Analyzing inflammatory response as excitable media

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The regulatory system of the transcription factor NF-κB plays a great role in many cell functions, including inflammatory response. Interestingly, the NF-κB system is known to up-regulate production of its own triggering signal—namely, inflammatory cytokines such as TNF, IL-1, and IL-6. In this paper we investigate a previously presented model of the NF-κB, which includes both spatial effects and the positive feedback from cytokines. The model exhibits the properties of an excitable medium and has the ability to propagate waves of high cytokine concentration. These waves represent an optimal way of sending an inflammatory signal through the tissue as they create a chemotactic signal able to recruit neutrophils to the site of infection. The simple model displays three qualitatively different states; low stimuli leads to no or very little response. Intermediate stimuli leads to reoccurring waves of high cytokine concentration. Finally, high stimuli leads to a sustained high cytokine concentration, a scenario which is toxic for the tissue cells and corresponds to chronic inflammation. Due to the few variables of the simple model, we are able to perform a phase-space analysis leading to a detailed understanding of the functional form of the model and its limitations. The spatial effects of the model contribute to the robustness of the cytokine wave formation and propagation.

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I. INTRODUCTION

Excitable media are naturally encountered in many biological systems. A typical excitable medium behaves in a manner much resembling spectators making a wave of raised hands at a sports game. The *excitable units* (or sections) get stimulated by their neighbors and amplify the exciting stimuli. At this stage the units are said to be in an excited state. Subsequent to excitation there is a recovery period in which new excitation is not possible, referred to as the refractory period. As a result of this behavior, spatially coupled excitable units are able to propagate undamped waves of high stimuli concentration through the system.

Some biological species have evolved to utilize the undamped waves that excitable media produce as a means of sending information through the system. Two well-known examples of biological excitable media are the neuron [1,2], which is able to propagate action potentials down the axon, and colonies of the social amoeba *Dictyostelium discoideum* [3,4], which propagate spiral waves of cyclic adenosine monophosphate (cAMP) and accordingly perform self-organized directed migration toward a common center. Both systems share the need for sending information through relatively large distances, where simple processes, such as, for example, diffusion, would not be adequate.

As recently shown by the authors, the regulatory system of nuclear factor κB (NF-κB) also contains the necessary components in order to exhibit "excitability," i.e., behave as an excitable medium [5].

NF-κB is present in all mammalian cells and is known to play an important role during inflammatory response [6–8]. The NF-κB system is triggered by inflammatory cytokines and in turn amplifies the cytokine signal, thus creating an excited state in which cytokine production is high. But because NF-κB also triggers production of its own inhibitors, the excited state will not last: eventually inhibitor concentration will become abundant and bind all NF-κB, making it inactive and hence cytokine production ceases. As long as inhibitors are plentiful, new activation of NF-κB cannot result in an excitation comparable to the initial one, although NF-κB has been shown to exhibit secondary small-amplitude peaks [6,7]. Thus the state with high inhibitor concentration constitutes a refractory period.

As a result of this behavior tissue cells containing NF-κB regulatory systems should theoretically be able to propagate traveling waves of high cytokine concentration through the tissue. Since cytokines also function as a neutrophil chemoattractant, this scenario is in good agreement with the current belief that neutrophils chemotax in a similar fashion as *Dictyostelium d.*, namely, through waves of chemoattractant.

As recently shown, a simple model of spatially coupled NF-κB units (cells) naturally leads to the propagation of cytokine waves in the tissue [5]. The model is a simplification of the real NF-κB system and provides a useful tool for investigating and understanding the underlying mechanisms of the complex regulatory system. In this paper we present and analyze the model in greater detail and obtain a better understanding of the many mechanisms that the simple model captures. The findings of this paper can hence contribute to the general understanding of inflammatory response—in particular, how different components of the immune system may send and transmit information through the organism. In addition, these findings contribute to the understanding of neutrophil recruitment during inflammatory response.

II. MODEL

In order to create an excitable medium it is important that the excitable unit responds with a transient amplification of the stimuli (opposed to persistent amplification). This means that the excitable unit must be an adapter in the sense that the system must adapt to the new surroundings after a transient phase. It is experimentally observed that the NF-κB system responds with a pronounced initial peak in nuclear NF-κB and thereafter, secondary oscillation of much smaller amplitude [6,7]. The damped oscillatory behavior arises due to several

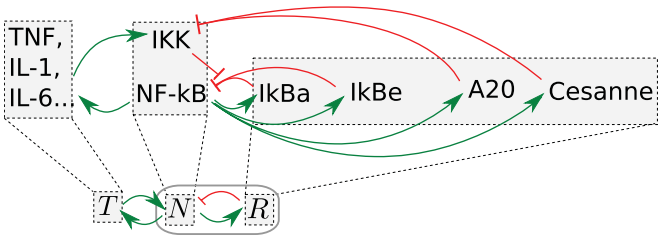

FIG. 1. (Color online) The NF-κB regulatory system is simplified as sketched here. Cytokines such as TNF, IL-1, and IL-6 activate the NF-κB system through the IKK pathway. The cytokines are simplified as a single variable denoted T. Active NF-κB is highly correlated to active IKK, and these two variables are thus also simulated as a single variable denoted N. Since cytokines activate IKK (and hence NF-κB) and NF-κB in return up-regulates production of cytokines, there is a positive feedback between the variables T and N. Inhibitors ($\mathrm{IkB}_{\alpha,\beta,\epsilon}$) and upstream regulators (A20, cesanne) all function to perform a negative feedback on either IKK or NF-κB and are hence simulated as the single regulating variable R, which performs a negative feedback on N. Activating interactions are sketched with $\rightarrow$ and inhibiting interactions are sketched with $\dashv$.

