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Review article

Mathematical modeling in necrotizing enterocolitis—a new look at an ongoing problem

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Abstract Necrotizing enterocolitis (NEC) is the most common and lethal disease that affects the gastrointestinal (GI) tract of the premature infant. The etiology of NEC remains undefined. The only consistent epidemiological precursors for NEC are prematurity and enteral alimentation. Various inflammatory mediators, including tumor necrosis factor (TNF)-a, interleukin (IL)-1, IL-6, IL-8, IL-10, IL-18, platelet-activating factor (PAF), and nitric oxide (NO) have been implicated in the pathogenesis of NEC, but the kinetics and role of these agents are ill-defined. Currently, there are no biomarker predictors of NEC risk and severity. Sera or tissue from early time points in the development of the disease may help delineate early inflammatory events that predispose an individual to NEC, thus providing an interventional opportunity. We suggest that the lack of diagnostic and therapeutic modalities for NEC are due to the absence of a systems view of the disease, which in turn is hindered by a lack of sensitive physiological measurements that predict perturbations in the intestinal tissue compartment and an inability to reliably test serial samples for the presence of inflammatory mediators in small volumes and in a high-throughput manner. Computational modeling is a useful tool in the study of complex systems such as the inflammatory process. Computation models provide an "existence proof" for a given mechanism, uncover subtle inconsistencies between the underlying hypotheses and quantitative data, and force one to ask how much is known. We suggest that a properly validated and calibrated mathematical model of inflammation and its pathologic consequences in NEC will be useful for predicting the physiologic and biologic response in infants suffering from the disease.

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1. Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a frequent and often lethal disease affecting the gastrointestinal tract of premature infants [1]. Advances in neonatology have resulted in the improved survival of premature and low-birth-weight infants, resulting in a growing population of infants at risk for NEC [2]. In its most severe form, destruction of the epithelial lining of the intestine may progress to full-thickness bowel necrosis, perforation, and peritonitis, accompanied by bacterial invasion and sepsis [3]. The overall mortality for NEC ranges from 10% to 50%, [4] and approaches 100% for patients with the most severe form of the disease [5]. In addition, infants that recover from NEC are at risk for the development of intestinal obstruction from strictures and short bowel syndrome from disruption of intestinal function, further prolonging hospitalization and impairing growth and development [6-9].

Although multiple risk factors including prematurity [10], age at initiation, composition and rate of enteral feeding [11,12] bacterial infection [13], and intestinal ischemia [14,15] have been implicated in the initiation and progression of NEC, sensitive indicators of infants predisposed to the development of disease or in the early stages of disease are not yet available. We present a mathematical model for early detection in infants at risk for NEC or in the early stages of disease based on a profile of proinflammatory cytokines as biomarkers of disease. Although disease pathogenesis in NEC is centered in the intestine, signs and symptoms of systemic sepsis accompany disease progression with alterations in the level of circulating cytokines and inflammatory mediators. This review focuses on clinical variables and inflammatory mediators that accompany NEC and provides an example of how a complex clinical problem can be deconstructed and studied using a computer algorithm based on clinical samples (Fig. 1). Early detection would allow a greater variety of management options, such as a longer period of bowel rest in infants at risk or in the early stages of NEC. More sensitive indicators as adjuncts to clinical signs and symptoms are likely to impact disease progression and outcome, thereby improving survival and reducing morbidity and mortality. Our hope is that mathematical models will validate novel and *early* biomarkers in NEC that are as discernable as the abnormal electrocardiogram for hyperkalemia or myocardial ischemia.

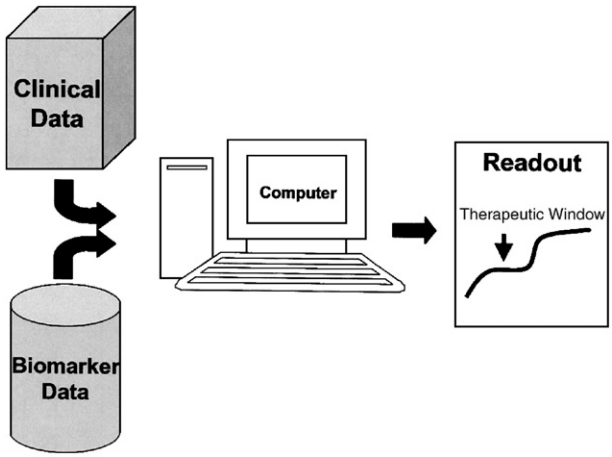

Fig. 1 Conceptual design for clinical mathematical modeling. This diagram depicts a general approach to taking clinical and biomarker data (eg, cytokines, complete blood counts, electrolytes) and entering the data into a computer algorithm that generates a simplistic representation of the data. The authors suggest that a simplistic pattern derived from complex data manipulations may reveal a pattern that can be identified and potentially manipulated for diagnostic or therapeutic purposes. For example, an electrocardiogram depicts heart rate and cardiac cycle. When derangements occur such as hyperkalemia, the electrocardiogram changes shape by showing peaked T waves. Peaked T waves are generally an early sign of hyperkalemia, whereas a sine wave is a very late sign. Recognition of the former peaked T wave offers a therapeutic window for effective therapeutic interventions.

