2007

A Hybrid Simulation Model for Studying Acute Inflammatory Response

Wayne W. Wakeland
Portland State University, wakeland@pdx.edu

Louis Macovsky
Dynamic BioSystems

Gary An
Northwestern University

A Hybrid Simulation Model for Studying Acute Inflammatory Response

Wayne Wakeland
System Science Ph.D. Program,
Portland State University, Portland,
Oregon, USA
wakeland@pdx.edu

Louis Macovsky
Dynamic BioSystems, Portland,
Oregon, USA
dynbiosys@verizon.net

Gary An
Division of Trauma/Critical Care,
Northwestern University, Chicago,
Illinois, USA
docgca@aol.com

Keywords: Hybrid model, agent model, system dynamics, inflammation, sepsis, MOF

Abstract
The modeling of complex biological systems presents a significant challenge. Central to this challenge is striking a balance between the degree of abstraction required to facilitate analysis and understanding, and the degree of comprehensiveness required for fidelity of the model to its reference-system. It is likely necessary to utilize multiple modeling methods in order to achieve this balance. Our research created a hybrid simulation model by melding an agent-based model of acute local infection with a system dynamics model that reflects key systemic properties. The agent based model was originally developed to simulate global inflammation in response to injury or infection, and has been used to simulate clinical drug trials. The long term objective is to develop models than can be scaled up to represent organ and system level phenomena such as multiple organ failure associated with severe sepsis. The work described in this paper is an initial proof of concept of the ability to combine these two modeling methods into a hybrid model, the type of which will almost certainly be needed to accomplish the ultimate objective of comprehensive *in silico* research platforms.

1. INTRODUCTION

1.1. Problem statement and significance

An important new research frontier is the use of agent based simulation (ABS) models and system dynamic (SD) or differential equation (DE) models to study complex biomedical phenomena such as the acute inflammatory response (AIR) and its disordered states of systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF) and sepsis [Neugebauer 2001 and Vodovotz et al 2004].

SIRS/MOF is an important problem domain in biomedical research [Buchman 1997, 2001]. It is considered one of the most significant and prevalent clinical problems in critical care medicine. SIRS/MOF is a disease process that is a byproduct of the success of initial therapies for infection and injury. It represents a behavioral region beyond the evolutionary "design parameters" of the inflammatory response, as patients previously suffering such a degree of initial insult would have died prior to manifesting SIRS/MOF. It is characterized by a systemic response to infection where, even though the original infection may be eradicated by the immune system and therapy, the collateral tissue damage results in system failure. The phenomenon involves interactions and feedback at multiple system levels (molecular, cellular, tissue, and organ) such that the complex interactions between components precludes the attribution of the systemic response, and therefore its control, to any single mediator or cell type. Since the inflammatory response is ubiquitous the manifestation of SIRS/MOF is distributed throughout the body's tissues and organs (hence Multiple organ failure), though the prevalent organs involved are the lungs, gut, kidneys and liver. While much has been learned in recent years, the translation of basic science knowledge to effective mechanism-based therapies for SIRS/MOF remains a largely unsolved problem.

While both ABS and SD/DE methods are considered to be complementary and appropriate for studying problems such as SIRS/MOF, very little has been published regarding hybrid approaches that integrate these two important methods into a single model that retains key advantages of each method and overcomes their individual drawbacks. For instance, ABS can be computationally expensive and much less efficient than SD/DE, whereas SD/DE requires the assumption of a well-mixed system and may not handle spatial heterogeneity as well as ABS. This paper presents an initial effort towards the development of an interface between ABS and SD/DE components within a single hybrid model.

1.2. Background and brief review of the literature

Wakeland et al [2004] used SD and ABS to study cellular receptor dynamics and compared the results from each method. This research indicated that the two paradigms are different and possess unique strengths and weaknesses. Guidelines were provided for assessing which of the two methods might be preferred in a given

situation. Both modeling paradigms were useful in communicating two- and three- state equilibrium (biochemical receptor) concepts. They are also useful in forcing the researcher to ask questions regarding the rules governing the system's dynamics. The ABS model allowed the researcher to question the rules at agent level but was not as convenient for modeling a system with a very large number of actors/agents when compared to the SD model. Neither modeling tool however was superior for obtaining new insights into receptor dynamics at the level of complexity for which the specific system was explored. The development of hybrid models was not addressed in this paper.

