Synergy of local, regional, and systemic non-specific stressors for host defense against pathogens

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HIGHLIGHTS

- An agent-based model validates and expands a conceptual model in which hosts can use non-specific stress for defense at local, regional, and systemic levels to preferentially harm pathogens.
- For pathogens to spread during an active infection, replication is necessary; but the replication process diverts resources for expansion that could otherwise be used for protection against stress.
- In our model, while localized non-specific stress has little efficacy as a host defense when used alone, it has strong synergy in combination with regional and systemic stressors.
- Systemic stress is particularly costly and risky as a defense since host cells throughout the body are harmed while the host attempts to kill localized pathogens.
- Based on the modeling results, host-induced non-specific stressors can provide a formidable defense in fighting pathogens, despite the detrimental effects on the host.

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ABSTRACT

The immune brinksmanship conceptual model postulates that many of the non-specific stressful components of the acute-phase response (e.g. fever, loss of appetite, iron and zinc sequestration) are host-derived systemic stressors used with the “hope” that pathogens will be relatively more vulnerable to non-specific systemic stress than the host and its cells. However, the conceptual model acknowledges the risk to the host in that the gamble to induce systemic self-harming stress to harm pathogens may not pay off in the end. We developed an agent-based model of a simplified host having a local infection to evaluate the utility of non-specific stress, harming host and pathogen alike, for host defense. With our model, we explore the benefits and risks of self-harming strategies and confirm the immune brinksmanship concept of the potential of systemic stressors to be an effective but costly host defense. Further, we extend the concept by including in our model the effects of local and regional non-specific stressors at sites of infection as additional defenses. These include the locally hostile inflammatory environment and the stress of reduced perfusion in the infected region due to coagulation and vascular leakage. In our model, we found that completely non-specific stressors at the local, regional, and systemic levels can act synergistically in host defense.

1. Introduction

While an ideal host defense should be targeted directly at pathogens and have no collateral host damage, infections are frequently accompanied by host-induced systemic effects that are costly and potentially harmful such as fever, anorexia, iron and zinc restriction, and low-grade anemia. These and other harmful components of the acute-phase response have been viewed as non-specific stressors actively used by the host to harm pathogens despite the obvious risk to self (LeGrand and Alcock, 2012). In this “immune brinksmanship” conceptual model, the host is seen as a risk taker, acting to suppress pathogens with the “hope” that pathogens will be relatively more vulnerable to harsh, generic stress than its own cells will be. This risky approach seems reasonable, considering that for a pathogen to successfully invade...
its host, it must grow and replicate, life history processes that are particularly vulnerable to stress. Additionally, pathogens are subjected to the infected site's localized specific (targeted) stresses coming from attacking inflammatory cells as well as non-specific (untargeted) stresses that are generally considered by-products of the "battlefield". These include low concentrations of nutrients (e.g., glucose, glutamine, iron, zinc) and oxygen along with high concentrations of lactic acid and reactive oxygen and nitrogen intermediates. The implications of this conceptual model, that non-specific stress can be an effective host defense, warrant further investigation.

In this work, we refer to stress as the deviation from homeostasis, which is harmful because of either direct damage or the cost of undergoing a protective stress response. The stress response can involve either the direct costs of providing protection or the lost opportunity costs, notably having to delay growth and replication while undergoing quiescence. Failure to respond appropriately to stress carries risks of the direct harm done by the stress. We define stressors as agents that cause harm by any means. Specific or targeted stressors would include those used to cause harm to pathogens while not incurring any collateral host damage (e.g., antibiotics killing bacteria while not harming host cells, based on molecular specificity). Non-specific stressors would harm all agents equally (e.g., antiseptics killing bacteria and host cells indiscriminately).

Previous mathematical and computational models have explored the acute-phase response and include inflammatory mediators that function to clear pathogens (e.g., macrophages, neutrophils, TNF-α, IL-1) but cause host tissue damage/dysfunction even while also producing self-regulatory anti-inflammatory mediators (e.g. IL-10, TGF-β) (An, 2008; Bauer et al., 2009; Vodovotz et al., 2009). Some of these models include more detailed information compared with those that present a more abstract view of the process; but, in either case, these models have elucidated many important dynamic features and driving forces in the inflammatory response to pathogens, leading to various states of disease outcome and resolution. These models are based on “first principles” of known biological processes of the mediators accepted to be most involved in the process. In addition, they emphasize the importance of considering collateral injury to the host. If the response is not properly resolved, either with self-regulatory mechanisms or via therapeutic intervention, the host experiences excessive tissue damage leading to organ failure and death. Most of the models include a variable or tracked quantity that represents tissue damage/dysfunction caused by the inflammatory mechanisms intended to protect the host.

