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The Inflammatory Twitch as a General Strategy for Controlling the Host Response

Joshua J. Pothen, Matthew E. Poynter, and Jason H. T. Bates

Allergic inflammation is a general host-defense mechanism for dealing with perceived foreign invaders. Although most effort has been directed toward understanding how this response gets turned on, how it gets turned off again when no longer needed is just as important to an organism’s survival. We postulate that the control of the allergic inflammatory response is achieved via frequency modulation whereby a sequence of self-resolving events is repetitively invoked only so long as Ag is present. This leads to the notion of a unitary inflammatory event that we argue has formal similarity to the skeletal muscle twitch, albeit manifest over a much longer time scale. To test the plausibility of this hypothesis, we created an agent-based computational model of the allergic inflammatory response in the lungs. Continual stimulation of the model results in cycles of tissue damage and repair interspersed with periods of nonresponsiveness indicative of a refractory period. These findings are consistent with the inflammatory twitch hypothesis and the notion that the allergic inflammatory response is controlled via frequency modulation. We speculate that chronic inflammatory diseases may represent a failure of the inflammatory twitch to resolve toward baseline. The Journal of Immunology, 2013, 190: 3510–3516.

The inflammatory response is a general biological defense mechanism for battling invading microbes or attending to injury. The symptomatic manifestations of inflammation, namely, tumor, rubor, calor, and dolor, reflect myriad molecular events that orchestrate a variety of resident and recruited cell types to operate in a specific manner within the affected tissue (1). Clearly, turning on an appropriate inflammatory response in time of need is critical to an organism’s survival. However, turning the response off again when it is no longer needed is equally important. Indeed, failure to resolve the inflammatory response is presumably behind the chronic inflammatory conditions that characterize many common diseases. This raises the general question as to what strategy the body uses to control both the upregulation and the downregulation of inflammation. Negative feedback has been proposed as a means for controlling the extent of allergic inflammation (2). However, recent work suggests this does not adequately explain the relationship between the stimulus and the allergic response (3). Another possibility is that these two events are subject to independent decision criteria; upregulation of inflammation is directed by a control mechanism that reacts to the appearance of a threat, whereas downregulation is controlled by a separate mechanism that detects the threat’s disappearance. This scenario is compelling because of its ready analogy to the fighting of military battles. However, it is not the only possibility.

An alternative control strategy for the inflammatory response is suggested by the formal similarity of its task structure to that of the skeletal muscle. The inflammatory response is something that an organism must be ready to invoke, without warning, any time a threat arises, and yet it must dissipate when no longer needed. The same applies to muscle activation; muscles must be ready to generate force whenever the brain decides to undertake some task, but must cease to do so as soon as the task is completed. However, force generation and cessation in muscle are not subject to separate decision processes, but rather are controlled jointly via frequency modulation. Specifically, a muscle continues to do the job asked of it so long as it receives a steady stream of periodic electrical impulses, the frequency of the impulses dictating the level of force. Once the need for the task has passed, however, the impulses stop and the muscle returns to quiescence. The functional unit of this control strategy is the muscle twitch, a transient manifestation of force driven by a sequence of events that includes not only those that cause force to escalate, but also those that bring about its subsequent resolution. In other words, the termination of a muscle twitch is an inevitable consequence of its initiation, so continuation and/or escalation of force is simply a consequence of how individual twitches summate when invoked in rapid succession. The singular advantage of this type of control strategy is that it obviates the need for additional decision resources beyond those involved in activation.

The above reasoning leads us to contemplate the possibility that inflammation might be controlled in a similar manner to skeletal muscle, that is, via the repetitive generation of self-limited, transient, unitary responses. This analogy implies the existence of an “inflammatory twitch,” an inevitably resolving sequence of cellular events having formal similarity to a muscle twitch, albeit over a greatly extended time scale. Further questions then arise as to the extent of this analogy. What is the duration of an inflammatory twitch? Can multiple inflammatory twitches summate in the same manner as muscle twitches? Do inflammatory twitches create a postinitiation refractory period? Answering these questions might elucidate important aspects of how the general host inflammatory response is controlled, and possibly even how its control might fail in disease.