2. Inflammatory mediators in NEC

Various proinflammatory cytokines and products of vasoactive substances are present in the serum and intestine of infants with NEC, including tumor necrosis factor α (TNF-α), interleukin (IL)–l, IL-6, IL-8, IL-10, IL-11, platelet-activating factor (PAF), and nitric oxide (NO) [16-24]. The significance and prognostic value of individual abnormalities in one or more of these substances is however unclear. Elevated plasma levels of TNF-α have been reported in infants with NEC [23,25,26]. Clinical trials demonstrate that pentoxifylline, which blocks TNF-α and IL-6 production, may provide a survival benefit in acute NEC [23,26], Other investigators, however, have been unable to detect any significant increase in plasma TNF-α levels in infants with NEC [27]. In fact, plasma TNF-α levels do not seem to correlate with either the severity or the duration of disease [28]. There are similar controversies regarding the presence and role of other proinflammatory cytokines in NEC including IL-1, a cytokine released early during an inflammatory response. In infants with NEC, plasma levels of IL-1 are initially elevated, although the IL-1 receptor antagonist, the endogenous inhibitor of IL-1 may be a better correlate of disease severity [24]. Similarly, although serum levels of IL-6 correlate with disease severity [23,28,29], no difference in intestinal IL-6 messenger RNA (mRNA) or protein was found in infants with advanced NEC and age-matched controls [30,31]. A similar controversy was found with IL-8, a potent chemotactic factor for neutrophils; although serum levels of IL-8 were increased in infants with severe NEC [24], no difference in IL-8 mRNA or protein in diseased intestine was detected in infants with acute NEC [30], whereas others have detected increased levels of IL-8, along with IL-11, and interferon γ mRNA in intestinal specimens from infants with acute NEC as

compared with controls [31,32]. Serial plasma levels of IL-6 and IL-10 were elevated among premature infants with NEC among those with suspected sepsis hours after clinical signs of sepsis were detected [33]. A similar study found serial elevations in IL-8, IL-1 receptor antagonist, and IL-10 with levels whose increase correlated with the severity of disease and the need for surgical intervention [24]. Plasma levels of other products of proinflammatory mediators also exhibit variability in NEC. Platelet-activating factor, a pro-inflammatory lipid mediator shown to directly cause intestinal mucosal injury and bowel necrosis in animal models of NEC [34,35], was elevated in neonates with NEC compared with age-matched controls [25]. Likewise, PAF-acetylhydrolase activity, responsible for the generation of PAF, was reciprocally decreased in infants with NEC. Increased levels of TNF-α may in addition result in increases in PAF production, although a PAF antagonist failed to limit the extent of mucosal injury in an ischemia/reperfusion model, suggesting that PAF alone is not sufficient to cause ischemic injury to the gut [36].

A challenge in NEC, as well as other tissue-specific inflammatory conditions, is in understanding the significance of pro-inflammatory mediators in the tissue as compared with those in serum or plasma. One possible explanation for the lack of unity in published studies is the high degree of variability among individual cases of NEC. However, we believe that the kinetics of cytokine production are highly dependent on patient-specific conditions [37], and fluctuations from an individual baseline are likely to yield important insight into the onset and progression of disease.

3. Experimental models of NEC

Previous authors have relied on a variety of animal models of intestinal inflammation, such as intraluminal administration of toxic agents, ischemia/reperfusion injury of the intestine, or intravenous administration of pro-inflammatory cytokines to reproduce the morphological and clinical changes seen in infants with NEC [38]. Administration of acidified casein and calcium gluconate into a surgically isolated loop of small intestine in rabbits, rats, or piglets has been shown to induce blunting and loss of villi, as well as increased intestinal permeability [39-41]. Sibbons et al used mesenteric artery and lymphatic ligation in newborn pigs to produce histologic lesions similar to those seen in NEC [42]. Krasna and Kim [43] reported that brief (15 minutes) occlusion of the superior mesenteric vessels followed by indomethacin administration could cause bowel necrosis in mice. Papparella et al [44] showed that occlusion of the superior mesenteric artery for 1 hour followed by reperfusion for 2 hours could induce ischemic (hemorrhagic) necrosis in newborn pigs. Others have used mesenteric ischemia/reperfusion in combination with intraluminal administration of formula, PAF, or both, to induce experimental NEC [45-47]. Lastly, some authors have combined 5 minutes of anoxia with intrarectal instillation of air to distend the bowel to induce intestinal injury in newborn rats [48,49].

Injection of PAF into the mesenteric vascular bed of rats has been shown to cause ischemic necrosis, prostanoid release, and oxygen free radical generation [50,51]. Platelet-activating factor and endotoxin synergistically induce bowel necrosis in rats [52]. Administration of PAF antagonists partially prevented the morbidity associated with endotoxin and hypoxia [34]. Furthermore, up-regulation of PAF-acetylhydrolase activity by steroid administration decreased PAF-induced injury, whereas inhibition of PAF-acetylhydrolase activity allowed small amounts of PAF to cause significant disease [53]. However, using a PAF antagonist, de Boissieu et al [36] failed to show any decrease in the intestinal injury after ischemia, although this inhibitor did attenuate peritoneal inflammation. Furthermore, Schriffin et al [54] showed that bacteria-free and specific pathogen–free adult rodents developed intestinal necrosis, but did not become septic when challenged with PAF, which suggest that PAF alone may not be sufficient to induce experimental NEC in absence of the intestinal microbial flora. These experimental approaches neither resemble nor parallel the human experience. Each one requires highly invasive artificial insults that often fail to take into consideration the multifactorial etiology of NEC. Furthermore, these models concentrate primarily on mucosal injury, often use adult animals, or use isolated loops of intestine. Clearly, a more suitable animal model is required.

Barlow et al [55] showed that formula-fed newborn rats subjected to brief but repeated periods of hypoxia in a plastic bag for 7 days developed morphological changes characteristic of NEC. In this model, ileal sections from breast milk–fed rats displayed normal intestinal architecture; however, sections from formula-fed rats revealed atrophic villi, ulcerations of the bowel wall, and, in some cases, full-thickness necrosis. The authors later demonstrated that repeated episodes of hypoxia correlated with increased incidence of NEC, and that cold stress, which alters intestinal blood flow, was as effective as hypoxia in inducing experimental NEC [56,57]. In Barlow's studies, the fraction of inspired oxygen was unknown because placing the animals in a plastic bag induced hypoxia. Furthermore, the only parameter assessed by the authors was intestinal morphology. Nadler et al [20] standardized the hypoxic insult, and evaluated mucosal inflammation and the expression of various pro-inflammatory cytokines in the intestine. Studies demonstrate a similar pattern of intestinal inflammation to that reported by Barlow and a cytokine profile that resembles that seen in human NEC [20]. The intestinal epithelium of formula-fed hypoxic rats shows elevated expression of mRNA of both the inducible NO synthase and interferon γ, increased nitrosative stress, increased enterocyte apoptosis, and decreased crypt cell proliferation [20]. Recently, Jilling et al [58] demonstrated similar inflammatory changes in a murine NEC model.