While there is a notion of stress and stressor-induced effects inherent to these previous models, they do not directly investigate the relationship between varying levels and types of stress and the characteristics of cells (pathogen and host alike) needed to function successfully under stress. Therefore, we explore this relationship further as well as expand the view of the previous models which primarily see the immune cascade from the perspective of a progression of local stressor events leading to systemic events. In fact, we were initially interested in whether or not systemic non-specific stressors used as an only defense against a variety of pathogens have any efficacy. In order to explore this, we used the NetLogo simulation platform (Wilensky, 1999) to develop an agent-based model of an intentionally simple host being infected by a locally invasive pathogen. Although the model was initially created to check the general feasibility of the immune bricksman-conceptual model as it relates to systemic stressors, our preliminary studies led us to extend the conceptual model to cover the utility of non-specific stressors for host defense in general. As such, we examine non-specific stressors acting locally and regionally, in addition to systemically, either alone or in concert with one another to ward off an invading pathogen. In this way, we can evaluate the effectiveness of stressors purposely acting at specific levels and not just as a byproduct of positive feedback from the local level.

The effect of each stressor is directed toward cutting off resources needed by the pathogens for survival irrespective of the overlap with the host cells’ own resource needs. While there does appear to be an apparent vulnerability of pathogens to stress because of their need to replicate for invasion, the trade-off for the host in using self-harming defenses may be too great. Therefore, we used our model to investigate the circumstances and cost for a host to use such stressors to fight off an invading pathogen and survive. Similarly, we explore when the pathogen is able to cope and succeed despite the host’s defenses. In addition, although these non-specific stressors are still seen during the acute-phase response, the apparent simplistic nature of their mechanism implies they may be merely remnants of a more fully mature and adapted immune response that now primarily makes use of specialized immune cells. Thus, since these non-specific stressors are still present, can they play a supportive role in host defense (and, if so, how?) or do these stressors generally cause more detrimental consequences to the host? We use our model to suggest answers to these questions as well as generate new questions and insights as related to the role of non-specific stressors in host defense. Lastly, as in most every modeling exercise, the model development process itself creates new questions for consideration, affecting the modeling cycle and generating new results and insights.

2. Materials and methods

Agent-based models consist of rules that define how various entities, called agents, interact within a specified environment over a given time period (Bauer et al., 2009; Bonabeau, 2002; Railsback and Grimm, 2012). Thus, they are also well known as individual-based models since the behavior of an individual can be observed rather than just the behavior of the population as a whole. In addition, spatial aspects of the environment can also be considered and, since interactions can occur at the local level, the need to assume a well-mixed system is not necessary. Therefore, the effects of stochasticity inherent to all biological processes can easily be considered. The freeware NetLogo (Wilensky, 1999) software provides ready accessibility for the non-mathematically trained individual to use agent-based modeling to explore theoretical hypotheses and generate new ones. One can simulate a large number of trials which generate data sets that contain information about how the rule settings affect outcome or other relevant features of the agent interactions. This numerical data can then be processed and mined to find interesting features and connections between settings and outcome.

The NetLogo computer interface consists of a number of adjustable settings, a view of the model world, and a number of monitors and instantaneous plots (Fig. 1). The definition of the host and the parameters associated with it as well as with the pathogens were chosen to create a biologically feasible infection in a primitive multicellular organism within the confines of the computer screen. It should be noted that some specific values for this highly generic model were arbitrarily chosen, but once a few were chosen, subsequent choices for other values became increasingly constrained. The model world representing the host is a 33×33 grid of patches (i.e. extracellular space) upon which initially sit the host cells (1057 regular host cells as gray circles and 22 key host cells as orange flags) and 10 pathogens (red triangles) (Fig. 1). The 1089 patches constituting the host was chosen to allow a large enough area to see interesting activity but
small enough to be computationally and visibly tractable (e.g. to be able to read the energy values of each cell on the screen). The 22 key host cells represent an essential organ, like the heart or brain, giving the host a vulnerable component to its makeup, though isolated from the surface of the host where infections are initiated. With this established, we then accordingly determined criteria for host survival. It was considered reasonable that a host should require about 2/3 of its (regular) cells to survive and could lose only very few of its key host cells; so 700 regular cells and at least 20 key host cells are required for host survival. If either of these cell types dips below the given threshold, then the host dies.