inhibitors performing negative feedback, but for our purpose it is sufficient to note that the secondary behavior is of much smaller amplitude than the initial peak, and hence the NF-κB system *is* an adapter.

In order to analyze the system we have constructed a simple model which captures the overall behavior of the NF-κB system. (We have verified our results by also simulating the system in greater detail, including several inhibitors and upstream regulators, and confirm that the qualitative behavior is also exhibited for this more sophisticated model.) The NF-κB system is simplified as sketched in Fig. 1. Cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) stimulate the NF-κB system though the IκB kinase (IKK). The cytokines are simulated by a single variable which we denote T. When IKK is activated inhibitors are degraded and NF-κB is released, translocating into the nucleus where it is active. Thus the concentrations of IKK and NF-κB follow each other and can be simulated by one variable, which we denote N. The inhibitors ($\mathrm{IkB}_{\alpha,\beta,\epsilon}$) and upstream regulators (A20, cesanne) all cause either IKK or NF-κB concentration to go down. These inhibitors and regulators are simulated by a "regulator" variable which we denote R.

The effect of inhibitors and other regulators is to perform a negative feedback on NF-κB, and is modeled by a simple negative feedback loop (see interactions between N and R in Fig. 1). These interactions can be described by the equations

$$\frac{dN}{dt} = k_{\mathrm{activate}} f(T) \times (N_T - N) - k_{\mathrm{inhibit}} R \tag{1}$$

$$\frac{dR}{dt} = r_{\mathrm{on}} N - r_{\mathrm{off}} R \tag{2}$$

The activation of N corresponds to translocating NF-κB into the nucleus. This term is proportional to some function of the cytokine concentration $f(T)$ and the amount of cytoplasmic NF-κB (we assume the total amount of NF-κB (N_T) is constant. Thus the amount of NF-κB which is available for activation is given by the amount of cytoplasmic NF-κB; $N_C = N_T - N$) [9].

Inhibition of NF-κB is proportional to the amount of inhibitors R and is considered to be saturated in N. (Simulations have shown that this approximation does not introduce an error of noticeable size.)

The activation of R is proportional to N and inactivation of R is modeled as a spontaneous degradation, only proportional to R. In order for this simple model to function as an adapter, it is important that the rate constant r_{off} is slow compared with the other rate constants of the system [5,10].

When the NF-κB network is stimulated by cytokines it responds by up-regulating hundreds of genes, including those coding for cytokine production. The newly synthesized cytokines are secreted into the extracellular matrix, where they can again stimulate the IKK pathway. Thus the interaction between NF-κB and cytokines constitutes a positive feedback (see interactions between N and T in Fig. 1).

The local concentration of cytokines (T) is modeled by the equation

$$\frac{dT}{dt} = p\frac{N^2}{N^2 + K^2} - \frac{T}{\tau} + S. \tag{3}$$

NF-κB–induced production of cytokines is proportional to the rate constant p (for positive feedback) and to the Hill function $N^2/(N^2 + K^2)$, because NF-κB is a dimeric transcription factor. As we shall see below, this term could also be modeled as a simple linear response (pN) and still give similar results. The cytokine degradation is modeled by a simple linear decay with a typical lifetime τ. The term S represents an additional cytokine production functioning as an external stimuli: during inflammatory response cytokines are secreted from nearby macrophages, which would correspond to a small flux of cytokines. This flux is "turned on" at time $t = 0$ and is modeled by a step function

$$S = \begin{cases} 0 & \text{for} \quad t < 0 \\ S_{\mathrm{on}} & \text{for} \quad t > 0, \end{cases} \tag{4}$$

where $S = 0$ corresponds to no stimuli. In the case of spatially coupled cells only the cytokines are secreted into extracellular space, and hence only the variable T is allowed to diffuse in between cells. In this case the equation describing cytokine concentration (at the ith cell) is given by

$$\frac{\partial T_i}{\partial t} = p\frac{N_i^2}{N_i^2 + K^2} - \frac{T_i}{\tau} + S_i + D\frac{\partial^2 T_i}{\partial x^2}, \tag{5}$$

with the only difference being the addition of the diffusion term.