We do not know the early events in the pathogenesis of human NEC. Human observations are derived from tissues resected from infants with the most advanced (end) stage of the disease because infants with early NEC are managed nonoperatively. Nonetheless, considerable information can be gained in understanding the pathogenesis of intestinal inflammation in this experimental model that incorporates some of the known risk factors for human NEC, including hypoxia and formula feeding. For example, Kelly et al [59] performed a time course microarray analysis on intestinal samples from our animal model of NEC and discovered 93 genes that are unregulated in NEC compared with breast-fed controls.

The foregoing discussion suggests that much data, but relatively few therapeutically useful insights, have been obtained over the past years of research on human and experimental NEC. This situation mirrors that of the sepsis and trauma fields. We reasoned that to break this logjam, we should begin to use approaches that treat NEC as a system rather than an isolated set of parts [60]. Recently, it has been suggested that statistically based methods may not achieve full usefulness in complex inflammatory diseases such as NEC because these methods require large amounts of data; in contrast, techniques such as mathematical modeling can derive insights from relatively small data sets such as those likely to be obtained from neonates [61]. Below, we describe our mathematical modeling approach and the strides we have made in bringing this technology closer to practical application in the setting of NEC.

4. A primer on mathematical modeling and applications to NEC

Systems biology is the integration of experimental and computational tools to characterize complex biological phenomena [62]. The experimental constituent allows for the development of a knowledge base, and can be derived from literature mining and classic quantitative experimentation. A hypothesis is then generated for the behavior of candidate elements of interest (genes, signaling pathways, cytokines, etc) based on consensus data. The computational constituent then uses a simulation-based analysis of the hypothesis using advanced software and machine-executable platforms. Drawn from these computations are predictions and conclusions governing the dynamics of the system in question. Returning to in vitro and in vivo models and applying the information garnered then verifies the validity of the findings. This approach has the potential for clarifying biological controversies [62], which abound in the study of NEC as described above.

Modeling the behavior of biological elements of interest allows for theoretical manipulation of the networks in which they take part. A robust and well-designed in silico systems approach to multifactorial diseases facilitates large-scale study of the mechanisms underlying certain pathologies in simulation without the need for large sample sizes. This approach circumvents the need for costly animal experimentation and limited human tissue [61]. In addition, once the models are established and shown to be robust, their data can be easily exchanged among scientific groups and applied to different research pursuits. The pathogenesis of NEC is a problem ideally suited to mathematical modeling approaches at various levels because of the unique combination of development, inflammation, and healing in a remote organ system with systemic manifestations.

Approaching intricate biological systems with a reductionist mentality falls short in that simple individual elements do not always act linearly, can proceed through sometimes-redundant pathways, and can be functionally diverse under variable circumstances [60,62]. This issue is best highlighted through our attempts to understand and modulate the multiorgan failure that may result from acute inflammatory responses to infection, traumatic injury, hemorrhagic/septic shock, and NEC. Although much has been learned about cellular mechanisms and molecular mediators that initiate and drive the inflammation and tissue repair in these disease states, treatment with multiple anti-inflammatory agents (anti-TNF, IL-1b, PAF, bradykinin, etc) showed no survival benefit in clinical trials [63]. The likely explanation for this is that acute inflammation is a complex process, and that pointed manipulation of single pathways or mediators within the system cannot be adequately predicted from knowledge of those pathways or mediators in isolation [64-66]. In addition, the correct therapy may depend on the exact chronological stage and trajectory of the disease.

Robustness is an essential part of biological systems and must be tested to ensure veracity. The properties exhibited by robust systems can be classified into 3 areas according to Kitano [62]: (i) adaptation, which denotes the ability to cope with environmental changes; (ii) parameter insensitivity, which indicates a system's relative insensitivity to specific kinetic parameters; and (iii) graceful degeneration, which reflects the characteristic slow degradation of a system's functions after damage rather than catastrophic failure. Such properties are achieved through feedback loops, modularity, redundancy, and structural stability, ensuring that robust systems remain homeostatic even when internal parameters or external environmental influences are altered.

Systems level analysis is being increasingly applied to a growing number of biological models, being made possible by a number of rapidly growing advances in quantitative molecular biology that make possible the large amount of high-throughout experimental data. Below, we describe our variation on this approach.

5. Systems biology approach to acute inflammation and NEC

To examine what cellular and molecular mechanisms contribute to the pathogenesis of NEC, we developed a mathematical model incorporating major components of the

acute inflammatory response using input elements from documented experimental data. We propose that modeling will characterize the complex interplay of the network and provide insight into the global consequences of manipulating individual components of inflammation.

Our NEC mathematical model is composed of 2 compartments, tissue and blood. Cell types include macrophages, neutrophils, dendritic cells, helper T cells (T_H1 and T_H2), and pathogens (with macrophages and neutrophils in either an active or resting state). Macrophages are confined to the tissue, whereas neutrophils are confined to the blood until they are activated. Dendritic cells travel to lymph nodes, where they activate T_H1 or T_H2 cells depending on the cytokine milieu. There are several diffusible compounds, namely, pro- and anti-inflammatory cytokines, free radicals (which cause tissue damage), and lipopolysaccharides (LPS) produced by the bacteria, all of which can cross through the endothelial boundary between the tissue and the blood. Furthermore, inflammation caused by pro-inflammatory cytokines leads to tissue death. This in turn results in an increase in the diffusivity of the small molecules. The aforementioned model is easily adaptable to previously described inflammation models. Notable differences include the normal acquisition of bacteria in the lumen of the intestine (a.k.a. succession) and the normal "leakiness" of the premature intestinal barrier that becomes less permeable over time.