In this model, we define energy as a single commodity that embodies all the necessary resources upon which host cells and pathogens depend to survive. For each host cell, the required amount of energy needed to survive (“survival energy”) was set at 40 units/cell. The survival energy setting for pathogens is adjustable, from 40 units/cell down to 10 units/cell. Values less than 40 increase the virulence characteristic of pathogens since it requires less energy to survive compared to host cells. At the beginning of every simulation, host cells and pathogens each start out at their maximal amount of energy: 300 units/cell for host cells and, for pathogens, 260 + “survival energy” units/cell. The value chosen for the maximal amount of energy for host cells (300) is arbitrary; but, based on this choice, a host cell’s survival energy was set at 40 units/cell (just over 10% of 300) to capture a cell’s inability to function once its energy reserves are sufficiently depleted from baseline. Pathogens, on the other hand, can have a lower survival energy, as mentioned, and therefore, have the potential to utilize this as a strategy to do better than host cells in unfavorable conditions. Since key host cells, representing an essential vulnerable organ, cannot replicate, we compensate for their irreplaceability by allowing them to survive at a 25% lower energy level than the regular host cells (30 vs. 40 units).

In the model rules, we also account for the normal energy expenditure of cells in maintaining basic functions, as well as the ability of cells to acquire energy from the surrounding environment. Both functions occur during each round of computation, or tick. Host cells and pathogens both derive energy from the patch on which each sits at approximately 14 units/cell of energy per tick and expend approximately 10 units/cell of energy per tick. These values were chosen since they fit reasonably with cells’ overall energy in having most of the simulations resolve in a reasonable timeframe (50 to 300 ticks). Randomness in the model is present due to a slight randomness factor whenever energy is gained or lost.

Energy is also the currency needed for replication in our model. The replication energy required of a host cell (180 units) is set relative to the value used for the maximal energy of host cells (300 units/cell) and is set to be slightly larger than half of this amount. The replication energy required for a pathogen (120 units) is set less than the amount required of host cells (180 units/cell) to allow pathogens the ability to replicate sooner. An empty patch adjacent to a replicating agent is also required in order for replication to occur. As the simplest and most fundamental implementation of a replication rule, the available energy of a cell (host or pathogen) is divided evenly between the two resulting daughter cells whenever the criteria for replication are met.

Pathogens have the ability to replicate more rapidly than host cells since (1) the pathogens require less energy to replicate (as discussed above), and (2) pathogens can gain energy from encountering host cells as part of their virulence (to be discussed). The trade-off, however, is that their progeny are closer to the 40 unit threshold for death (i.e. survival energy setting) since they would have only approximately 60 units of energy, while host cell progeny would have 90 units of energy. When the survival energy value for pathogens is set lower (e.g. 10 units/cell), then the energy level of pathogen progeny is comparable to that of host cell progeny relative to the survival energy of 40 units/host cell, i.e. each has a safety margin of 50 units of energy. We also explore a variety of values for the required energy of pathogens to replicate, as will be discussed in the results.

Virulence of the pathogens is based on four adjustable factors, while the host has a variety of basic adjustable defenses based on
energy deprivation (Tables 1 and 2). Two of the pathogen virulence factors are (1) the ability to remove a portion of energy from host cells with which they are in direct contact, thereby harming these host cells (“harm host”) and (2) the ability to gain energy proportional to the number of host cells with which they are in direct contact (“gain energy”), with a slight randomness factor. Although these two processes might be expected biologically to go hand-in-hand, we chose to model them separately to determine if one or the other had greater impact on overall outcome in the various scenarios explored. Values for the portion of energy removed (“harm host”) and gained (“gain energy”) were varied over the ranges given in Table 1, and the specific values tested in combination with other defenses can best be categorized as low, medium, and high relative to the energy currency.

As mentioned previously, the amount of energy needed for a pathogen to stay alive (“survival energy”) can vary between a high value (e.g. 40) corresponding to a less virulent pathogen and a low value (e.g. 10) corresponding to a more virulent pathogen. Through the pathogen mobility setting, pathogens can be equipped with the ability to wander around the grid if they are adjacent to a vacant patch (On) or stay put in their initial location (Off). Thus, a combination of varying levels of the first three virulence factors, along with the choice of On or Off for the mobility setting, can lead to a variety of pathogen virulence settings. The values shown in Table 1 were chosen to create 36 different pathogen types for our host to encounter. Among these 36 types, 9 were chosen for additional tests. Throughout the text, combinations of these settings are given in groupings such as [5, 5, 40, stay] which indicate the settings chosen for the factors listed in the order of Table 1.