For the system to react as an excitable medium the activation of the excitable units must be strongly thresholded. This threshold is in accordance with recent experimental findings [11,12]. We implement this by modeling the activation of NF-κB with a sigmoidal response to T (and Hill coefficient = 3):

$$f(T) = \frac{T^3}{T^3 + K_A^3}. \tag{6}$$

The variables have been renormalized in the following way: $N \rightarrow N/N_T$ and $T \rightarrow T/K_A$, which is equal to putting the parameters N_T and K_A [Eqs. (1) and (6)] equal to unity (and redefining the remaining parameters [5]). This also means that

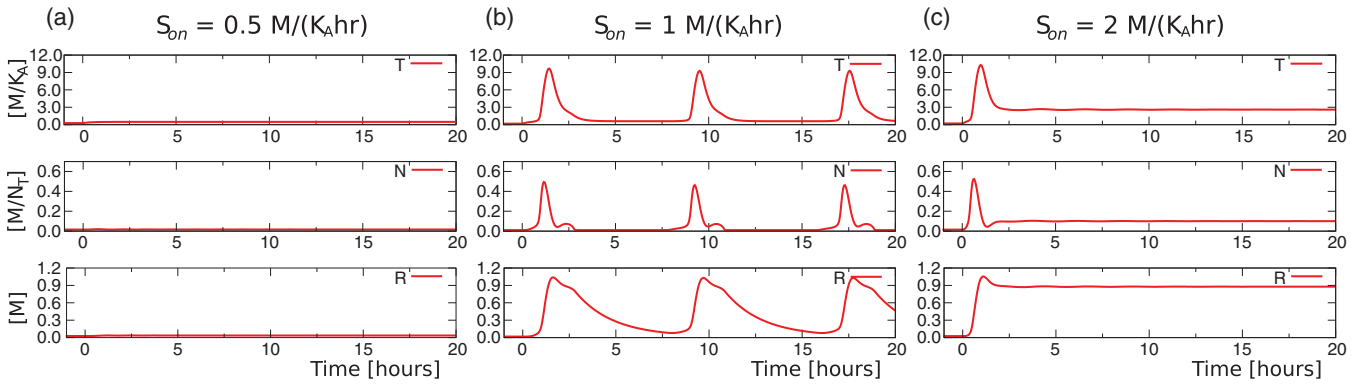

FIG. 2. (Color online) Simulation of reaction to stimulus using Eqs. (1), (2), and (3). The stimulus is turned on at time $t = 0$ [see Eqs. (3) and (4)]. Top panels: cytokine concentration (T). Middle panels: Active NF-κB concentration (N). Bottom panels: regulator concentration (R) (representing the combined effect of all inhibitors). The unit [M] stands for molar concentration. K_A and N_T are normalization constants of T and N, respectively. (a) The weak stimulus ($S = 0.5\,M/(K_A\ \mathrm{hr})$) causes T to increase a little but not enough to activate N. The system comes to rest in a new steady state with low concentrations of all three variables. (b) The intermediate stimulus ($S = 1\,M/(K_A\ \mathrm{hr})$) causes the system to oscillate. The increase in T exceeds the triggering threshold for activating N and consequently, all variables rise to high levels. The high level of R inhibits N, which decreases back to almost prestimulation levels after approximately 2 hours. At low N level T and R will begin to decrease; R decreases slowly because of the slow degradation rate r_{off} [see Eq. (2)]. After approximately 9 hours R has decreased back to prestimulation levels and the system spikes again. (c) At high stimulus ($S = 2\,M/(K_A\ \mathrm{hr})$) the system will not settle back to prestimulation levels, because the inhibition from R is not enough to drive N back down, once the positive feedback is present. As a result, the system comes to rest in a new steady state in which both N and T levels are much higher than triggering levels. R is sustained at a high level, creating an infinite refractory period.

the cytokine triggering threshold for activating N is reached when T exceeds $T^* \approx 1$.

The parameter K [Eqs. (3) and (5)] describes the NF-κB positive-feedback threshold for internal transcription of cytokines. To achieve maximal sensitivity to N this parameter was chosen to match approximately half-maximum of the initial N peak, which gave $K = 0.3$. (N reaches a maximum of $\approx$0.6 in our simulation.)

The rate constants k_{activate}, k_{inhibit}, r_{on}, and r_{off} have been fitted to match the typical time scale of the NF-κB initial peak ($k_{\mathrm{activate}} = k_{\mathrm{inhibit}} = r_{\mathrm{on}} = 5.0\ \mathrm{hr}^{-1}$ and $r_{\mathrm{off}} = 0.5\ \mathrm{hr}^{-1}$). The lifetime and diffusion constant of TNF have previously been estimated [13] and are used here as the cytokine lifetime, $\tau = 25$ minutes, and diffusion constant, $D = 2 \times 10^{-7}\,\frac{\mathrm{cm}^2}{\mathrm{min}}$. Thus the only free parameter of our model is the parameter p. This parameter sets the strength of the positive feedback, and as we shall see in the Results section, this parameter can be varied to be both too small, not obtaining an adequate feedback, or too large, making the system incapable of returning to resting state.