We initially approached this complex model by breaking the system into smaller pieces that are amenable to mathematical analysis. For example, consider the damage-induced increase in cross-endothelial diffusion of small molecules. This increase in diffusion allows more cytokines to enter the blood from the tissue pool, which activates more resting neutrophils. The active neutrophils enter the tissue, causing damage that increases the diffusion. This is a key positive feedback loop, which we think underlies the inflammatory response. To see what is needed for diffusion-dependent bi-stability (between healthy and inflamed states), we extract the part of the full model involving neutrophils and inflammatory cytokines; this is a time-tested approach in dynamical systems modeling, which allows for analysis of otherwise essentially impenetrable differential equation-based models. We have used this approach successfully in the past to address aspects of the biology of sepsis [67-69]. Eq. 1 demonstrates this approach. By setting some of the variables to their steady states, we obtain a simple 2-variable model involving the active neutrophils in the tissue n_a and the blood level of inflammatory cytokines c_b, where $f(c_b)$ is a sigmoidal function representing the combined production of activated neutrophils by inflammatory cytokines and transport of activated neutrophils to the tissue compartment. The parameters ν, γ, and η are degradation rates of the neutrophils, blood cytokines, and tissue cytokines, respectively; r is the rate of production of cytokine by the neutrophils in the tissue in response to initial tissue damage (eg, from hypoxia) and bacterial translocation. The parameter d is the diffusivity of the cytokines from the blood to the tissue. When d is small, the only state is the resting state. As d increases, the system is bi-stable with a new inflamed state. A simulation of these interactions is shown graphically in Fig. 2.

$$\begin{aligned} n_a' &= f(c_b) - \nu \cdot n_a \\ c_b' &= -\gamma c_b + \tfrac{d}{d+\eta}(r \cdot n_a - \eta c_b) \end{aligned} \tag{1}$$

The inflamed state is very easy to achieve once the diffusivity passes a critical value. Another submodule in the large model involves the transient production of TNF-α in the tissue by the macrophages. This product is transient because of the inhibitory effect of IL-10 on the macrophage activation. Thus, the module involves resting macrophages, activated macrophages, TNF-α, and IL-10. As above, by setting both macrophage populations to steady-state levels,

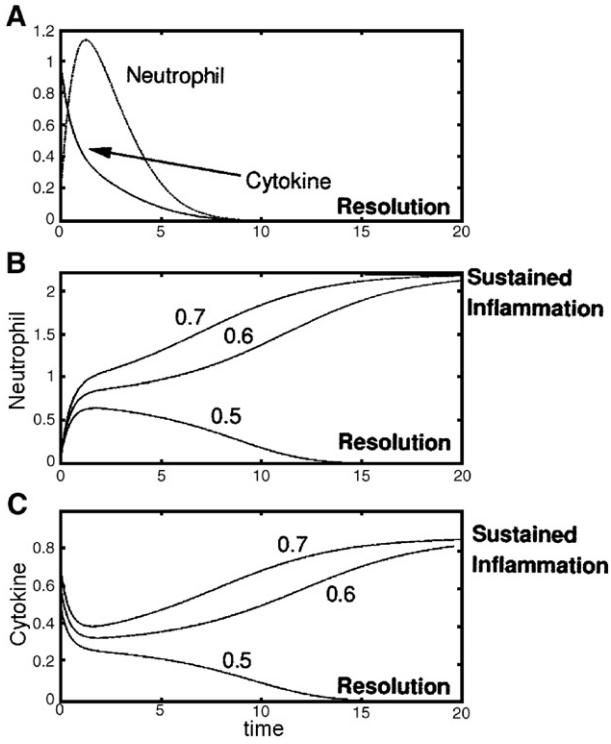

Fig. 2 Modeling neutrophil-cytokine interactions and their effect on intestinal permeability. Panel A, A representation of the interaction among neutrophils and cytokines in a setting of low damage. Low damage is associated with a low level of leakiness of the intestinal epithelial barrier after exposure to a small amount of pro-inflammatory cytokines (prototypically TNF-α). At higher damage, a larger degree of epithelial barrier leakiness occurs after exposure to a small amount of pro-inflammatory cytokines, leading to increased activation of neutrophils (panel B) and consequent production of pro-inflammatory cytokines (panel C). These events lead to bi-stable behavior (sustained inflammation vs resolution of inflammation) depending on exact conditions. The numbers (0.5, 0.6, 0.7) are the initial values of the pro-inflammatory cytokine. For low values there is resolution, but for higher values, there is enough feedback to cause a sustained response. Panels A to C, All numbers are unitless.

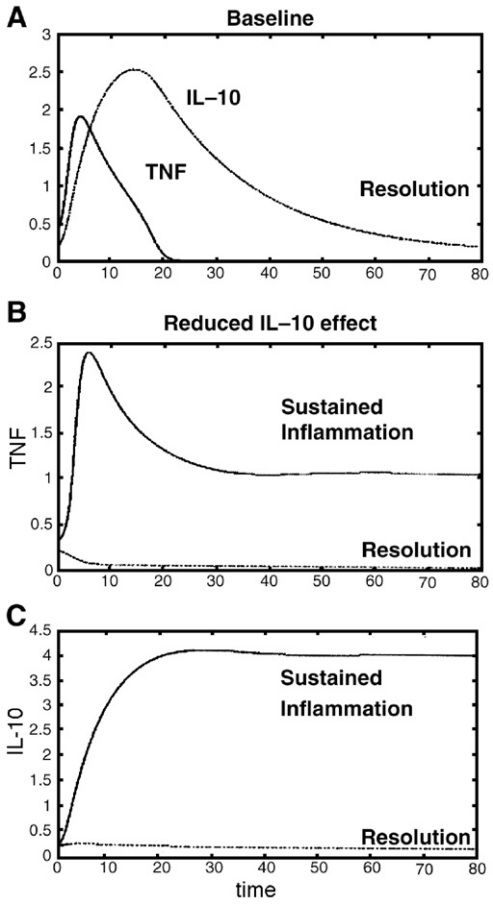

Fig. 3 Modeling interactions between TNF-α and IL-10 in the setting of intestinal inflammation. Panel A, At baseline, exposure to a small amount of TNF-α can lead to an amplification of the inflammatory response, but the anti-inflammatory actions of IL-10 result in a return to the baseline state. Panels B and C, The effectiveness of IL-10 on suppressing TNF-α is simulated to be degraded by half. The result is bi-stability between a stable rest state and an inflamed state, differing only by the initial amount of TNF. Panel B shows the effects of this scenario on TNF-α. Panel C shows the effects of this scenario on IL-10. Panels A to C, All numbers are unitless.