We then defined and tested various host defenses, alone or in concert with each other, against the various pathogen virulence types. There is one host defense strategy (local specific stressor) that acts at the local level and specifically targets only pathogens, without doing harm to host cells. The other local strategy (local non-specific stressor) harms the pathogen and surrounding host cells equally. Two regional stressors are also considered (tourniquet/amputation and clotting), as well as a systemic strategy (systemic stressor) that was automated. Table 2 describes how we defined the mechanisms of the host defenses that were considered. The test values listed in Table 2 that were used when exploring combinations of the defenses were chosen based on efficacy of each defense alone against a cohort of pathogens. As will be seen in the Results section, the values of 0, 2, and 10 for the specific and non-specific stressors represent no or very low efficacy when used alone; the values of 0, 20, 35, and 50 for the regional clotting stressor represent no (0 and 20) to moderately (35 and 50) effective. The regional tourniquet/amputation stressor was not used in combination with the others. An intensity setting of 60 (i.e. all cells/pathogens gain 60% less energy at each time tick than usual) was decided on for the Off setting of the systemic stressor since it showed low efficacy against 36 pathogen types on its own. These defense strategies are further discussed in the Results section.

Of the host defenses and pathogen offenses, only the systemic host stressor was intended to be manipulated during the course of a simulated infection, such as when a host voluntarily modifies its systemic stress by altering its feeding or behaviorally modifying its temperature. When manually adjusting the slider for the systemic stressor during test simulations, it was readily apparent that the concept worked (it can indeed be a useful defense), but that proper timing and intensity was essential to get the ideal balance between pathogen overgrowth and host death due to excessive stress. Thus, we automated the timing of the systemic stressor to

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**Table 1**

<table>
<thead>
<tr>
<th>Pathogen virulence effect/characteristic</th>
<th>NetLogo model name</th>
<th>Description</th>
<th>Values tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harm host</td>
<td>Pathogen-harm-host</td>
<td>Removes $x$ units of energy from each host cell in contact with a pathogen</td>
<td>5, 20, 50</td>
</tr>
<tr>
<td>Gain energy</td>
<td>Pathogen-take-up-energy</td>
<td>A pathogen gains $x$ units of energy for every host cell with which it is in contact (host cells unharmed)</td>
<td>5, 20, 50</td>
</tr>
<tr>
<td>Survival energy</td>
<td>Pathogen-survive-low energy</td>
<td>Sets the survival energy threshold (values below 40 increase virulence)</td>
<td>40, 50</td>
</tr>
<tr>
<td>Mobility</td>
<td>Pathogen-wander?</td>
<td>If On, move randomly to a vacant patch (without costing energy)</td>
<td>On (wander), Off (stay)</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Host defense strategy</th>
<th>NetLogo model name</th>
<th>Description</th>
<th>Tested values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local specific stressor</td>
<td>Local-host-directed</td>
<td>Removes $x$ units of energy from each pathogen in contact with a host cell (host cell not harmed)</td>
<td>0–50 when used alone; 0, 2, 10 when combined with other defenses</td>
</tr>
<tr>
<td>Local non-specific stressor</td>
<td>Local-host-stressor</td>
<td>Removes $x$ units of energy from each cell (host cell or pathogen) that is adjacent to a host cell that is in contact with a pathogen; i.e. a host cell in contact with a pathogen blindly harms every cell around itself, up to 8 potential targets.</td>
<td>0–50 when used alone; 0, 2, 10 when combined with other defenses</td>
</tr>
<tr>
<td>Tourniquet/amputation (a regional stressor)</td>
<td>Regional amputation</td>
<td>Reduces energy of all cells in the lower right region of the host by $x$ % per tick</td>
<td>0, 30, 100 (not used with other defenses)</td>
</tr>
<tr>
<td>Clotting (a regional stressor)</td>
<td>%-reduced-clot-patch-energy (auto-) Systemic-host-stressor</td>
<td>Reduces $x$ % of the energy gained at each tick to all cells within 2 cell widths of a host cell in contact with a pathogen (shown as olive-green patches)</td>
<td>0, 20, 35, 50 when used alone or combined with other defenses</td>
</tr>
<tr>
<td>Systemic stressor</td>
<td>Reduces $x$ % of the energy gained at each tick to all cells within the host. When set to “auto,” it automatically sets the systemic stress to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>${95$ cells $&lt; 95$ regular host cells $&lt; 1030$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Off otherwise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: With this definition, once the host cell # gets down to 925, the stressor switches Off and then On again when above 925.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
come on (at a fixed intensity of 60) and then off again, basing the
design of the automation process on thresholds of host cell
numbers: On when the host was down to 1030 regular host cells
and Off when the host had 925 or less regular host cells remaining,
followed by On again when the host recovered more than
925 cells. The initial On threshold of 1030 host cells (down from
an initial 1057 host cells and corresponding to about 30 pathogens,
up from an initial 10 pathogens) was considered a reasonable
compromise between causing unnecessary self-harm and waiting
too long to fight a growing infection.