III. RESULTS: TEMPORAL BEHAVIOR OF A SINGLE CELL

The system described by Eqs. (1), (2), and (3) is simulated starting from an initial steady state where all concentrations are low and there is no stimuli ($S = 0$). At time $t = 0$ the system is stimulated by "turning on" the small cytokine flux ($S = S_{\mathrm{on}}$). Had there been no interaction with NF-κB, T would increase to a steady-state level given by a balance between S_{on} and τ [see Eq. (3)]. But if the stimulation S_{on} is strong enough (roughly speaking, if T exceeds the threshold $T^* \approx 1$), the system will respond with an up-regulation of N, which in turn amplifies T to values manyfold larger than the initial stimulation. Depending on the value of S_{on}, three qualitatively different scenarios can be achieved: if S_{on} is too small the increase in T will not activate N [Fig. 2(a)]. If S_{on}, on the other hand, is large enough to make T exceed the triggering threshold, $T^* \approx 1$, N will increase and cause T to increase further [Figs. 2(b) and 2(c)]. As a result N will also increase to a high level and consequently activate production of its own inhibitors: R begins to increase. As R peaks the negative feedback causes N to decrease and settle back to lower values. If S_{on} is large, [Fig. 2(c)] a new steady state will be obtained in which R is high and both N and T are balanced at levels significantly higher than prestimulation values [Fig. 2(c)]. Interestingly, intermediate values of S_{on} [Fig. 2(b)] will lead to situations where N and T settle back to prestimulation values when R is high. Because N decreases to such low values, the inhibitor R will also start to decrease, although this is a slow process because of the slow degradation rate r_{off} [see Eq. (2)]. When R decreases sufficiently N is no longer inhibited and after some time N and T can peak again [Fig. 2(b)].

A. Phase-plane analysis of the system

The intermediate S_{on}, leading to oscillatory behavior, is of course a very interesting situation. The system has many things in common with classical excitable media, such as, e.g., the Belousov-Zhabotinsky reaction, and we follow an approach similar to the one described in the review by Meron [14].

Notice that N and T are fast variables whereas R is a rather slow variable. Thus the model contains two effective time scales and we can assume that N and T will effectively reach steady state and follow changes in R adiabatically. In order to understand the system in greater detail, we plot the nullclines of N and T for fixed values of R. The nullclines are plotted in N-T space (see Fig. 3). Before stimulation ($t < 0$) the