the model reduces to 2 equations for TNF (X) and IL-10 (Y) tissue concentrations (Eq. 2). Here, several variables have been rescaled to eliminate some of the parameters. This is a classic activator-inhibitor system, where X is self-activating and Y inhibits. The main point is that if the IL-10 production is slower than that of TNF, then with appropriate choices of k_x and k_y, this system is excitable and there is a transient increase in TNF before returning to rest. If IL-10 is blocked or reduced, then bi-stability between a healthy state and an inflamed state is possible, as depicted graphically in Fig. 3.

$$\begin{aligned} \frac{dY}{dt} &= -\mu X + k_X F(X,Y) \\ \frac{dY}{dt} &= -\nu Y + k_Y F(X,Y) \\ F(X,Y) &= \frac{X^2}{(1+X^2)(1+Y)} \end{aligned} \tag{2}$$

6. Calibration of inflammation models to experimental data

A central part of our long-term modeling approach is the calibration of existing mathematical models of inflammation [37] to data in rodent and human NEC. In previous work, Vodovotz et al [61] hypothesized that the machinery linking different components of the early inflammatory was "hard-wired" and independent of the specific stress encountered. However, the response would have different expressions given that different types of stress provided different initial conditions to an otherwise unified system. With this underlying assumption, the mathematical model was calibrated to various species of preclinical relevance: mice (subjected to LPS, surgical trauma, and surgery followed by hemorrhagic shock [37]), rats (subjected to endotoxemia, surgical trauma, and surgery + endotoxemia [Lagoa et al, manuscript in preparation], as well as true bacterial sepsis [Lagoa et al, manuscript in preparation]), and swine (based on literature data and including interactions of inflammatory cytokines with matrix metalloproteases in the setting of endotoxemia) [70]. We have also begun to calibrate the inflammation model in humans, using data on human endotoxemia [69]. We have begun to collect dense inflammatory biomarker data in our neonatal rat model of NEC described above, which will be used to calibrate our mathematical model for systemic inflammation in the setting of NEC [71].

7. Spatial modeling of NEC

In addition to its dynamic behavior, the inflammation process in NEC exhibits a number of spatial characteristics, such as diffusion of inflammatory agents, chemotaxis, and epithelial cell migration. We have developed a model for NEC, which includes 4 compartments—lumen, epithelial layer, organ tissue, and blood (Sullivan et al, submitted for publication). The model allows for spatial distribution and movement of the system components. This is achieved by including spatial derivatives in the differential equations. Each compartment is assigned specific diffusion parameters, which affect the movement of inflammatory agents.

The ability of lumen bacteria to infiltrate the organ tissue critically depends on the integrity of the epithelial wall, which is affected by factors such as cell migration and strength of tight junctions. The ability of a damaged wall to heal depends on the level of infection via the amount of LPS present in the system. Tight junction proteins can be destroyed by the presence of NO, which is produced by the inflammatory reactions.

We present simulation results that show that even normally harmless bacteria in the lumen can penetrate damaged epithelial wall and cause serious infection. The level of damage may affect the outcome, leading to either healthy state or persistent inflammation. In Fig. 4, we show

