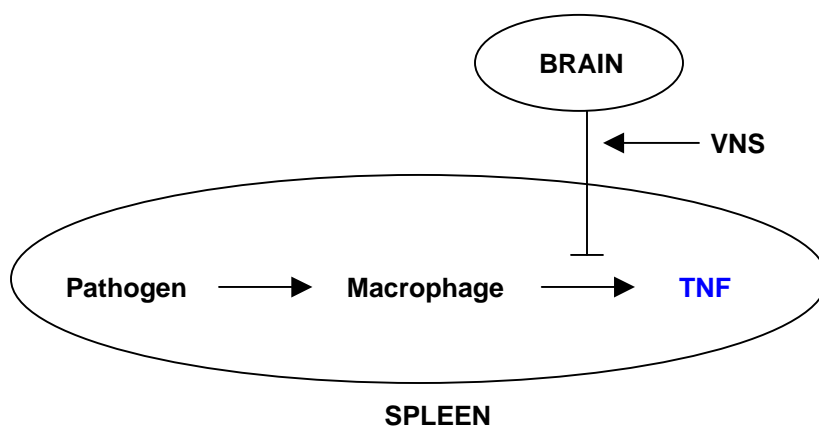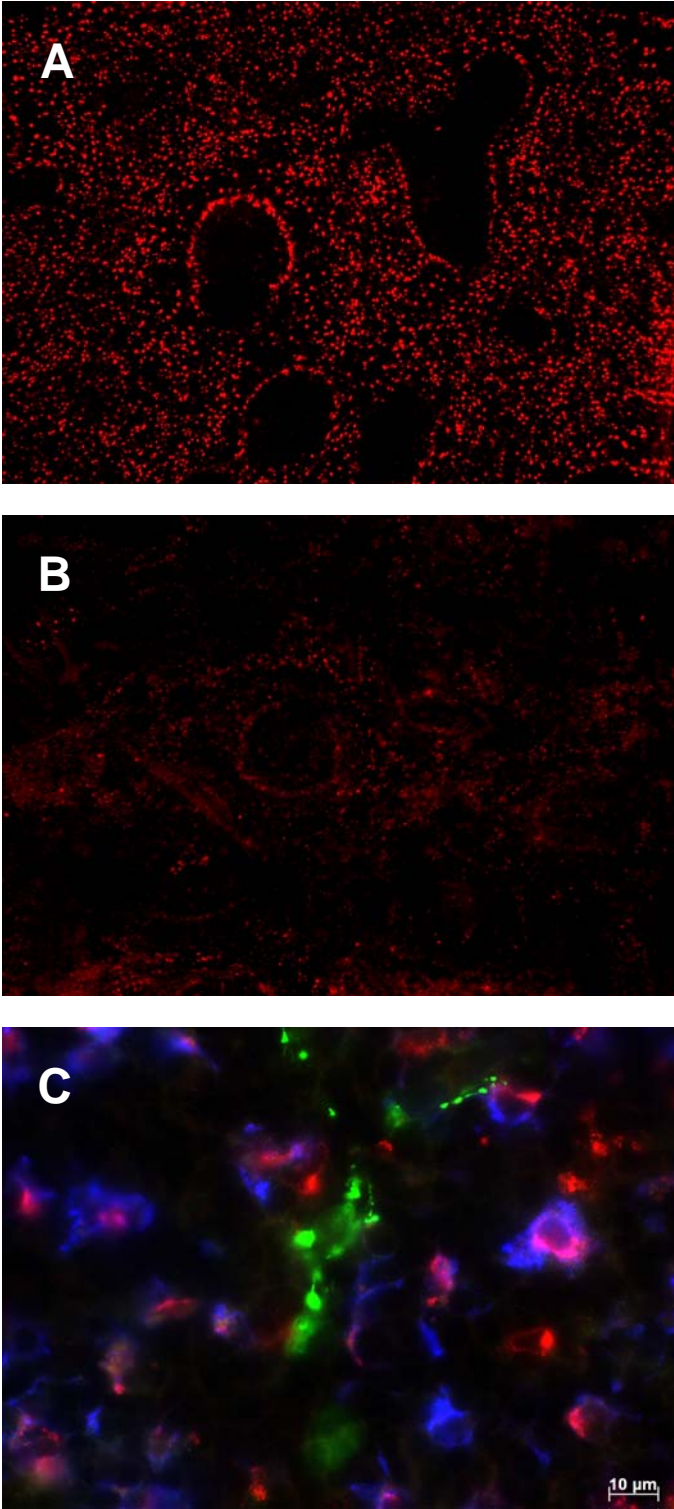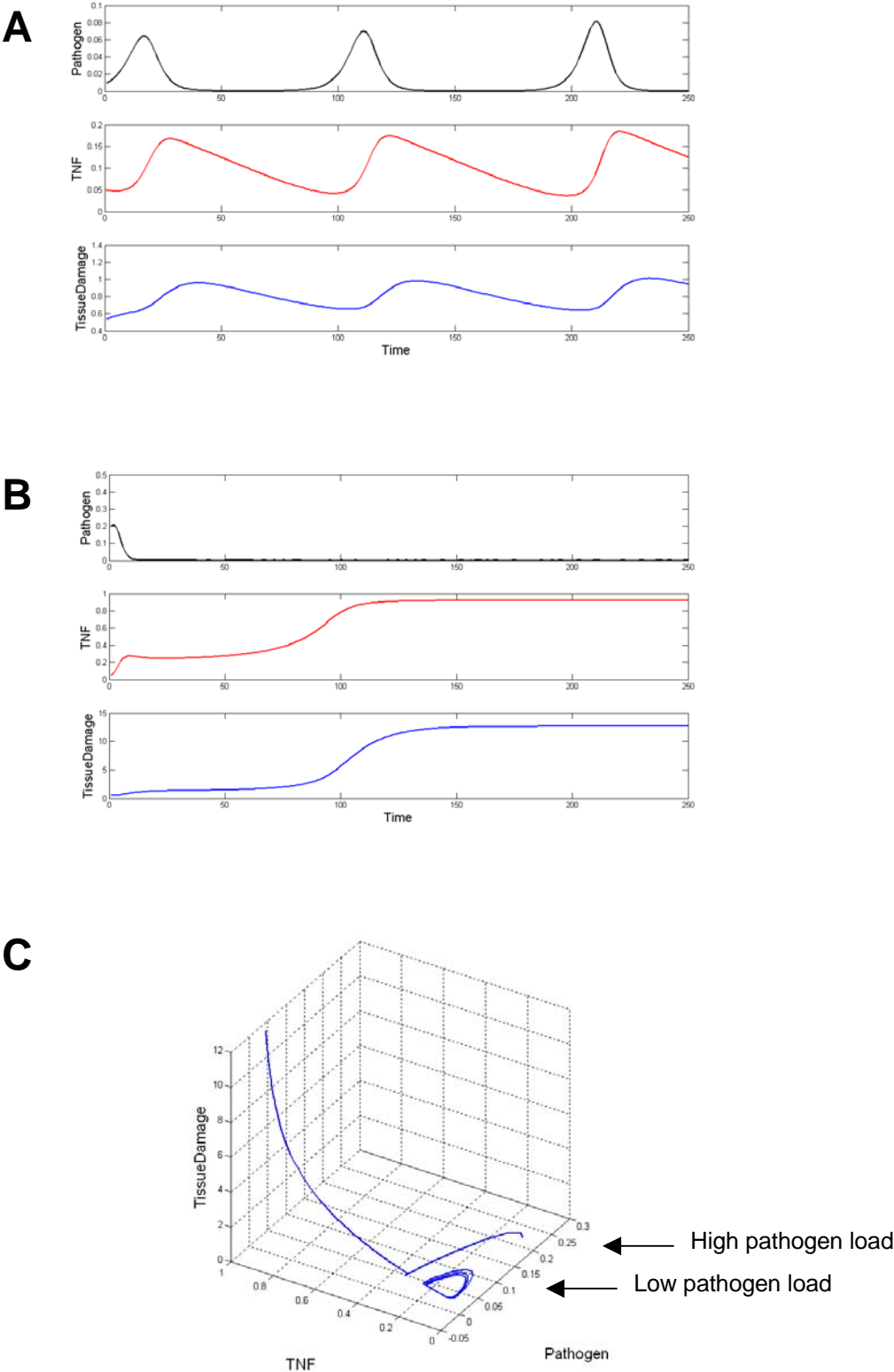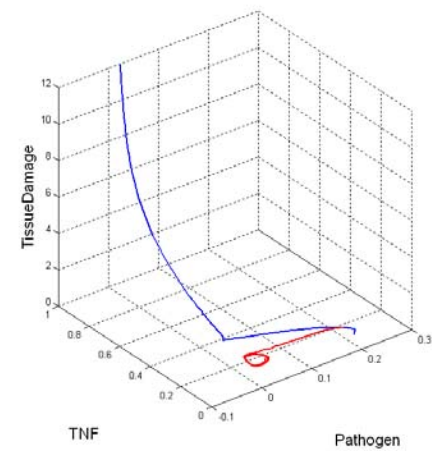
Figure 1

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B

Figure 2

Figure 3

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