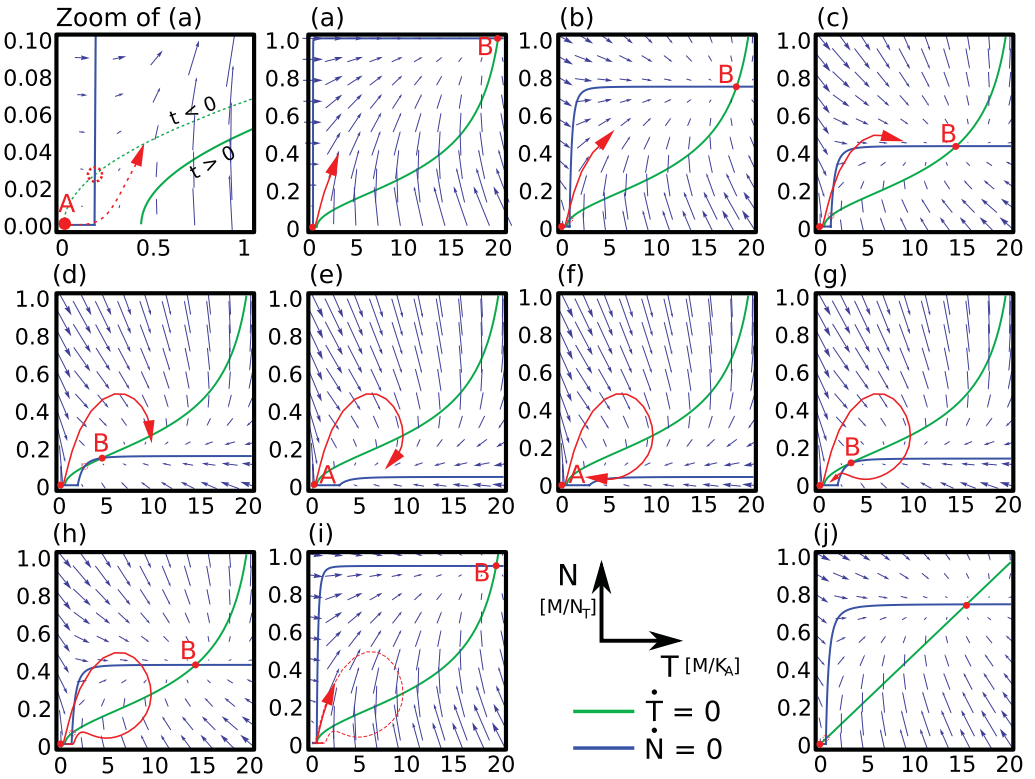

FIG. 3. (Color) The situation shown in Fig. 2(b) is here shown in the phase plane of N and T. Nullclines are plotted in blue ($dN/dt = 0$) and green ($dT/dt = 0$) lines. Stable fixed points are indicated with solid red dots. Unstable fixed points are indicated with dashed red circles. Initially ($t < 0$) the system has three fixed points located at the intersections of the nullclines. Two of these fixed points have low N and T values and are shown in the zoom of panel (a) (first panel). The system is at rest in the low stable fixed point, which we refer to as fixed point A. At time $t = 0$ the stimulus S is turned on [see Eqs. (3) and (4)], causing the T nullcline to shift to the right as shown in the zoom of panel (a) (first panel). Consequently, fixed point A and the unstable fixed point disappear in a saddle-node bifurcation and the system starts to evolve toward the fixed point with high N and T, which we refer to as fixed point B [see panel (a)]. As N increases, R will also increase [see Eq. (2)], causing the N nullcline to move as shown in panels (b)-(e). The system will dynamically change and always evolve toward the stable fixed point, eventually causing N and T to decrease [panels (d)-(f)]. At some point R becomes so large (the N nullcline has moved so far) that fixed point B disappears in a saddle-node bifurcation [panels (d) and (e)], and the system will now evolve toward fixed point A which has been re-established [since panel (b)]. At this point N has decreased back to a relatively low level and R will consequently begin to decrease, causing the N nullcline to move back [panels (f)–(h)]. Meanwhile, the system is caught in the basin of attraction of fixed point A [see panel (g)] and will move toward this fixed point [panel (h)]. Eventually, R has decreased sufficiently and the N nullcline has moved such that fixed point A disappears again and the system begins a new round in phase space [panel (i)]. The times corresponding to the panels are: (a) $t = 0.0$ to $t = 1.0$, (b) $t = 1.1$, (c) $t = 1.2$, (d) $t = 1.4$, (e) $t = 1.6$, (f) $t = 2.0$, (g) $t = 2.7$, (h) $t = 3.7$, and (i) $t = 9.1$ hours. Panel (j) shows the nullclines as they would look if cytokine production (up-regulation of T) had been modeled with a simple linear term pN instead of the sigmoidal term $(N^2/(N^2 + K^2))$ used in Eq. (3).

nullclines intersect in three distinct fixed points—two stable fixed points separated by an unstable fixed point in between. We refer to the two stable fixed points as fixed point A and fixed point B. For $t < 0$ fixed point A and the unstable fixed point lie very close to each other in N-T space, and both have relatively low levels of N and T. [See intersections of dotted green line and blue line in the first panel of Fig. 3 (zoom of panel (a))].

When S is shifted from $S = 0$ to $S = S_{\text{on}}$, the T nullcline is shifted to the right by an amount $\delta = \Delta S\tau$. Hence, if S_{on} is large enough, fixed point A and the unstable fixed point will disappear in a saddle-node bifurcation, and the only fixed point of the system is now fixed point B [Fig. 3(a)]. As the system begins to evolve toward fixed point B, N increases and causes R to increase correspondingly. As this happens the N nullcline will begin to move, dynamically changing the phase space as shown in Figs. 3(a)–3(c). The system will continuously evolve toward fixed point B as it moves "down" [Figs. 3(a)–3(c)], eventually making N and T decrease [Fig. 3(d)]. While the N nullcline moves, fixed point A and the unstable fixed point have re-established in a new saddle-node bifurcation [since Fig. 3(b)]. Eventually R will increase to such high values that fixed point B coalesces with the unstable fixed point and disappears in a second saddle-node bifurcation [Figs. 3(d) and 3(e)]. Now the system will evolve toward fixed point A, causing N and T to decrease back to almost prestimulation values [Figs. 3(e) and 3(f)]. As N is no longer high, R will no longer be up-regulated and will begin to decrease because of spontaneous degradation. This will cause the N nullcline to move "back" [as shown in Figs. 3(f)-3(h)], although as mentioned above this is a slow process (because of slow r_{off}). As the N nullcline moves, fixed point B and

the unstable fixed point are re-established [Fig. 3(g)], but now the system is caught in the basin of attraction of fixed point A [Fig. 3(g)]. As R slowly decreases, the system rests in fixed point A [Fig. 3(h)]. Eventually, the N nullcline has moved such that fixed point A and the unstable fixed point once again disappear in a saddle-node bifurcation, and the system will once again make a round in the phase space [Fig. 3(i)].

The three qualitatively different scenarios of Fig. 2 can be well understood from an investigation of the phase space. In order to exhibit oscillations the system must be able to undergo the two saddle-node bifurcations described above: first, fixed point A and the unstable fixed point coalesce, and second, fixed point B and the unstable fixed point coalesce. The value of S_{on} sets the size of the T-nullcline shift, $\delta = \Delta S\tau$ (recall Fig. 3, first panel). A too-small S_{on} will not cause the first bifurcation because the T nullcline is not shifted far enough. A too-high S_{on} will inhibit the system from undergoing the second bifurcation because the shift is too large and the system will come to rest in fixed point B.