One of the first studies to report the use of ABS to model SIRS/MOF was An [2001]. This paper synthesized a wide variety of basic science results into an overall model that illustrated the complex dynamics seen in the clinical environment. The underlying processes (rules) were discussed in some detail, but the actual code was not provided in this initial paper. In addition to parametric variation, the degree of randomness could also be easily varied. Many graphical results were presented, some of which showed the behavior over time for different cases, while others compared the results of multiple runs with parameters being varied over a wide range, or with parameters fixed but with many different randomly generated cases. The paper was organized as a biomedical basic science research paper to illustrate how an *in silico* model could be used in the much the same fashion as a traditional laboratory model.

A recent issue Critical Care Medicine included two relevant papers on this topic [An 2004, Clermont et al 2004] as well as a short editorial article [Marshall 2004] contrasting *in silico* modeling with *in vivo* and *in vitro* research. The editorial is both encouraging and cautionary, warning that the potential benefits of these new approaches rest heavily upon the correctness of model algorithms and model data. It asserts that ABS models have yet to prove themselves by predicting effects that were not previously known.

An [2004] extends his earlier work, using the ABS model of SIRS to conduct *in silico* experiments that generally replicate the [disappointing] results of several large-scale clinical trials of cytokine-directed anti-mediator agents. The author also evaluated several hypothetical clinical trials, and found that they too would be likely to not achieve statistically significant results. He also showed how ABS models could be used to help design more effective clinical trials. In addition to the graphical and tabular results, extensive model details were provided, including a link to a website containing the actual model used to conduct the experiments.

Clermont et al [2004] developed a DE-based model to study immunomodulatory strategies for treating cases of severe sepsis. Their focus was to assess the feasibility of using DE models to improve the design of clinical trials. The model was used to simulate 1000 patients that were subjected to various simulated treatments. The results were presented in much the same fashion as results would be presented from an actual clinical trial. This research replicates *in silico* the general findings from actual clinical trials--that it is very difficult to design a treatment strategy that is effective over a broad range of sepsis patients. The authors provide an appendix that gives the model equations and parameters.

Other recent publications of interest include Vodovotz et al [2004], which reviewed mathematical models of the acute inflammatory response. This paper emphasized the need for non-reductionist approaches, and featured results from both An [2004] and Clermont et al [2004]. The paper also looked closely at the validation strategies used to assure the correctness of the model logic and model data. Further, Vodovotz et al [2006] outlined the process of model development as integrated into general biomedical research. This paper was directed primarily at the traditional "wet lab" research community, but also outlined the process of integrated, iterative model development for both ABS and SD/DE models in conjunction with ongoing standard laboratory research.

2. RESEARCH METHODOLOGY

Our research was carried out in three phases. Phase I consisted of reproducing An's ABS model, and then reducing it to facilitate parameter testing and improve computation efficiency. Phase II attempted a similar process with the Clermont et al ODE model. Phase III involved developing a new SD/DE model using the same modeling tool used by An to implement his ABS model (Netlogo 2005), and a means of linking these two submodels together. Inherent in Phase III was the determination of the "interface edge" between the ABS submodel and the SD submodel. The determination of this "edge" has consequences with respect to the aspects of model logic that are translated into the SD component, as well as further implications for further development of the resulting hybrid model.

2.1 Phase I

We used the Netlogo implementation of An's 2004 SIRS model as a starting point. This model simulates inflammation with interactions between endothelial cells and circulating inflammatory cells at the blood/blood vessel-lining interface. It is a 2-d grid torus ABS with the grid populated with static endothelial cell agents, over which move inflammatory cell agents. Injury or infection is simulated as a spatially discrete pattern of endothelial cell damage that can be varied by size. Therefore, at initial perturbation there is portion of the ABS that is

"damaged" and a portion that is still "normal." The relative sizes of these areas changes as the system either "heals" or progresses to "death." Even though the ABS is extremely abstract compared to reality, is still quite complex, requiring 14 pages of procedures. There are 14 different "breeds" of agents representing different cell types, each of which has unique logic. First, minor differences between the logic documented in the paper and the model provided on the web were rectified, and then a variety of experiments were run in order to confirm the prior results, and ascertain the feasibility and potential value of creating a hybrid version of the model. Observing the graphical display during several short model runs indicated that most of the simulated tissue was either healthy or severely "damaged," with a "sharp" boundary between the two regions. This suggested that the model could probably be scaled down without loss of utility. The area of the modeled region was scaled down factor of 4. Also, the number of "cases" per experiment was reduced from 100 to 10, and the time per run reduced from 28 to 7 simulated days. Other minor changes were also made, including modifying the logic so that the iteration number would not be incorrectly reset to zero, and to force the initial infection to be automatically invoked for the first iteration instead of requiring the user to remember to push a particular button at the start of the run.