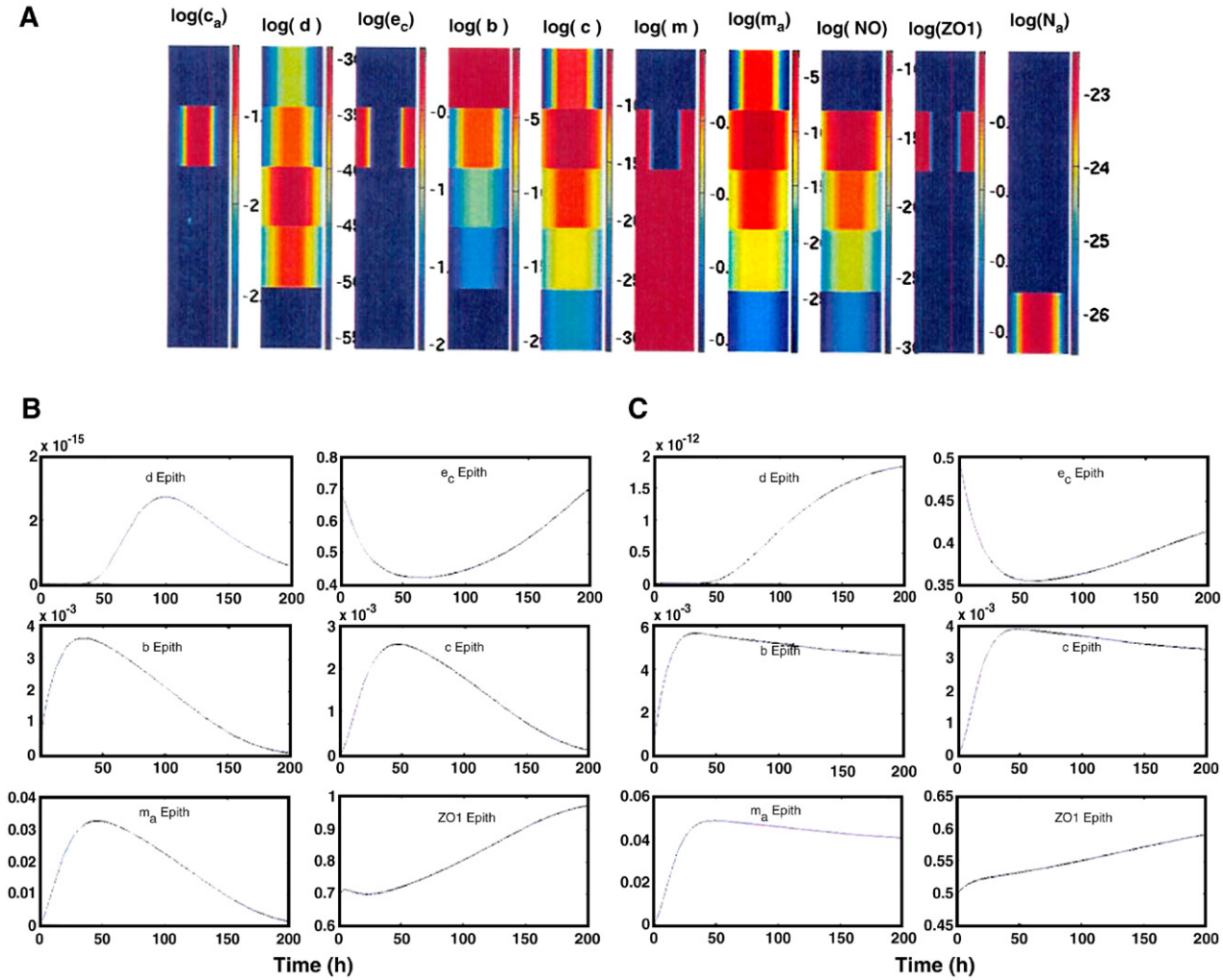

Fig. 4 Spatial model of NEC. Panel A, System state at time = 50 for case 1. The simulation domain is a cross section consisting of a lumen layer (top), an epithelial layer, 2 tissue layers, and a blood layer (bottom). The system components are c_a, anti-inflammatory cytokines; d, damage; e_c, epithelial cells; b, bacteria; c, cytokines; m, resting macrophages; m_a, activated macrophages; ZO1, tight junction protein; and N_a, activated neutrophils. At $t = 50$, the bacteria have diffused through the tissue and has started an inflammation process. Damage-dependent endothelial barrier controls the movement into and out of the blood compartment. Some cytokines have penetrated into the blood, activating a small amount of neutrophils. Panel B, Time history of averaged component values in the epithelial layer for case 1 (partial hole). The initial level of epithelial cells is 1 of 3 in the hole and 1 for the rest of the layer. The inflammation process in the epithelial peaks around time = 50. At that time the bacteria and inflammatory agents (c, m_a) start to decrease and the epithelial layer starts to heal (e_c, ZO1). The damage starts to decrease at around $t = 100$. The system is close to a healthy state at the end of the simulation. Panel C, Time history of averaged component values in the epithelial layer for case 2 (completely missing portion of the wall). The initial level of epithelial cells is 0 in the hole and 1 for the rest of the layer. The hole is closing much more slowly in this case. The inflammation persists and the damage increases throughout the simulation. Panels A to C, All numbers are unitless.

results from 2 simulations, first with a partially damaged epithelial wall and second with a completely missing portion of the wall. In the first case, although some bacteria enter the tissue and start an inflammatory process, it is eventually killed and the wall is healed. In the second case, a larger amount of bacteria penetrates into the tissue, leading to a more severe inflammatory response, which in turn affects the ability of the wall to heal. Fig. 4A shows the state of the system in the first case, at the peak of the inflammatory process.

8. Summary

Necrotizing enterocolitis is a multifactorial disease largely affecting premature infants. Many inflammatory mediators are implicated in NEC pathogenesis, but no set of biomarkers delineates infants that will go on to develop NEC. Animal models shed some light on mediators that may contribute to NEC, but fall short in predicting biomarkers that may determine susceptibility. Mathematical modeling is an alternative strategy used in understanding

complex inflammatory diseases, and it holds promise in helping investigators understand the pathogenesis of NEC.