In the process of establishing the thresholds for the systemic
stressor, it became clear that there was a trade-off between having
the Off threshold too high or too low. If too high, the host did not
utilize its available energy for defense. In other words, the host
would die from pathogen overgrowth yet still have plenty of
stored resources (energy) that could have been utilized for
defense. If the Off threshold were set too low (i.e. too close to
the 700 host cells needed for host survival), the host would
frequently kill itself in trying to kill the pathogens. In the model,
due to the ordering of events, the feedback to the host regarding
available host cells is slightly delayed, as would be in real life.
Because of this brief lag time in communication during which
systemic stress is still ongoing (in addition to ongoing pathogen-
induced harm), there was a clear need for a margin of safety with
respect to the 700 host cells needed for survival. The balance of
this trade-off in terms of setting the threshold to 925 was
determined through simulations with these various settings. In
real life, such a threshold would be determined by natural
selection. It is notable that the host was never able to recover to
1030 host cells while pathogens were still present, since host cells
were still being killed by pathogens. So, 1030 was no longer a
reasonable On threshold once the infection had progressed.

For each setting of pathogen virulence characteristics and host
defense characteristics, ten replicate simulations were run. The
primary criterion for efficacy of host defense was whether or not
the host won by completely eliminating the pathogens. Secondary
efficacy parameters were the time (number of ticks) required to
win or die and the cumulative energy deficit (total potential host
energy minus current total host energy, summed over each tick).

The agent-based model can be accessed by downloading NetLogo
from http://ccl.northwestern.edu/netlogo/ and separately download-
ing the file from the NetLogo Modeling Commons website http://
modelingcommons.org/browse/one_model/3973#. Documentation
accompanies the file in the Info section and in the code.

3. Results

3.1. Local stressors as a defense

As expected, the local specific stressor strategy of host defense that
directly harms the pathogens without doing any collateral harm to
host cells showed increasing efficacy as the intensity of the defense
increased (Fig. 2). The other local strategy, local non-specific stressor,
causes a host cell adjacent to a pathogen to harm not only the
contacted pathogen, but also equally harm any other pathogens and
host cells to which it is adjacent, as if depriving all adjacent cells of
nutrients. In sharp contrast to the straightforward efficacy of directly

![Fig. 2. Efficacy (% host wins) of local specific stressor defense against nine pathogen types. The virulence of each pathogen type is ranked from least (1) to
most (9). Efficacy was identical for the pathogen types [5, 50, 40, stay] (listed 3rd)
and [50, 20, 40, stay] (listed 8th). There was no efficacy against the 9th pathogen
type, [50, 50, 40, stay], at any of the tested defense intensities. Proportion ± S.E. of
10 replicates.](image1)

![Fig. 3. (A) Efficacy (% host wins) of local non-specific stressor defense against nine pathogen types. The virulence of each pathogen type is ranked from least (1) to
most (9). There is limited efficacy against the least virulent pathogen type, [5, 5, 40, stay] and no efficacy against the other 8 pathogen types. Proportion ± S.E. of 10 replicates.
(B) Recheck of efficacy of local non-specific stressor defense against the least virulent pathogen type, [5, 5, 40, stay], seen in Fig. 3(A). Proportion ± S.E. of 100 replicates.](image2)
targeting pathogens without collateral damage (local specific stressor), the strategy of using completely non-specific harm against cells in all directions (local non-specific stressor) had very little efficacy against pathogens when used alone. Fig. 3 shows that there was only limited efficacy against the least virulent of the 9 pathogen types tested, [5, 5, 40, stay].

Additionally, there was evidence of an unusual multiphasic dose response for the local non-specific stressor when used as the only defense against this pathogen type. This was confirmed by including more data points at the higher levels of intensity and by using 100 replicates per data point, rather than only 10 (Fig. 3(B)). Close examination of multiple simulations in real time did not help explain the basis for the variation in efficacy with increasing intensity of this undirected local stressor. Given that it is hard to determine whether it is more beneficial or harmful to have a host cell harm all adjacent cells just to hurt the target pathogen, perhaps it is not surprising that this efficacy response is complex and apparently depends on multiple factors that were not easily identifiable for this particular pathogen type.

3.2. Non-specific regional stressors as defenses

Besides inducing non-specific stress locally at the contact zone of the host with the pathogen, another approach was to induce stress around a larger region around the infection. At one extreme would be amputation of the infection site along with surrounding unaffected cells. This regional stressor was modeled like a tourniquet such that varying degrees of energy could be removed from a 5 x 5 patch area in the lower right corner of the host surrounding the 2 x 5 patch area of initial infection. As expected, if sufficient stress were applied before pathogens had spread beyond this region, then the infection could always be eliminated, though at the cost of losing essentially all host cells there (results not shown).