Sufficient experiments were run to reproduce the results reported in Figure 1 of An [2004]. These were run on multiple computers to reduce the total elapsed time. The total elapsed computer time was nearly 30 hours. The computers used included three laptops with processor speeds varying from .4 to 1.2 GHz. The results clearly showed the Initial Injury Number (IIN) values that demark the lower and upper boundaries of the "region of interest" (ROI) described by An [2004].

The next set of experiments required many more runs than the first set, so it was necessary to further optimize the model logic. The primary changes were: 1) reducing small values of biochemical agents to zero in order to lessen the number of calculations required in the diffusion process, 2) moving the "divide OXY by 100" from inside the SUM operation to outside the SUM, and 3) calculating the SQRT function used in the injure-sterile and injure-infection procedures one time instead of calculating this function IIN times for each iteration. These changes speeded up model execution by a more than a factor of two, dropping the computer time required to run the new experiments from 70 to 30 hours.

Procedures were also added to facilitate experiments that incrementally removed the effect of individual model components and combinations of components. These experiments test the impact of removing T-cells and T-cell germinators from the model. First, the five initial values associated with T-cells were set to zero, one at a time. Next, selected combinations of two or three initial values were set to zero simultaneously. Finally, all five initial values were set to zero. For each parameter set, 10 cases were simulated, for each of two different values of IIN. The IIN values were chosen to reflect the lower and upper bound of the ROI where the uncertainty in outcome is the highest.

The data collection procedures provided within An's original model were not enhanced, leading to a degree of experimental inefficiency. This was rectified later, as described below.

2.2 Phase II

In preparation for adding SD/DE logic to the ABS model, the DE model published by Clermont et al [2004] was reviewed. This mechanistic model of an acute inflammatory process was a systematic series of differential equations one for each inflammatory component chosen for simulation. Each equation describes the state level or concentration of components and, based on the principle of mass-action, the interactions of these components. Components were selected for their accepted correlation to clinical outcomes. Rate constants were extracted from the pertinent literature or empirically determined to simulate reported data.

An attempt was made to independently reproduce their results by implementing the equations and parameters provided in the appendix of the paper. We entered the 18 differential equations and more than 80 constants into Matlab [2005]. A few minor typographical errors in the published paper were corrected as seemed most reasonable, and assumptions were made regarding omitted values. We attempted to perform experiments with this model by specifying values for the 11 floating parameters and using Matlab's DE solver to solve the differential equations. However, despite interaction with Clermont and his team we were never able to reproduce the fundamental results. Since this was not the primary thrust of our research we did not persist in this endeavor, however, a significant benefit of this activity was increasing our understanding about representing the target system's interactions in equation form.

2.3 Phase III

A hybrid model was created using the System Dynamics tool within Netlogo. We use the term "edge" for the interface and boundary between the ABS and SD submodels. The determination of the "edge" can be made on a series of system characteristics: structural, behavioral, hierarchical, etc. To determine the "edge" in this case we focused on those aspects of the biological

system that fell into either a *local* process or a *systemic* process. Since the base ABS focused on the expansion of an initial localized infection towards systemic effects, the area of initial insult and subsequent interactions at this point were left to the ABS. However, certain aspects of the biological response, primarily related to the production and life-cycle of circulating inflammatory cells, occur "off-screen" from the ABS, and it was felt that these dynamics could be better modeled using a SD/DE methodology. Accordingly, we implemented a simple DE submodel that focused on systemic polymorphoneutrophils (PMN) production, maturation, sequestration, and release. This submodel had three state variables: PMNs_mature_marrow, PMNs_circ, and PMNs_sequestered. Many of the flows between these compartments were modeled strictly within the SD submodel, but others were influenced by the conditions within the ABS submodel. Figure 1 shows the flow diagram for the SD submodel. Additional integration between the SD and ABS submodels included modifications to the logic in the ABS submodel regarding the creation of PMNs. In the purely ABS version of the model, the logic was to randomly generate PMNs at a rate the corresponded to the need. In the hybrid model, the "availability" of PMNs (modeled in the DE submodel) was used to modulate the PMN creation rate with the ABS submodel. In the SD submodel, the maturation of PMNs was influenced by total granulocyte colony stimulating factor (GCSF) from the ABS model. The primary role of the SD model was to manifest a delay between the elevation of the cytokines and the increase in PMNs. The experiments described previously were then rerun using the hybrid model. Further details of this model may be found in Wakeland et al [2006].