As mentioned in the Model section, we could also choose to model the positive feedback from NF-κB on cytokine production as a simple linear response, pN, instead of the sigmoidal response, $N^2/(N^2 + K^2)$, which is only valid if NF-κB is truly a dimeric transcription factor [see Eq. (1)]. In this case the T nullcline would be a straight line and the N nullcline would remain unchanged. We plot this situation in Fig. 3(j), from which it can be inferred that such a simplification of the model would lead to similar results. From this plot we conclude that at least one of the nullclines must have a sigmoidal form in order to obtain a bistable system. This means that a smaller Hill coefficient, $H = 2$, would suffice in Eq. (6). Hence a minimal model could be obtained by modeling N and T dynamics by the equations

$$\frac{dN}{dt} = k_{\text{activate}} \frac{T^2}{T^2 + K_A^2} \times (N_T - N) - k_{\text{inhibit}} R$$
$$\frac{dT}{dt} = pN - \frac{T}{\tau} + S.$$

Compare with Eqs. (1), (3), and (6).

B. The excitability of the system depends on the strength of positive feedback p

The effect of the positive feedback can be understood by investigation of the nullclines upon variation of p [see Eq. (3)]. The slope of the T nullcline is roughly set by p [see dashed green lines in Fig. 4(a)]. Qualitatively there are three distinct behaviors with weak, intermediate, and strong feedback being similar to the three states with weak, intermediate, and strong stimuli in Fig. 2.

If p is small ($p \approx 10$) the slope of the T nullcline is very steep and hence fixed point B will have a small T value. The system cannot get excited as even a small increase in R will move fixed point B down to low N and T values and the system will have only a very small round in the phase space before reaching this fixed point. The system comes to rest in fixed point B, because R will never become large enough to cause the second saddle-node bifurcation. The resulting situation is very similar to the one in Fig. 2(a).

On the other hand, a strong positive feedback ($p >\approx 100$) allows for a single excitation followed by an infinite refractory period. Large p makes the slope of the T nullcline flatter [Fig. 4(a)]. Right after the stimulus is induced the system follows a long trajectory in the phase space, resulting in a spike in N and T. However, the system comes to rest in fixed point B because the maximal R value is not high enough to move the N nullcline sufficiently far down for fixed point B to disappear in a saddle-node bifurcation. In this case fixed point B has significantly higher N and T levels, meaning that the cytokine concentration is sustained high above the triggering level. The relatively high N level causes R to be sustained at a high level, hence creating an infinite refractory period. This situation will be very similar to the one shown in Fig. 2(c). We refer to this situation as a locked state because the nullclines are locked in fixed point B, even when the stimulus is removed. In the picture of inflammatory response the locked situation would correspond to *chronic inflammation*.

The nullclines of the system can of course also be altered by other parameters of the model, and in order to explore changes in cytokine production we have varied the parameter τ which determines the typical lifetime of the cytokines before they are degraded (the inverse degradation rate). Hence a high

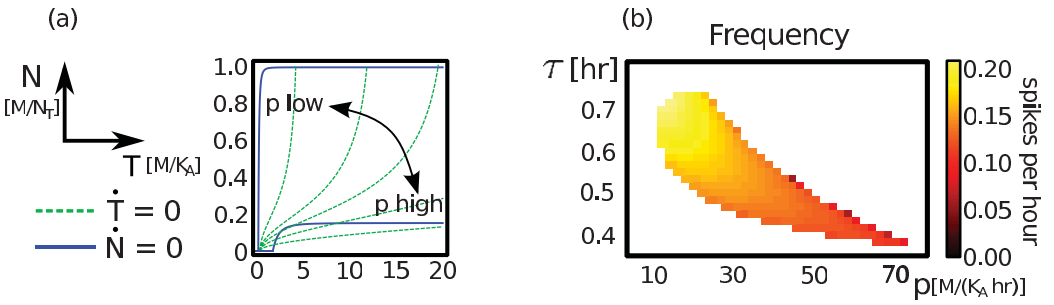

FIG. 4. (Color online) (a) The slope of the T nullcline (dashed green line) becomes steeper as p decreases and flatter when p increases. The N nullcline (solid blue line) is shown for two different values of R and will move from the high plateau to the low plateau as R increases (recall Fig. 3). In the case of small p the N nullcline will not need to move very far before fixed point B (recall Fig. 3) has moved to relatively low levels of N and T, hence creating a situation as shown in Fig. 2(a), where the system comes to rest in fixed point B. In the case of high p the system will also come to rest in fixed point B, which in this case is created at high T levels. R will never become large enough to make fixed point B disappear in a bifurcation and the system is locked in fixed point B. (b) Combinations of the parameters p and τ which lead to oscillatory behavior. The color of the graph indicates the frequency of the oscillations.

p (a high production rate of cytokines) should be counteracted by a low τ in order to keep the cytokine concentration balanced such that it can repeatedly transcend the triggering threshold at $T^* \approx 1$, corresponding to repetitive rounds in phase space as shown in Fig. 3. In other words, the nullclines must lie such that they are able to undergo saddle-node bifurcations both at fixed point A and at fixed point B. Whereas p sets the slope of the T nullcline, τ sets the size of the shift to the right when the stimulus S is introduced. The frequency at which the system can spike depends on how fast the system will undergo the two bifurcations. In Fig. 4(b) we show a plot of the combinations of p and τ which lead to self-oscillatory situations together with their spiking frequencies.