Another form of regional stressor, clotting, was designed to mimic the reduced blood flow in the region of an inflammatory focus due to coagulation, leukocyte trapping, extravascular fibrin deposition, and fluid leakage as edema (Engelmann and Massberg, 2013; Opal and Esmon, 2003; Saadi et al., 2002). The reduced blood flow and subsequent stress from impared nutrient influx and waste efflux was modeled by reducing incoming energy to an area two patches out in all directions from a host cell encountering a pathogen. The clot-like impairment remained in effect until the infection was cleared locally and host cells had replicated back into the site. In a series of contests against a set of 36 pathogen types, increasing efficacy was noted at values of 35% and 50% reduction of energy, but with no efficacy at a setting of 20% (Fig. 4). Fig. 4 also shows that the local non-specific stressor alone at an intensity setting of 10 had almost no efficacy against the 36 pathogen types (confirming Fig. 3), but was remarkably synergistic with the regional clotting stressor.

3.3. Non-specific systemic stressor as a defense

The acute-phase response has a number of potentially harmful components such as fever (heat) and nutrient restriction (loss of appetite and sequestration of iron, zinc, and manganese) which can act as stressors, potentially harming every cell in the host including the pathogens within the host. We modeled the systemic stress of the acute-phase response by reducing the energy that each agent, host and pathogen alike, gains per tick by the same amount (See Table 2, last row). Because pathogens could directly harm host cells, vacant patches available for replication were limited to the leading edge of the infection, at least before the systemic stressor was applied. Pathogens preferentially replicated into the vacant patches, in part because they tended to gain energy from encounters with the host cells, while the host cells were losing energy from their encounters with pathogens. Further enhancement of the invasiveness of pathogens at the leading edge of the infection site occurred since our model allowed pathogens to replicate with lower amounts of energy than host cells. In essence, the pathogens were dividing their energy resources among a large number of progeny while most host cells merely stored their energy resources. This difference can be seen on the right-hand side of Fig. 1, where the 63 pathogens (up from an original 10) have a “mean pathogen energy” of only 85.8 while the 988 host cells (down from an original 1057) have a “mean host cell energy” of 208.8. In this figure, the systemic host stressor setting of 60 is On and is causing a net loss of approximately 4.4 energy units/tick to all host cells and pathogens. Thus the pathogens’ “strategy” of rapid replication leaves them with less energy to counter stress.

Fig. 5 illustrates the high cost of defense using systemic stress, even intermittently, in terms of both the loss of host cells distant from the infection site (Fig. 5(A)) and the reduced energy levels in remaining host cells (Fig. 5(B)). As a sole defense, the automatic systemic stressor set at 60 had only modest efficacy when tested against the standard set of 36 pathogen types of varying virulence (Fig. 6). However, it was notably synergistic with the non-specific local stressor set at 10. The systemic stressor was also synergistic with the regional clotting stressor, most notably when the regional stressor was set at 20 (compare blue curves in Fig. 6), a level where the regional stressor alone had no efficacy (cf dashed red curve in Fig. 4 or in Fig. 6). The combination of the non-specific local, regional, and systemic stressors was effective in eliminating approximately 90% of the infections among the 36 pathogen types tested.

Table 3 provides a detailed example of contests involving the systemic stressor with both types of local defenses (specific and non-specific local stress) against four pathogen types of medium virulence. Overall, each of the two local defenses (each tested at 2 and 10) performed well in combination with the systemic stressor. They tended to have only additive effects together

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Footnote: The systemic stressor setting of 60 is causing a 60% reduction in the 14 units of energy gained per tick, allowing 5.6 units of energy to be gained; but the 10 units/tick in metabolic costs are still being lost. Thus, there is a net loss of 4.4 energy units/tick to each host cell and pathogen. (Recall that each gain/loss of energy is also subject to a randomness factor.)
without the systemic stressor (i.e. the local non-specific stressor defense provided little help). Furthermore, note in the table that the local non-specific stressor defense, when set at 2, had good efficacy (80% winning) in combination with the systemic stressor; whereas, the local specific stressor defense, when set at 2, had no efficacy with the systemic stressor (boldfaced cells of Table 3(A)).

3.4. Pathogen virulence factors

Although not the primary purpose of the model, we did gain insight into the pathogens’ utility of several potential virulence factors. Biologically it might be expected that when a pathogen contacts a host cell it would both harm the host cell and
simultaneously extract the host cell’s resources/energy. As mentioned in the Methods section, we chose to model these two processes separately as “harm host” (the first number listed in the pathogen type) and as “gain energy” (the second number listed). In doing so, we found that the relationship between these two virulence factors is not straightforward since it appears that gaining energy is generally more beneficial to the pathogen than harming the host, as shown in Fig. 2 (see virulence rank order). In particular, increasing the second factor’s number increased the virulence rank more than increasing the first factor by the same amount. In our model, where replication can only occur if an adjacent vacant patch is available, host cells must be killed to clear space for pathogen replication to occur. However, excessive harm to host cells, relative to energy taken in from them, cleared out a barren zone ahead of the pathogens thereby depriving the pathogens of energy they could have otherwise gained from contacting viable host cells.