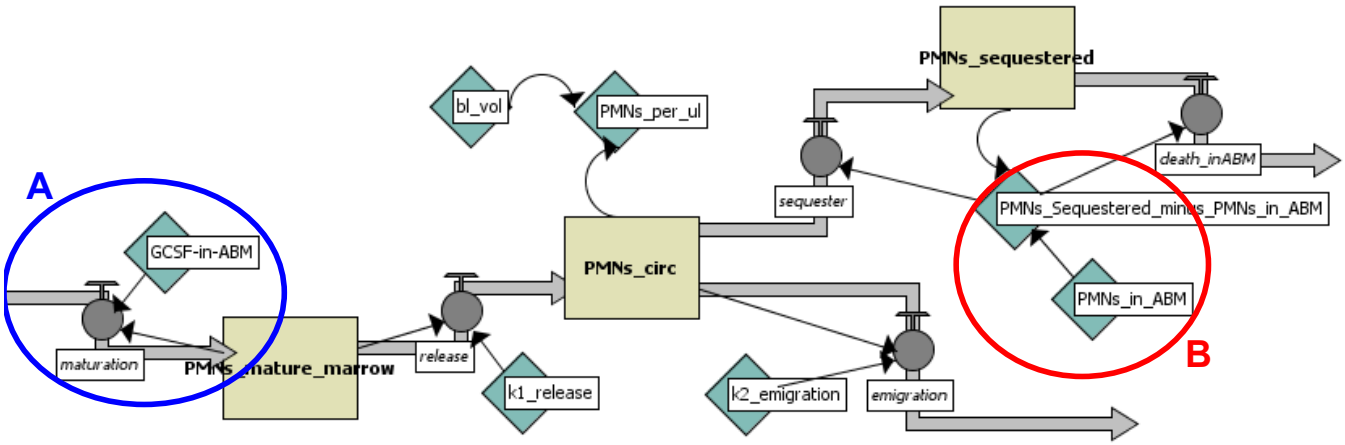

Figure 1: Flow diagram of the system dynamics submodel, showing the compartments and their interconnections. Inflammatory responsive cells, in this case polymorphic neutrophils (PMNs), are produced and mature in the bone marrow (PMNs_mature_marrow). These are released into general circulation (PMNs_circ) at a basal rate, which is responsive to chemical signals produced elsewhere in the body. Circle A highlights the location of feedback *from* the inflammatory process represented by the agent-based model. PMNs are sequestered (PMNs_sequestered) from circulation by local chemical signals and attractants produced locally by the inflammatory process. Circle B highlights the location of feedback (the transition of circulating PMNs into inflammatory tissue) *to* the agent-based model. Emigration of PMNs is the "normal" homeostatic loss of PMNs to other tissues.

3. RESULTS

3.1 Phase I Results

Figure 2 shows the initial experimental results using the ABS model. The x-axis indicates the initial injury number IIN, from 0 to 1000. For each value of IIN two points are shown, the end infection (EIN) and the end oxygen deficit (EOD), where end refers to the end of the simulation run (considered to be one week after the initial injury/infection in our case vs. four weeks used by An). Each point represents the result from one of the 10 simulated cases. The mean values for each of these 10 cases is also shown, in a larger font, with line segments connecting these points. When IIN is small, both the mean EOD and mean EIN are also small, indicating a favorable prognosis. When IIN is very large, both EOD and EIN are large, indicating an almost certain unfavorable outcome. An [2004] describes the center region of Figure 2 as the "region of interest," where EIN is small, but EOD remains large. In this region, the inflammatory response has ameliorated the initial infection, but the "collateral damage" to otherwise normal tissue prevents recovery.