IV. RESULTS: SPATIOTEMPORAL MODEL OF THE TISSUE LEADS TO PROPAGATING WAVES

When the cells are coupled in space and cytokines are allowed to diffuse between them, waves of high cytokine concentration arise [see Fig. 5(a)]. We have constructed a spatial model consisting of a one-dimensional lattice of cells. Every cell is able to regulate cytokine production as described in Eqs. (1), (2), and (5), and only the variable T is allowed to diffuse between cells. We use open boundaries representing the bloodstream in which the cytokines (T) will be absorbed. During inflammatory response only cells at the site of infection would be subject to the external stimulus S [see Eq. (5)], and we simulate this by adding the external stimulus S only to the central cell of the one-dimensional lattice; $S_i = S \times \delta(i,0)$. Adding the diffusion term [see Eq. (5)] causes the effective removal of cytokines to become larger, and in order to counteract this we have increased S tenfold compared to the above ($S_{\text{on}} = 10\ \text{hr}^{-1}$).

At time $t = 0$ the central cell is stimulated and starts to amplify the cytokine concentration. The cytokines will diffuse to neighboring cells which consequently also get stimulated, and thus a wave is created. We stress that the second (and later) waves arise because of the oscillatory behavior of the central cell which will initialize new waves that can propagate through the system. The cells which do not feel the external stimulus S will only get stimulated when they feel a spillover of cytokines from their neighbors. Hence the situation is indeed cooperative in the sense that the cytokine wave is truly propagated from one cell to the next; the cells are not oscillating individually. If the external stimulus S is removed from the central cell, no new waves will be initialized and the system will settle back to rest as soon as the last wave has reached the absorbing boundary.

A. Space contributes to the robustness of the model

An interesting observation is that the spatial model seems more robust toward creating repetitive waves. In Fig. 5(d) we plot the combinations of p and τ which lead to propagating

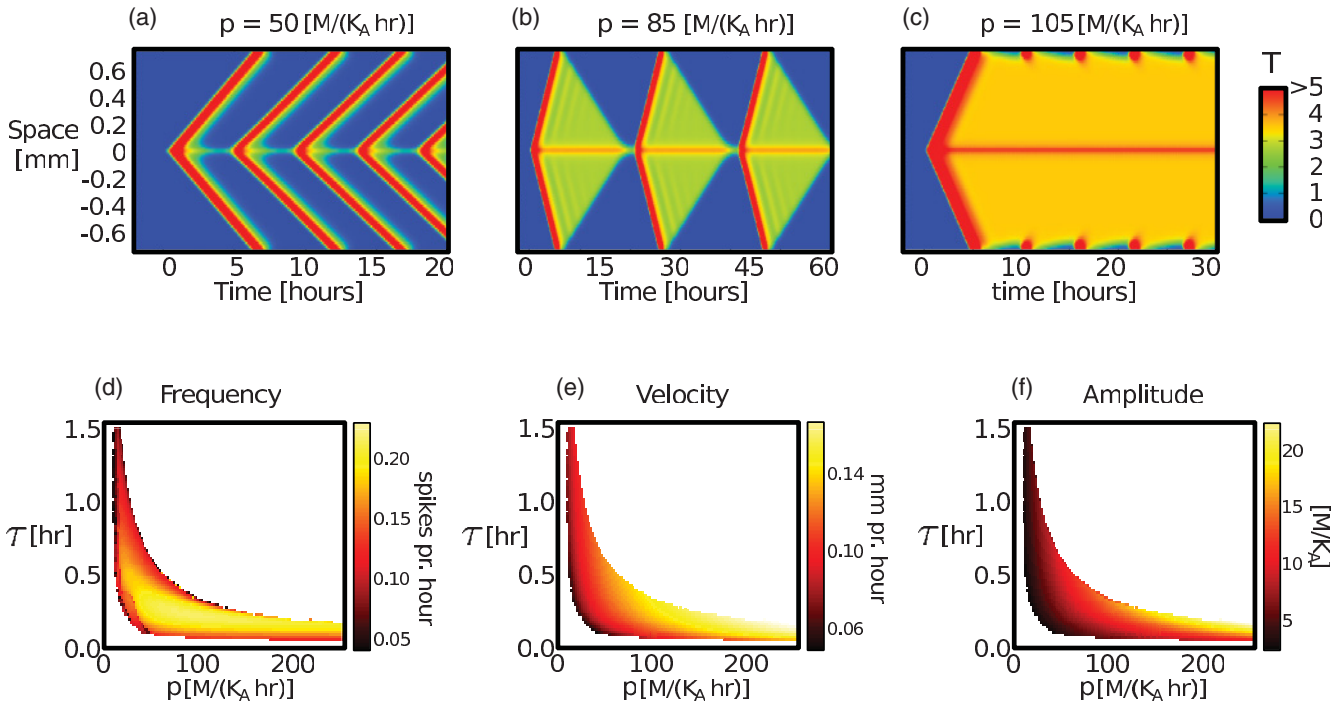

FIG. 5. (Color) (a)–(c) Space-time plots of the cytokine (T) concentration for three different values of the parameter p, which describes the strength of the positive feedback between N and T [see Eqs. (1), (2), and (5)]. (a) The central cell is stimulated at time $t = 0$ and initializes waves of high cytokine concentration which are propagated through the spatial system. (b) At higher values of p the positive feedback is so strong that the system becomes flooded with cytokines (the cells are in the locked state described in the text). But diffusion effects from the absorbing boundaries enable the system to resettle to prestimulation values and new waves can propagate. (c) If p is very large only the cells close to the boundary will be able to escape the locked state. Here oscillations will arise even though p is very high. Notice the different time scales. (d)–(f) Combinations of p and τ which lead to repetitive waves in the spatial model. We plot frequency (d), velocity (e), and amplitude (f).