Another virulence factor, “survival energy”, when set below the default energy threshold for survival of 40 (same as the host cells), provided little benefit to rapidly invasive pathogens. However, the ability to survive at low energy levels made it more difficult for the host to completely eliminate infections, as seen in Table 3(C) versus (A). The other potential virulence factor, “mobility”, has the pathogens randomly wander to adjacent vacant patches without costing energy. Wandering increased virulence when the pathogens had a relatively more aggressive offense than the host cells had defense, since the pathogens gained additional energy from the frequent encounters with host cells and cleared patches for future replication. However, wandering pathogens that were less individually aggressive at energy extraction than were the host cells tended to lose more energy than they gained from their encounters with host cells, making mobility an “anti-virulence factor” in those settings. This is italicized in the entries of Table 3(A) versus (B), in the cases of the host defense stressor settings 2-2-On and 10-10-Off (with no regional stressor). In the case of 2-2-Off, wander makes the pathogen more virulent, with the host winning only 1 out of 10 times vs. 9 out of 10 times in the same case without wander; while the opposite effect is seen with 10-10-Off.

3.5. Effect of pathogen replication energy

The experiments discussed to this point had the amount of energy pathogens needed for replication set at 120 units, 2/3 of the 180 units that host cells required, as a way of ensuring that pathogens had a replication, hence virulence, advantage. However, in our model we found that rapid replication comes with the vulnerability to stress. Therefore, we explored the effect of the setting of the pathogens’ energy needed for replication (keeping the host’s needs constant). Fig. 7 shows that the less energy pathogens need for replication, the more vulnerable they are to systemic stress, recalling that they have devoted their energy to replication, leaving little in reserve to withstand host-induced systemic stress. Notable is that even when pathogens require the same (or more) energy to replicate as host cells they are still somewhat vulnerable to systemic stress, since they still replicate faster than host cells. This is due to the pathogens’ virulence directed against the contacted host cells which provides the pathogens with energy while depleting energy from the host cells.

4. Discussion and Conclusions

Our host-pathogen model is extremely basic, most notably lacking a vascular system, specialized defense cells, and a means of pathogen transmission. However, we view the simple host, simple pathogens, and limited rules of behavior as promoting the generality of the model’s results. Further, each of the model components such as pathogen virulence factors (Table 1) and host defenses (Table 2) are based on premises and produce behaviors that are biologically reasonable (as discussed in Section 2 and presented throughout Section 3). The assumptions that went into the construction of the model rules and features were based on these biologically reasonable and foundational principles. The subsequent model behavior that emerged from the dynamic interaction of those principles confirmed the concepts put forward in the immune brinksmanship conceptual model (LeGrand and Alcock, 2012) regarding how non-specific stressors might work to successfully fight pathogens. The rules and features of the model were not a priori defined to have pathogens be more susceptible to the effects of stress nor to have host cells more metabolically efficient than pathogens; rather, it was as a consequence of the model dynamics that the pathogens tended to be more susceptible to stress. If the model is viewed as an extremely primitive multicellular organism, one can begin to envision how such an organism might have used strictly non-specific stressors as defenses. Additionally, the model likely highlights defenses still present in host organisms but which have been overshadowed by more sophisticated defenses studied by most immunologists today.

Our model was originally intended to demonstrate the utility of completely non-specific systemic stressors, typical of the potentially harmful components of the acute-phase response, in helping control localized infections. As proposed by the immune brinksmanship model (LeGrand and Alcock, 2012), we have shown that host-derived non-specific systemic stressors can indeed eliminate simulated infections of low virulence alone. Because the immune brinksmanship model notes that many of the stresses occurring locally at infected sites are apparently reinforced systemically by the acute-phase response, we used our agent-based

![](image)
model to examine the potential efficacy of local non-specific stressors in controlling infections. It is not intuitively clear that a defense can be based upon host cells in contact with pathogens “lashing out” in all directions harming every adjacent cell equally. Indeed, because glucose and glutamine are nutrients used in large quantities by inflammatory cells to kill pathogens, some authors suggest that limitation of these nutrients at infectious sites may be detrimental (Krawczyk et al., 2010; Newsholme et al., 1996; O’Neill and Hardie, 2013; Pearce et al., 2013). At first glance, our results would seem to suggest little benefit of local non-specific stress since, when used alone, this strategy showed only slight efficacy against even our least virulent pathogen type tested (Fig. 3). In sharp contrast however, our non-specific local stressor, at a level that had no efficacy alone, was strongly synergistic with both the non-specific systemic stressor and the non-specific regional stressor (Fig. 6).