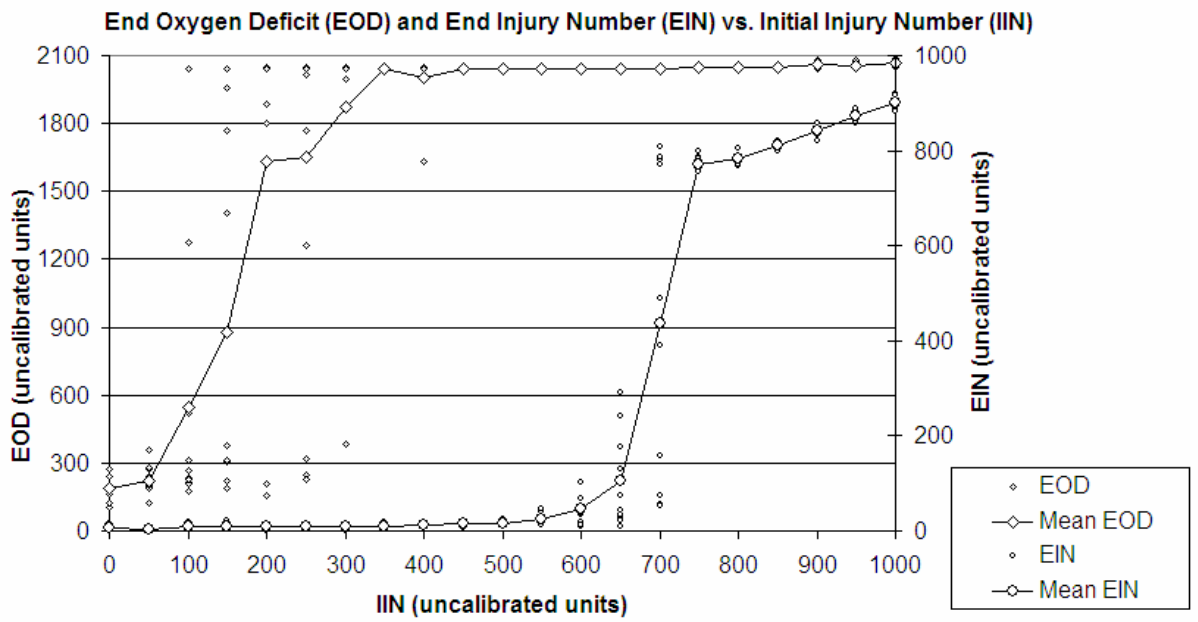

Figure 2: Reproduction of the "Region of Interest" (An 04). The left hand curve shows the mean value of end oxygen deficit for each given value of Initial Injury (IIN). Each mean value is calculated from 10 cases (runs) for each particular value of IIN. The individual values for each case are also shown as a scatter-plot. The right hand curve is similar, but shows the end injury number (EIN) for the corresponding IIN values. The region of interest is between the two curves.

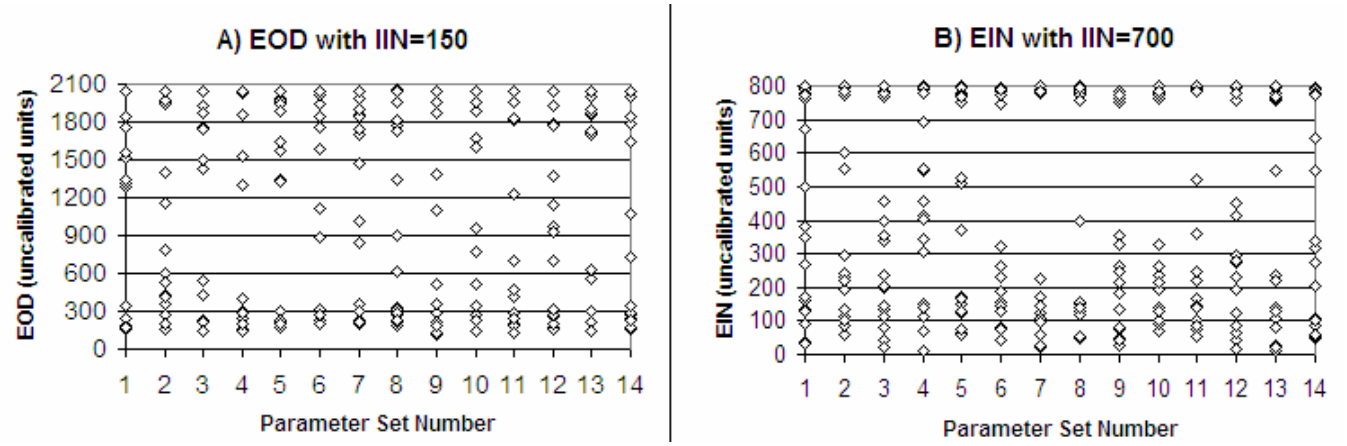

Figure 3. Graph A shows the end oxygen deficit (EOD) and graph B shows the end injury number (EIN) for each of 14 different sets of parameter values. 10 simulated cases are shown for each parameter set and for each of the two values for initial injury (IIN).

Figure 3 shows the results of the second set of experiments focusing on the effects of the T-cell components. Parameters associated with T-cells were varied significantly, essentially "turning off" their pro-inflammatory and/or anti-inflammatory effects. Ten cases were run for each set of parameter values. Figure 3A shows EOD when IIN = 150, near the left hand boundary of the region of interest (see Figure 2).

Figure 3B shows EIN when IIN = 700, which is near the right hand boundary of the region of interest. One might expect that the pattern of outcomes in terms of EOD and EIN would be correlated to some degree with which set of parameters was used. However, as can be seen in Figure 3 the overall impact of these particular processes is essentially "lost in the noise," since the variation in the results for a given set of parameter values is much larger than the variation between different sets of parameter values. When IIN = 150, EIN is near the minimum in all cases, and when IIN = 700, EOD is at the maximum in all cases; hence graphical results for these experiments are not shown.