Accordingly, the goal of this study was to investigate the plausibility of the inflammatory twitch hypothesis. We focused in...
particular on allergic inflammation in the lung because this condition has been well characterized in terms of the cell types involved (1). Also, we have previously observed that when allergically sensitized mice are continuously challenged with Ag over a period of weeks, their inflammatory response does not continue indefinitely, but instead eventually resolves toward baseline (4). Furthermore, if Ag challenge is interrupted for several weeks and then reinitiated, the animals again mount a vigorous inflammatory response (5). These observations appear to be consistent with our notion of the inflammatory twitch, but they do not demonstrate that it is feasible relative to the interactions of all the cells involved. Allergic inflammation in the lung is a complex event, localized around the lung and its associated mediastinal lymph nodes, yet involving a variety of cells recruited at different times from outside the organ (6, 7). It is not immediately obvious that a twitchlike, self-limited response to the appearance of an Ag in the lung could actually manifest from a system with these features.

Testing the plausibility of the inflammatory twitch hypothesis thus requires that the spatiotemporal dynamics of the various cellular players and chemical signals involved be taken into account. In this study, we undertook this task computationally, using a modeling technique that appears ideally suited to this purpose. This technique, known as agent-based modeling, allowed us to simulate the dynamic environment comprising the pulmonary capillary and its associated alveolar compartment, and to determine whether something akin to an inflammatory twitch is indeed possible, and what its temporal and spatial morphology might look like.

Materials and Methods

Model structure

We used NetLogo 4.1.3 freeware (8) to design an agent-based model that simulates allergic inflammation in an alveolus of the lung in response to Ag stimulation. The model uses two types of variables known, respectively, as patches and agents. Patches represent fixed pieces of the local environment, and thus contain local variables representing information about that area. In our model of the allergic inflammatory response, the patches represent the capillary and alveolus, as well as the endothelial barrier between them. (The sizes of the capillary and alveolar spaces are arbitrary.) Each alveolar patch has a local parameter known as “tissue-life,” which indicates the amount of tissue damage that has occurred on that patch. It is set between 0 and 100, where 100 corresponds to full health and 0 indicates a fully destroyed patch. Thus, the average health of all alveolar patches represents the average health of the alveolus.

In contrast with patches, which represent the environment, agents represent individual entities capable of moving across patches and interacting with surrounding patches and with other agents. The agents in our model are the multiple cell types involved in the allergic inflammatory response in the lung. The behavior of the model is thus determined by the collection of rules that each agent obeys.

Our overall schema for the allergic response is shown in Fig. 1, and it includes what we believe to be the key mechanisms and cell types that are involved. To increase the manageability of the model, we have not included all known details of allergic inflammation in this schema. For example, following the approach of Brown et al. (8), we lumped dendritic cells, B cells, and macrophages together into a single APC type. Similarly, neutrophils, eosinophils, and other cell types that cause local tissue damage are merged into a single proinflammatory cell (PIC) type, whereas fibroblasts and other cells that are involved in tissue repair are combined into a single anti-inflammatory cell (AIC) type. This grouping of cell types is, of course, a gross oversimplification of reality, but we believe it represents the greatest degree of coarse graining that could reasonably be applied to the cellular community so that it still retains the ability to exhibit competition between inflammatory and reparative processes.

The inflammatory response is initiated by the sudden presence of particles at random locations in the alveolar space. Both mast cells and APCs are involved in this initiation. We focus on these two cell types because in vivo, mast cells release both preformed and synthesized chemical mediators such as PGs, leukotrienes, and vasoactive amines upon cross-linking of their receptors by bound Ag (1). This leads to many of the symptoms that are observed in the allergic inflammatory response, such as vasodilation and bronchoconstriction (1). In addition, APCs process Ag particles via pattern-recognition receptors, such as TLRs, and subsequently become activated. This affects cytokine production in such a way as to ultimately affect the T cell population, leading to amplification and perpetuation of the inflammatory phase of the allergic response (9).

The model was initialized with 5 mast cells, 10 APCs, and 10 PICs in the alveolus, and the same numbers in the capillary. The alveolus was also initialized with 20 AICs, 10 Th cells, and 10 regulatory T cells, whereas the capillary contained 5 Th and regulatory T cells. The capillary acts as a reservoir of cells, so if any of these cells dropped below these baseline values in the capillary, the appropriate number of additional cells was randomly added to the capillary space. The capillary effectively acts as a source and sink for cells in the alveolus. Thus, although the volume of these spaces is arbitrary, changing the size of the capillary would not have a significant effect on the model.

The model includes an endothelial barrier between the capillary and the alveolus. Pores in the barrier allow restricted cell movement between the two spaces. If the sum of various PI chemical signals, namely, granules and PI cytokines in the lung space, is greater than a critical value (arbitrarily set to 200 for this model), the barrier is removed. This simulates the effects of vasodilation and endothelial leak by allowing unhindered cell movement between the capillary and alveolar spaces.