The utility of the modeling process became apparent when we were forced to classify different stressors so that they could be modeled appropriately. An obvious intermediate category between local and systemic is regional. We then simulated the regional stress associated with a therapy such as applying a tourniquet around a limb to control an infection (or invasive tumor). The extreme example of regional stress-related resource deprivation is amputation or surgical excision. Our model showed the obvious benefits of early regional resource deprivation in infection control as well as its equally obvious costs in terms of lost tissue (and resulting lost functionality). In wondering if there might be a more biologically relevant regional stressor than amputation/excision, we recognized that inflammation and coagulation are tightly linked through a number of pathways (e.g. overlapping components of the coagulation and complement cascades, inflammatory platelet activity, and inflamed endothelium becoming pro-coagulant). Inflammation is commonly noted for its increased blood supply due to vasodilation. However, an infected site typically has reduced blood inflow due to regional thrombosis, plugging of capillaries by leukocytes, neutrophil extravascular traps (NETs), and extracellular fluid causing vascular compression and increased distance from patent vessels. The evolutionary benefit of this reduced blood flow has been ascribed to reducing the spread of pathogens from the infected site and localization of antimicrobial factors (Alcock and Brainard, 2008; Engelman and Massberg, 2013; Opal and Esmon, 2003; Saadi et al., 2002). However, since reduced blood flow to an infected site would also provoke stress due to resource restriction and reduced clearance of toxic products, we developed a “clot” as a regional stressor in which we applied varying severities of energy restriction (stress). Recall that in our model the clot extends out two patches in all directions from each host cell in contact with a pathogen. We found that this form of regional stress was synergistic with both non-specific local stress and systemic stress in controlling infection (Fig. 6).

Although the model most resembles an infection of a surface wound by large invasive organisms such as yeasts, once obvious differences are accounted for, we believe the model and its findings have wide applicability due to their fundamental nature. Most notably the model closely mimics invasive tumors, and each of the host defenses modeled has therapeutic correlates. For instance, the local specific stressor corresponds with newer therapies based on tumor-specific surface molecules, the local non-specific stressor corresponds with precision radiation therapy, regional amputation corresponds with wide surgical excision, the regional clotting stressor corresponds with less precise radiation therapy or anti-angiogenesis therapy to inhibit the tumor’s vascular supply, and the systemic stressor corresponds with classical systemic chemotherapy that primarily targets rapidly growing or dividing cells.

Based on the results of our model, we propose that the host also benefits from the local non-specific stressors at infection sites that are induced by inflammatory cells. Therefore, a likely additional function of inflammatory cells is to deliberately waste and use up resources that the pathogens, as well as themselves, might use (e.g. glucose, glutamine, oxygen) and to deliberately make the site more stressful by increasing lactic acid and free radical exposure. Additionally, we propose that an evolved function of the linkage of coagulation with inflammation is not only to impair pathogen spread, but is also to provide additional stress to the infected region, further harming the pathogens relatively more than the host. Understanding the potential benefits of self-induced stress may provide better insight into appropriately treating patients presenting complex immune responses such as sepsis. It has also become clearer as to why therapies that provide less specificity may have greater longevity as therapeutic agents (e.g. antiseptics) compared to molecularly specific therapies to which pathogens can more quickly adapt and subvert (e.g. antibiotics).

In summary, our agent-based model of a simple host infected by an invasive pathogen shows how host-derived stressors that are completely non-specific can provide surprisingly effective pathogen control. We see that pathogens and host cells face a trade-off in whether to use resources (energy) for growth and population expansion or to maintain resources in reserve to counter possible stress. Pathogens require growth and replication to be pathogenic and, therefore, preferentially divert resources toward this effort. In contrast, the host cells in our fully developed host simply stored their resources, which were then available to counter stress. In our generalized model, we have demonstrated synergy for host defense among local, regional, and systemic stressors. In absolute terms, each affected host cell is harmed to the same extent as each affected pathogen, though in relative terms the host is typically harmed less. However, systemic stress is particularly costly since even host cells distant from the infection site are harmed. Localized stress owes part of its efficacy against pathogens to the relative expendability of local host cells in relation to the host as a whole; that is, distant host cells are spared the costs of the stress. Through the use of our agent-based cell-level infection model, we have seen that even the application of completely non-specific stress can provide a formidable, though costly, host defense.

References