Statistical tests of the data shown in Figure 3 indicated that the hypothesis that these samples were all drawn from the same population cannot be rejected ($p<.05$). Therefore, the removal of the T-cell effects did not appear to have a statistically significant effect.

A subjective result from Phase I was the sense that the ABS model may be unnecessarily complex. The fact that it takes many hours or even days to run a full set of experiments limits the practical utility of such a model, an acknowledged drawback of ABS. The high degree of complexity also makes it difficult to assure that the logic matches the modeler's intent, much less reality. However, a major strength of the ABS model is its transparency—it shows exactly how the researcher believes that the various mechanisms actually work. We will address the implications of the balance between these two issues in the discussion.

3.2 Phase II Results

No objective results were produced in Phase II. However, the experience demonstrated the difficulty in utilizing prior published papers and models, particularly in systems with very complex dynamics. The complexity of the resulting models represents a significant barrier for other researchers to overcome in order to replicate, and, ideally, extend published results.

3.3 Phase III Results

Testing the hybrid model prompted a sequence of modifications in the hybrid logic, and revealed problems with the model scaling that was done in Phase I. This was corrected, and the experiments were rerun. Figure 4 shows a recreation of Figure 2 using the hybrid model results. The mean values from the ABS model (Figure 2) are also shown for comparison. The results are very similar, except that slope of the EOD graph is steeper for the hybrid model.

The hybrid model was then used to rerun the 14 parameter set experiment from Phase I. The results are shown in Figure 5. Only one graph is shown, EIN with IIN=700, for comparison to Graph B from Figure 3. In Figure 5, most of the points are located at the extreme values for EIN, whereas in Figure 3, Graph B, this is not the case. The results for EOD are not shown in Figure 5 because they do not differ appreciably from Figure 3, Graph A.

4. DISCUSSION

This study demonstrated that the use of agent based simulation modeling, system dynamics modeling, and differential equation-based modeling is not only complementary, as had been suggested by others, but can in fact be unified into a single hybrid model, with the benefit of able to optimize the balance between the strengths and weaknesses of each method.

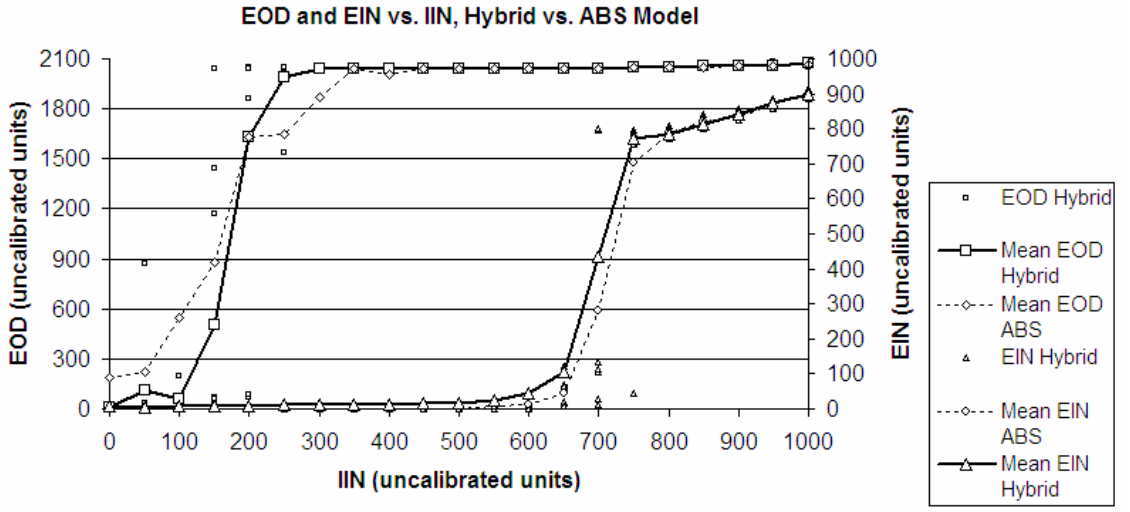

Figure 4. Comparison of hybrid model results (Region of Interest) with ABS model results from Phase I. The solid line with hollow squares indicates the mean ending oxygen deficit (EOD) vs. initial injury number (IIN). The smaller hollow squares show the results from each of ten runs from which the mean was computed. The solid line with diamonds shows the mean ending injury number (EIN) vs. IIN, with the smaller diamonds showing the results for individual runs. The dashed lines show the mean values from the ABS model runs for comparison (taken directly from Figure 2).

.