Model dynamics

Inflammation in the model is initiated by random placement of a certain number of particles throughout the alveolus (8). Additional particles may be placed at any later time point to simulate continual stimulation of the inflammatory response.

Cell movement in the model is probabilistic. If there is no reason for a cell to move in any particular direction, the cell moves a distance of one patch either forward, back, right, or left with equal probability at each time step. However, if a patch contains a signal to which the cell is responsive, the probability of movement to that patch will be increased proportional to the strength of the signal. Thus, although, on average, a cell will move in the direction of the strongest signal, the movement at any particular time step may not be in this direction.

Diffusion of chemical signals (i.e., granules, PI cytokines, and AI cytokines) is simulated by randomly distributing a specified fraction of each signal on a patch to its adjacent patches at each time step. Removal of these substances by enzymes, blood flow, and so on is simulated by having their strengths decrease by set proportions (specific for each substance) at each time step (8).

The life span of each cell type in the model follows an exponential distribution that is implemented as follows. At each time step, and for each cell in the model, we generate a random number on the uniform distribution between 0 and 1. If this number is greater than a critical value specific for the cell type in question, then the cell is eliminated. Mast cells and APCs are relatively long-lived compared with most of the other cell types (6, 10). Although the most abundant CD4 T cell type, memory T cells, are also long-lived, we do not consider them long-lived in our model because they may only be active for a shorter period after Ag exposure.) We thus chose the critical value for mast cells and APCs to be 0.9997, giving them an estimated mean life span of 2500 time steps, whereas the critical value for all the other cell types except the AICs was 0.9978, giving them an estimated mean life span of 200 time steps. The AICs were allowed to live indefinitely because fibroblasts are known to be very long-lived relative to the other cell types (11, 12).

Agent rules

The overall behavior of the model is a consequence of the way in which its agents (the cell types described earlier) behave. The behavior of each agent is governed by a set of rules designed to capture the essential elements of actual biological behavior. The various agents in our model are listed in Table I, together with the signals to which they respond and the rules that govern their behavior. The rulesets for each cell type are generally similar in that they cause the cells to move toward and become activated by certain chemical signals, and then release chemical signals of their own into the surrounding patches.

The initial cell types involved in the allergic response are mast cells and APCs, which both respond to particles and release chemical signals that initiate the subsequent events of the allergic response. Mast cells accomplish this task by binding on the particle to the mast cell membrane and then releasing a user-specified number of granules into the surrounding patches for 10 time steps (1). Mast cells initially exist in an immature state until a user-specified amount of time has passed, at which point they be-
Table I. Summary of all agents and their respective rules included in the model

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Moves Toward</th>
<th>Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell (immature)</td>
<td>Attractants</td>
<td>Moves preferentially to attractants, or randomly if there are no attractants</td>
</tr>
<tr>
<td>Mast cell (mature)</td>
<td>Particles, attractants</td>
<td>Upon binding particle, releases granules for 10 time steps; becomes inactive for user-set number of particles</td>
</tr>
<tr>
<td>APC</td>
<td>Particles</td>
<td>While digesting particle, releases APC signals; becomes inactive for user-set time if encounters user-set number of particles</td>
</tr>
<tr>
<td>Th cell</td>
<td>Granules, APC signals</td>
<td>If moving toward granules and/or APCs, releases PI cytokines for 10 time steps; afterward, if there is a strong chemical signal on the current patch, converts into a PIC</td>
</tr>
<tr>
<td>PIC</td>
<td>PI cytokines, granules</td>
<td>If moving toward PI cytokines and/or granules, become activated for 10 time steps; while activated, release PI cytokines and attractants; also, subtract 5 from the health of the patch currently on if cell has moved toward a chemical signal, or 2 if not; at the end of activation, the cell dies</td>
</tr>
<tr>
<td>T regulatory cell</td>
<td>PI cytokines</td>
<td>If moving toward PI cytokines, release AI cytokines</td>
</tr>
<tr>
<td>AIC</td>
<td>Patches with health &lt; 100</td>
<td>If on a patch with health &lt; 100, add 1 to that patch’s health parameter at each time step</td>
</tr>
</tbody>
</table>
Finally, Fig. 6 shows what happens to tissue health when the interval between application of particles is varied. Stimulation periods of 0.5, 1, and 2 wk yield rather similar results, characterized by an initial pronounced dip in tissue health followed by decreasing dips and more chaotic behavior as time progresses. By contrast, an application interval of 10 wk produces regular marked oscillations in tissue health that continue unabated.