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References

[1] Kliegman R.M, Fanaroff AA. Neonatal necrotizing enterocolitis: a nine-year experience. II. Outcome assessment. Am J Dis Child 1981;135(7):608-11.
[2] Holman RC, Stoll BJ, Clarke MJ, et al. The epidemiology of necrotizing enterocolitis infant mortality in the United States. Am J Public Health 1997;87(12):2026-31.
[3] Kliegman RM, Fanaroff AA. Necrotizing enterocolitis. N Engl J Med 1984;310(17):1093-103.
[4] Blakely ML, Lally KP, McDonald S, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: a prospective cohort study by the NICHD Neonatal Research Network. Ann Surg 2005;241(6):984-9 [discussion 989-94].
[5] Rowe MI, Reblock KK, Kurkchubasche AG, et al. Necrotizing enterocolitis in the extremely low birth weight infant. J Pediatr Surg 1994;29(8):987-90 [discussion 990-1].
[6] Petty JK, Ziegler MM. Operative strategies for necrotizing enterocolitis: the prevention and treatment of short-bowel syndrome. Semin Pediatr Surg 2005;14(3):191-8.
[7] Barksdale EM, Stanford A. The surgical management of short bowel syndrome. Curr Gastroenterol Rep 2002;4(3):229-37.
[8] Warner BW, Vanderhoof JA, Reyes JD. What's new in the management of short gut syndrome in children. J Am Coll Surg 2000;190(6):725-36.
[9] Goulet O, Ruemmele F. Causes and management of intestinal failure in children. Gastroenterology 2006;130(2 Suppl 1):S16-S28.
[10] Horton KK. Pathophysiology and current management of necrotizing enterocolitis. Neonatal Netw 2005;24(1):37-46.
[11] McKeown RE, Marsh TD, Amamath U, et al. Role of delayed feeding and of feeding increments in necrotizing enterocolitis. J Pediatr 1992;121(5 Pt 1):764-70.
[12] Anderson DM, Kliegman RM. The relationship of neonatal alimentation practices to the occurrence of endemic necrotizing enterocolitis. Am J Perinatol 1991;8(1):62-7.
[13] Krediet TG, van Lelyveld N, Vijlbrief DC, et al. Microbiological factors associated with neonatal necrotizing enterocolitis: protective effect of early antibiotic treatment. Acta Paediatr 2003;92(10):1180-2.
[14] Kliegman RM. Models of the pathogenesis of necrotizing enterocolitis. J Pediatr 1990;117(1 Pt 2):S2-S5.
[15] Martinez-Tallo E, Claure N, Bancalari E. Necrotizing enterocolitis in full-term or near-term infants: risk factors. Biol Neonate 1997;71(5):292-8.
[16] Upperman JS, Potoka D, Grishin A, et al. Mechanisms of nitric oxide–mediated intestinal barrier failure in necrotizing enterocolitis. Semin Pediatr Surg 2005;14(3):159-66.
[17] Ren Y, Lin CL, Li Z, et al. Up-regulation of macrophage migration inhibitory factor in infants with acute neonatal necrotizing enterocolitis. Histopathology 2005;46(6):659-67.
[18] Harris MC, D'Angio CT, Gallagher PR, et al. Cytokine elaboration in critically ill infants with bacterial sepsis, necrotizing enterocolitis, or sepsis syndrome: correlation with clinical parameters of inflammation and mortality. J Pediatr 2005;147(4):462-8.
[19] McCloy MP, Roberts IA, Howarth LJ, et al. Interleukin-11 levels in healthy and thrombocytopenic neonates. Pediatr Res 2002;51(6):756-60.
[20] Nadler EP, Dickinson E, Knisely A, et al. Expression of inducible nitric oxide synthase and interleukin-12 in experimental necrotizing enterocolitis. J Surg Res 2000;92(1):71-7.
[21] Ford HR, Sorrells DL, Knisely AS. Inflammatory cytokines, nitric oxide, and necrotizing enterocolitis. Semin Pediatr Surg 1996;5(3):155-9.
[22] Muguruma K, Gray PW, Tjoelker LW, et al. The central role of PAF in necrotizing enterocolitis development. Adv Exp Med Biol 1997;407:379-82.
[23] Lauterbach R, Pawlik D, Kowalczyk D, et al. Effect of the immunomodulating agent, pentoxifylline, in the treatment of sepsis in prematurely delivered infants: a placebo-controlled, double-blind trial [see comments]. Crit Care Med 1999;27(4):807-14.
[24] Edelson MB, Bagwell CE, Rozycki HJ. Circulating pro- and counterinflammatory cytokine levels and severity in necrotizing enterocolitis. Pediatrics 1999;103(4 Pt 1):766-71.
[25] Caplan MS, Sun XM, Hseuh W, et al. Role of platelet activating factor and tumor necrosis factor–alpha in neonatal necrotizing enterocolitis. J Pediatr 1990;116(6):960-4.
[26] Lauterbach R, Zembala M. Pentoxifylline reduces plasma tumour necrosis factor-alpha concentration in premature infants with sepsis. Eur J Pediatr 1996;155(5):404-9.
[27] Harris MC, Costarino Jr AT, Sullivan JS, et al. Cytokine elevations in critically ill infants with sepsis and necrotizing enterocolitis [see comments]. J Pediatr 1994;124(1):105-11.
[28] Morecroft JA, Spitz L, Hamilton PA, et al. Plasma interleukin-6 and tumour necrosis factor levels as predictors of disease severity and outcome in necrotizing enterocolitis. J Pediatr Surg 1994;29(6):798-800.
[29] Morecroft JA, Spitz L, Hamilton PA, et al. Plasma cytokine levels in necrotizing enterocolitis. Acta Paediatr Suppl 1994;396:18-20.
[30] Viscardi RM, Lyon NH, Sun CC, et al. Inflammatory cytokine mRNAs in surgical specimens of necrotizing enterocolitis and normal newborn intestine. Pediatr Pathol Lab Med 1997;17(4):547-59.
[31] Ford H, Watkins S, Reblock K, et al. The role of inflammatory cytokines and nitric oxide in the pathogenesis of necrotizing enterocolitis. J Pediatr Surg 1997;32(2):275-82.
[32] Nadler E, Stanford A, Zhang X, et al. Intestinal cytokine gene expression in infants with acute NEC: IL-11 mRNA expression inversely correlates with extent of disease. J Pediatr Surg 2001;36(8):1122-9.
[33] Romagnoli C, Frezza S, Cingolani A, et al. Plasma levels of interieukin-6 and interleukin-10 in preterm neonates evaluated for sepsis. Eur J Pediatr 2001;160(6):345-50.
[34] Caplan MS, Kelly A, Hsueh W. Endotoxin and hypoxia-induced intestinal necrosis in rats: the role of platelet activating factor. Pediatr Res 1992;31(5):428-34.
[35] Caplan MS, Sun XM, Hsueh W. Hypoxia causes ischemic bowel necrosis in rats: the role of platelet-activating factor (PAF-acether). Gastroenterology 1990;99(4):979-86.
[36] de Boissieu D, Canarelli JP, Cordonnier C, et al. Effect of BN 50727 on pathological findings and tissue platelet activating factor levels during ileal ischemia in newborn piglets. J Pediatr Surg 1996;31(12):1675-9.
[37] Chow CC, Clermont G, Kumar R, et al. The acute inflammatory response in diverse shock states. Shock 2005;24(1):74-84.
[38] Crissinger KD. Animal models of necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 1995;20(1):17-22.