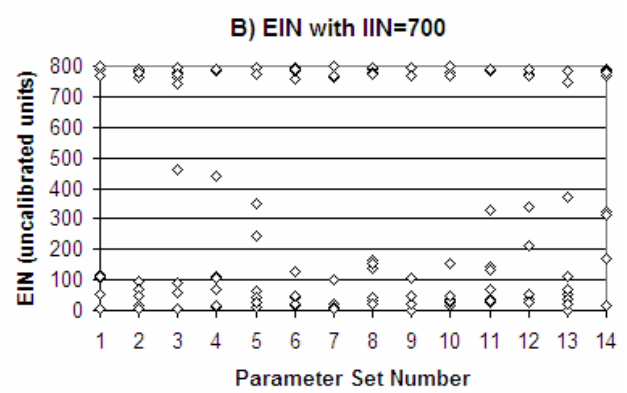

Figure 5. Results of running the 14 parameter sets experiment using the hybrid model. The results are shown only for EIN with IIN=700 because they differ appreciably from Figure 3, whereas the results using the hybrid model for EOD with IIN=150 look exactly the same.

Phase I results indicate that some aspects of the original ABS model may not impact the primary behavioral responses to the degree seen clinically. Specifically, the lack of effect of completely removing T-cells suggests that further work is needed to verify that the logic correctly captures the phenomena to an appropriate degree of approximation.

Phase II results underscore how difficult it is for biomedical researchers to replicate and on the work of others, even with full disclosure of all aspects of the prior research and full cooperation of the original research team.

Phase III results show that differential equations, specified using system dynamics (SD) notation, can be easily added to an ABS model implemented in Netlogo, and that the resulting submodel can be integrated easily into the ABS logic and vice versa. The behavior of the AIR model with a simple SD submodel replacing some of the agent-based logic was nearly identical to the behavior of the pure ABS model. The region of interest (Figure 2) was modified only slightly (Figure 4) in that the slope of the EOD as a function of the initial injury becomes steeper. One possible explanation for this result is that since the SD submodel is deterministic, and it replaced a portion of the ABS model that was stochastic, the overall uncertainty of the response became smaller, and thus the transition from recovery to non-recovery as a function of initial injury occurred more rapidly.

Challenges in the present study included the high degree of complexity in both the ABS model and the published DE model. This complexity made it very difficult to achieve a high degree of confidence that the model logic/equations/parameters were correctly implemented. The ABS and DE models provided by the original researchers both contained errors or other types of discrepancies, from missing parentheses to equations with entirely different terms. It was necessary to correct these inconsistencies before the primary research activities could commence. The high degree of complexity also dramatically increased the time required to run experiments with the ABS model and the hybrid model.

One conclusion we reached is that researchers should strive to find ways to reduce the complexity of their models to the irreducible minimum. The present research suggests that both the ABS model and the hybrid model contain non-essential components. The problem with this is not simply that the models contain logic or variables that are superfluous and can be ignored. Rather, these additional components may actually be a liability because they obscure important relationships and make it much more difficult for other researchers to confirm and/or extend the research.

There is, however, also recognition of the potential pitfalls in the selection of modeling ontologies if "efficiency" and "simplicity" are the overarching modeling goals, particularly in systems as complex as the inflammatory response. One of the benefits of using models to study the AIR is the ability of mathematical/simulation models to function as tools for knowledge representation and integration. A major difficulty in current attempts to characterize the AIR is the sheer volume of mechanistic data present; it is virtually impossible to determine which aspects of the system are "critical" and which areas are not. In fact, it is most likely that it is combinations and clusters of cells and mediators that are the true determinants of system behavior, rather than individual mediators or cell types. Therefore, at least initially models of the AIR need to be quite inclusive and comprehensive, even at the initial expense of efficiency and simplicity. However, through reductive processes as demonstrated in this paper, some degree of determination of "criticality" may be accomplished by manipulation of the *in silico* mirror of the reference system, thus leading to insights with respect to the reference system that may have implications for more traditional laboratory research by identifying potential targets for manipulation.

Our results suggest that caution should be exercised in the interpretation of the behavior of these models with respect to clinical practice. The parameter changes tested using the ABS model could have easily represented the potential impact of a pharmacological intervention. However, the research also demonstrated that the impact of other variations appear to almost entirely mask the potential impact of this type of targeted intervention. While the idea of *in silico* clinical trials is very intriguing, much more research is called for. Parameter testing of the type we have shown should be a necessary component in the analysis of these models, not only to delineate the

"true" effect of an intervention, but to also facilitate the criticality determination of the system's components as described above. Given these cautions, it should be emphasized that the use of these mathematical modeling techniques are not intended as replacements for standard methods of biomedical research, but rather are intended to be adjuncts to the general research process.