Discussion
From the perspective of survival, turning off the body’s defense mechanisms when they are no longer needed is just as important as turning them on in response to a threat. Indeed, many chronic and debilitating diseases appear to be manifestations of a host response that fails to resolve when it should. Elucidating the pathophysiology of these diseases amounts, in large part, to finding out why control of the host response fails, and this begins with consideration of how it is controlled normally. Our thinking on this subject tends to be influenced by the common view of the host response as the waging of a military battle in which an army of defenders attempts to vanquish an invading foe. The danger with this analogy, however, is that it leads to the supposition of top-down command, yet there is no obvious analogue in living organisms of the general who surveys a battle scene and gives orders based on what is perceived to be happening.

Even more problematic, the military analogy suggests separate command and control systems for turning on the host response when it is needed, and then turning it off again when its job is done. Turning the response on presents no particular conceptual difficulty because it merely requires detection of a threat, and the various specialized cells of the immune system do just that. Turning the response off again via a separate controller is a problem, however,
action potentials, which themselves are instigated by the brain’s desire to move some part of the body. Control of muscle force thus requires only detection of the presence of something, but not detection of the absence of something.

This line of reasoning leads immediately to the notion of the inflammatory twitch as the unit of response of the immune system to an invader, but it brings with it some ancillary analogues. Most obvious is refractoriness, a period after twitch initiation during which a second twitch cannot be instigated. Refractoriness is a necessary requirement for twitch morphology because it ensures that the events involved in generating the twitch are properly turned off before they can be reinitiated. In the case of muscle force, the timing of refractoriness may allow for stacking (summation) of the forces from multiple twitches as occurs in skeletal muscle, or it may prevent stacking, as is the case for cardiac muscle. It is not obvious a priori which strategy the immune system would choose, but we have previously obtained evidence from sensitized mice treated with sequential daily exposures to a foreign protein (OVA) that the latter situation may pertain to allergic inflammation of the lung. Inflammation peaks after ~3 d of exposure and then gradually abates as exposures are continued over days to weeks (4, 5). This is referred to as tolerization (16) and is thought to involve T regulatory cells. Resting the animals for several weeks, however, allows them to once again respond vigorously to a subsequent OVA challenge (5), suggesting that the inflammatory twitch in these animals lasts on the order of weeks and has a refractory character that prevents them from stacking.

These findings are recapitulated by the agent-based model we developed in this study, which exhibits clear oscillatory behavior after continual stimulation with particles (the analog of Ag exposure in mice). Of particular note is the fact that, for most stimulation regimens, the largest and most well-defined oscillations are observed early on, and these are followed by more behavior that becomes progressively more chaotic uniform with less well-defined peaks (Figs. 3, 6). In contrast, if the period between successive stimulations is long enough to allow resolution of the response to each challenge, the oscillations remain sharply defined with time (Fig. 6D), resembling a series of well-separated twitches that are unable to summate. The twitch hypothesis is further supported in the model by our finding that allowing the model to rest for a period of time results in a reiteration of the initial peaked response (Fig. 5), similar to our previous observations in mice (5).

Inferences drawn from the behavior of our agent-based model must be viewed in light of its numerous simplifying assumptions, which were made in the interests of conceptual and computational tractability. Many of these assumptions relate to the way in which individual biological entities behave. For example, we assume that cells have infinite stores of the chemical signals they release, and that the epithelium plays a purely passive role as a physical barrier to cell movement, which from our own work (17) and that of others (18) is clearly an oversimplification. Furthermore, the alveolar airspace in the model is inaccessible to any cell type, whereas in reality, cells do cross the epithelial barrier and some are cleared via the airways, which creates an additional sink for cells from the alveolar compartment. This might affect the prediction of the early inflammatory oscillations but could result in greater suppression of the subsequent inflammatory activity (such as beyond 5000 time steps in Fig. 3).
The most important assumptions in the model, however, relate to the way in which we coarse-grain the myriad details of reality into a much smaller number of independent components. Coarse graining is a hierarchical process, the success of which depends on appropriate binning of the underlying biological details into their various model groups. In our model, for example, we coarse-grain the dynamics of cell movement by lumping all the different movement velocities into a single average rate of random movement from location to location, regardless of whether the cells are activated. This presupposes that cell movement per se is the most important feature of the ability of cells to travel between different locations, and that different cells moving at different velocities is of secondary importance, following the approach taken by Brown et al. (8). In reality, for example, fibroblasts tend to remain localized to fixed locations within the lung, which could mean that nearby regions could be maintained, on average, in a more healthy state compared with regions far from a fibroblast, which could increase the topographical heterogeneity of predicted tissue damage in the model. We also coarse-grain cell size by assuming it is the same for all cell types.