[39] Miller MJ, Adams J, Gu XA, et al. Hemodynamic and permeability characteristics of acute experimental necrotizing enterocolitis. Dig Dis Sci 1990;35(10):1257-64.
[40] Gollin G, Bell C, Dubose R, et al. Predictors of postoperative respiratory complications in premature infants after inguinal herniorrhaphy. J Pediatr Surg 1993;28(2):244-7.
[41] Di Lorenzo M, Bass J, Krantis A. An intraluminal model of necrotizing enterocolitis in the developing neonatal piglet. J Pediatr Surg 1995;30(8):1138-42.
[42] Sibbons P, Spitz SP, van Velzen D. The role of lymphatics in the pathogenesis of pneumatosis in experimental bowel ischemia. J Pediatr Surg 1992;27(3):339-42 [discussion 342-3].
[43] Krasna IH, Kim H. Indomethacin administration after temporary ischemia causes bowel necrosis in mice. J Pediatr Surg 1992; 27(7):805-7.
[44] Papparella A, DeLuca FG, Oyer CE, et al. Ischemia-reperfusion injury in the intestines of newborn pigs. Pediatr Res 1997;42(2):180-8.
[45] Crissinger KD, Burney DL, Velasquez OR, et al. An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing piglets. Gastroenterology 1994;106(5):1215-22.
[46] Musemeche CA, Baker JL, Feddersen RM. A model of intestinal ischemia in the neonatal rat utilizing superior mesenteric artery occlusion and intraluminal platelet-activating factor. J Surg Res 1995;58(6):724-7.
[47] Bhatia AM, Feddersen RM, Musemeche CA. The role of luminal nutrients in intestinal injury from mesenteric reperfusion and platelet-activating factor in the developing rat. J Surg Res 1996;63(1):152-6.
[48] Okur H, Kucukaydin M, Kose K, et al. Hypoxia-induced necrotizing enterocolitis in the immature rat: the role of lipid peroxidation and management by vitamin E. J Pediatr Surg 1995;30(10):1416-9.
[49] Kazez A, Kucukaydin N, Kucukaydin M, et al. A model of hypoxia-induced necrotizing enterocolitis: the role of distension. J Pediatr Surg 1997;32(10):1466-9.
[50] Cueva JP, Hsueh W. Role of oxygen derived free radicals in platelet activating factor induced bowel necrosis. Gut 1988;29(9):1207-12.
[51] Hsueh W, Gonzalez-Crussi F, Arroyave JL. Release of leukotriene C4 by isolated, perfused rat small intestine in response to platelet-activating factor. J Clin Invest 1986;78(1):108-14.
[52] Musemeche C, Caplan M, Hsueh W, et al. Experimental necrotizing enterocolitis: the role of polymorphonuclear neutrophils. J Pediatr Surg 1991;26(9):1047-9 [discussion 1049-50].
[53] Furukawa M, Lee EL, Johnston JM. Platelet-activating factor-induced ischemic bowel necrosis: the effect of platelet-activating factor acetylhydrolase. Pediatr Res 1993;34(2):237-41.
[54] Schiffrin EJ, Trop M, Schroeder S, et al. Platelet-activating factor induces intestinal necrosis, but not septic shock, in germ-free and specific-pathogen–free rodents. Burns 1991;17(4):276-8.
[55] Barlow B, Santulli TV, Heird WC, et al. An experimental study of acute neonatal enterocolitis—the importance of breast milk. J Pediatr Surg 1974;9(5):587-95.
[56] Barlow B, Santulli TV. Importance of multiple episodes of hypoxia or cold stress on the development of enterocolitis in an animal model. Surgery 1975;77(5):687-90.
[57] Powell RW, Dyess DL, Collins JN, et al. Regional blood flow response to hypothermia in premature, newborn, and neonatal piglets. J Pediatr Surg 1999;34(1):193-8.
[58] Jilling T, Simon D, Lu J, et al. The roles of bacteria and TLR4 in rat and murine models of necrotizing enterocolitis. J Immunol 2006; 177(5):3273-82.
[59] Kelly N, Friend K, Boyle P, et al. The role of the glutathione antioxidant system in gut barrier failure in a rodent model of experimental necrotizing enterocolitis. Surgery 2004;136(3):557-66.
[60] Csete ME, Doyle JC. Reverse engineering of biological complexity. Science 2002;295(5560):1664-9.
[61] Whitcomb DC, Aoun E, Vodovotz Y, et al. Evaluating disorders with a complex genetics basis, the future roles of meta-analysis and systems biology. Dig Dis Sci 2005;50(12):2195-202.
[62] Kitano H. Systems biology: a brief overview. Science 2002; 295(5560):1662-4.
[63] Freeman BD, Natanson C. Anti-inflammatory therapies in sepsis and septic shock. Expert Opin Investig Drugs 2000;9(7):1651-63.
[64] Buchman TG, Cobb JP, Lapedes AS, et al. Complex systems analysis: a tool for shock research. Shock 2001;16(4):248-51.
[65] Tjardes T, Neugebauer E. Sepsis research in the next millennium: concentrate on the software rather than the hardware. Shock 2002;17(1):1-8.
[66] Vodovotz Y, Clermont G, Chow C, et al. Mathematical models of the acute inflammatory response. Curr Opin Crit Care 2004;10(5):383-90.
[67] Kumar R, Clermont G, Vodovotz Y, et al. The dynamics of acute inflammation. J Theor Biol 2004;230(2):145-55.
[68] Reynolds A, Rubin J, Clermont G, et al. A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation. J Theor Biol 2006;242(1):220-36.
[69] Day J, Rubin J, Vodovotz Y, et al. A reduced mathematical model of the acute inflammatory response II. Capturing scenarios of repeated endotoxin administration. J Theor Biol 2006;242(1):237-56.
[70] Nieman G, Bartels J, Wei J, et al. Mathematical simulation of inflammation in porcine septic shock and ARDS. Shock 2005; 23(Suppl 3:3).
[71] Zamora R, Vodovotz Y, Ford H, et al. Plasma cytokine levels in experimental necrotizing enterocolitis: a mathematical model is needed. 4th International Conference on Complexity in Acute Illness, Cologne Germany 2005.