The present study was exploratory in nature, and focused on a single ABS model and its extension to a hybrid version. Consequently, these findings are only suggestive in nature; further examination of multiple examples from multiple modeling disciplines is warranted. The concept of "articulated" models involving multiple, interchangeable components has been raised by Ropella et al [2005] as a means of advancing model design and use. In this manuscript we use the term "edge" to refer to this articulating interface, "edge" as opposed to "point" insomuch the interface is multi-dimensional, "edge" as opposed to "surface" as "edge" connotes, simultaneously, both a boundary and a transition. We believe that the general application of modeling to biomedical research will be greatly enhanced by the development of hybrid models that will utilize the respective advantages of different modeling methods.

Specific opportunities for further research suggested by the present research include: 1) creating a simplified version of the model that retains its behavioral richness, 2) the combination of different ABS submodels representing specific organs linked together within an SD "wrapper" to simulate organ-organ crosstalk and total body behaviors, 3) the identification and investigation of different "edges" of ABS and SD/DE interfaces that would best utilize the respective strengths of each method, and 4) the development of a means of dynamically "shifting" the edge between the ABS and the SD/DE submodels with the intent of improving computational efficiency in large scale models.

5. REFERENCES

An, Gary (2001) "Agent-based computer simulation and SIRS: building a bridge between basic science and clinical trials" *Shock 16*(4):266-273.

An, Gary (2004) "*In silico* experiments of existing and hypothetical Cytokine-directed clinical trials using agent based modeling" *Crit Care Med 32*(10):2050-2060.

Buchman TG (1997) "Awash in data: the problem of predicting physiology in severe sepsis" *Shock 8*(3):232.

Buchman TG, Cobb JP, Lapedes AS, Kepler TB (2001) "Complex systems analysis: a tool for shock research" *Shock 16*(4):248-51.

Clermont, Gilles, John Bartels, Rukmini Kumar, Greg Constantine, Yoram Vodovotz, Carson Chow (2004) "*In silico* design of clinical trials: A method coming of age" *Crit Care Med 32*(10):2061-2070.

Dancey JT, Deubelbeiss KA, Harker LA, Finch CA (1976) "Neutrophil kinetics in man" *J. Clin. Invest*. 58: 705-715.

Kumar R, Clermont G, Vodovotz Y, Chow CC (2004) "The dynamics of acute inflammation" *J Th. Bio 230*:145-155.

Mantovani, A., C. A. Dinarello, et al. (2000). *Pharmacology of Cytokines*. Oxford, Oxford University Press

Marshall, John C (2004) "Through the glass darkly: The brave new world of *in silico* modeling" *Crit Care Med 32*(10):2157-2158.

Matlab (2005) see The Mathworks http://www.mathworks.com/ (software verion 7)

Netlogo (2005) see Northwestern University http://ccl.northwestern.edu/netlogo/ (software version 2.1)

Neugebauer EA, Willy C, Sauerland S (2001) "Complexity and non-linearity in shock research: reductionism or synthesis?" *Shock 16*(4):252-8.

Ropella, GEP, Hunt, CA, Sheikh-Bahaei, S (2005) Methodological Considerations of Heuristic Modeling of Biological Systems. Proceedings of The 9th World Multi-Conference on Systemics, Cybernetics and Informatics, July 10-13, 2005 - Orlando, Florida; available at http://biosystems.ucsf.edu/Researc/RecentPapers.html

Suwa T, Hogg JC, English D, Van Eeden SF (2000) "Interleukin-6 induces demargination of intravascular neutophils and shortens their transit in marrow" *A J P Heart Circ Physiol 279*:H2954-H2960.

Vodovotz, Yoram, Gilles Clermont, Carson Chow, Gary An (2004) "Mathematical models of the acute inflammatory response," *Current Opinion in Critical Care 10*:383-390.

Vodovotz Y, Clermont G, Hunt CA, Lefering R, Bartels J, Seydel R, Hotchkiss J, Ta'asan S, Neugebauer EA and An G. Evidence-based Modeling of Critical Illness: An Initial Consensus from the Society of Complexity in Acute Illness. *Journal of Critical Care,* in press.

Wakeland W, Macovsky L, Gallaher E, Aktipis A (2004) "A Comparison of System Dynamics and Agent-based Simulation Applied to the Study of Cellular Receptor Dynamics," Proc. of the 37th Ann. Conf. of the Hawaii Int'l Complex System Society, pp 1381-1390.

Wakeland W, Macovsky L, An G (2006) Details of SD submodel in hybrid model of Airs, http://www.sysc.pdx.edu/faculty/Wakeland/papers/HybridAIRSmodel.pdf