Similarly, by lumping multiple cell types into only two functional groups, the PICs and AICs, we assume that it is simply the existence of competing cell types that is paramount, whereas the existence of different phenotypes within each cell group is of secondary importance. For example, we ignore differences in life span and behavior between cells neutrophils and eosinophils, both of which are lumped together into the PIC category. The question thus remains whether such a crude level of coarse graining causes us to miss some crucial detail of overall system behavior that would have become apparent had we divided the PIC and AIC groups into subgroups with different properties. There are many known details of cell behavior that could be investigated in this regard. For example, the alveolar macrophage has a life span between that of other PICs and AICs, and can initially behave as a PIC, but then switch to a AIC depending on environmental stimuli (20), and indeed we invoked the macrophage data from Tanaka et al. (3) as representative AIC types in Fig. 4. Mast cells and APCs can signal to cells such as monocytes and macrophages, causing them to turn into PICs (21, 22). Some PI cytokines, such as TNF-α, inhibit their own synthesis, which self-limits their production (23). Thus, the number of ways in which we might delve into a finer level of model coarse graining is enormous. For the time being, however, we will let ourselves be guided by the notion that it is the competition between temporally offset PI and A1 processes that gives rise to the inflammatory oscillations predicted by our model and that support the inflammatory twitch hypothesis. Accordingly, we take the position that the details alluded to earlier, although clearly important for refining the details of the model predictions, are not crucial to the actual existence of these predictions.

The behavior of our model can thus not be taken to constitute proof or otherwise of a biological theory. However, it does provide a test of plausibility to an extent that is impossible to achieve through any other approach. The allergic inflammatory response involves a large number of mutually interacting cell types, each playing different roles. Determining the details of the ensemble behavior of such a complex system defies human intuition. Agent-based computational modeling, in contrast, allows one to estimate how the system might possibly behave within its spatiotemporal constraints. This approach has been exploited convincingly by Brown and colleagues (8) in the exploration of a number of aspects of the inflammatory response. In the case of this model, we have demonstrated that the major cell types involved in the allergic inflammatory response could indeed conspire to produce collective behavior in the lung that is formally similar in many important respects to the muscle twitch.

An important feature of the muscle twitch is the fact that it manifests in cycles when stimulation is continual. Support for the inflammatory twitch hypothesis in this regard comes from the experimental work by Tanaka et al. (3) showing that mice undergoing chronic Ag challenge have cytokine levels that peak 1 wk after initiation of the Ag challenge and then decrease for up to 3 wk afterward. These cyclic biomarkers are consistent with the waxing and waning of the hyperresponsive phenotype that occurs in allergic mice (5). Such observations are consistent with the duration of this inflammation and resolution event being on the order of 4 wk, allowing us to propose that this time period corresponds to ~400 time steps in our model (Figs. 3, 4).

Another key feature of the muscle twitch is its refractory period, and this is borne out in our model simulations (Figs. 3 and 6D illustrate this most clearly). Experimental support for the existence of a refractory period in the inflammatory response is evident in the data of Dienz et al. (24), who showed that when mice deficient in IL-6 are exposed to influenza virus, they experience significantly higher mortality around day 9 of the infection, with fewer neutrophils recruited to the lung compared with wild-type controls. In both deficient and wild-type strains, however, neutrophils disappeared from the lung around day 9. These data are consistent with the triggering of an immune response twitch having an active duration of ~9 d. Interestingly, mice (and humans) are known to be particularly susceptible to secondary bacterial infections after this initial immune response to virus (25), suggesting that they enter a period of refractoriness to reinitiation of the immune response beginning around the time when the neutrophils disappear. The duration of the combined active inflammatory phase and the subsequent refractory phase in our model appears to be on the order of weeks (Figs. 5, 6). Interestingly, we have previously observed (26) that OVA challenge in mice results in cyclical levels of NF-κB in bronchial epithelial cells with a period of 2–6 h. Although this is clearly far too short to correspond to the period of the inflammatory twitch, it does suggest that frequency modulation may be a strategy used by living organisms to control metabolic processes manifesting over a wide range of length and time scales.

In conclusion, we have advanced a hypothesis in which a unitary self-resolving event that we call the inflammatory twitch serves as the basis for control of the host response to an insult from the environment. We developed an agent-based model of allergic inflammation in the lung that exhibits key features predicted by this hypothesis, including refractoriness to continued stimulation leading to cycles of inflammation and repair. An implication of this hypothesis is that chronic inflammatory diseases may reflect a failure of the inflammatory twitch to resolve toward baseline.

Disclosures
The authors have no financial conflicts of interest.

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