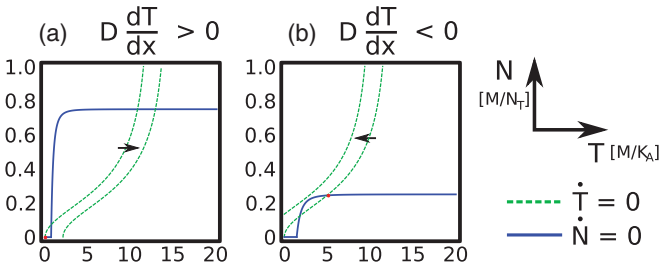

FIG. 6. (Color online) Diffusion effects will shift the T nullcline (dashed green line) horizontally and can be both positive (corresponding to a shift to the right) and negative (corresponding to a shift to the left). (a) The N nullcline (blue line) is plotted for a relatively low R level. If the diffusion term is positive, it can cause the system to bifurcate such that fixed point A disappears. This stimulates the system to move around in phase space as shown in Fig. 3. (b) The N nullcline (blue line) is plotted for a relatively high R level. If the diffusion term is negative, it can cause the system to bifurcate such that fixed point B disappears and the system is unlocked from the locked state. This effect contributes to the ability to bifurcate at both fixed points and makes the spatial model more prone to exhibit repetitive waves than the single isolated cell.

waves. As can be seen from the plot, there are far more p-τ-combinations that lead to repetitive waves than in the case of a single isolated cell [recall Fig. 4(b)]. Figures 5(d)–5(f) also display how typical wave characteristics such as frequency, velocity, and amplitude change with p and τ. Velocity and amplitude of the waves grow with increasing p (and decreasing τ), which leads to strong and fast cytokine production. On the other hand, the frequency is highest where p and τ are correctly balanced, in order to be able to undergo the saddle-node bifurcations, described above, as fast as possible.

The reason why the spatial model is more robust can be found in the effects of diffusion. In the nullcline picture, the diffusion term [see Eq. (5)] acts to shift the T nullcline horizontally (see Fig. 6). As opposed to the external stimulus S, which also shifts the T nullcline horizontally, the diffusion term can become both positive and negative. A positive diffusion term corresponds to cytokines diffusing in from the neighbors, leading to an increased positive flux of cytokines and hence a shift of the T nullcline to the right [see Fig. 6(a)]. In this situation the diffusion terms acts as a stimulus just like S, but a stimulus which travels through space and stimulates the cells one by one, creating a wave. On the other hand a negative diffusion term, meaning that cytokines diffuse away, leads to a shift of the T nullcline to the left. The spacial organization increases the chance that somewhere between the source and the absorbing boundary there will be a cell where the positive and negative diffusion terms balance such that cells can undergo saddle-node bifurcations at both fixed points. If, for example, p is high, a group of cells near the center become locked in fixed point B (locked state). For cells further away from the source a large negative diffusion term will shift the T nullcline to the left [see Fig. 6(b)]. In this situation the diffusion term unlocks the system so that it will again be able to undergo the bifurcation; hence the diffusion term expands the parameter space that can undergo both bifurcations and hence create waves.

Of course, some combinations of the parameters p and τ will lead to situations where most cells in the system cannot undergo bifurcations because diffusion is not strong enough. This can lead to situations where almost all cells become locked in fixed point B [see Fig. 5(c)]. In this situation we still observe oscillations but only close to the boundaries. These oscillations arise because the diffusion term will be very large and negative close to the boundary. Hence the cells which are close enough to the boundary will always be able to undergo bifurcations and oscillate. In Fig. 5(b) we show an intermediate situation where diffusion into the absorbing boundaries also plays a large role — it enables the system to oscillate, although with a smaller frequency.

V. CONCLUSION

The simple model presented in this paper captures many of the most important features of the NF-κB system, although it is highly simplified and consists of only three variables. The model essentially consists of a coupled positive and negative feedback, which makes it able to transiently amplify a signal of high cytokine concentration. This simple system provides a good tool for investigating and understanding the interactions between NF-κB and cytokines, especially because it makes it possible to explore the phase space, thereby achieving a greater understanding of the parameters.

The model captures how a single unit (cell) can become an oscillator if it is stimulated appropriately (close to the site of infection), but also how it can simply pass on the signal if it is stimulated transiently (in tissue farther away).

From phase-space analysis we conclude that the system is bistable and able to oscillate because it can undergo bifurcations, shifting the system between low and high fixed points [14]. The phase-space analysis also provides a useful understanding of the unknown parameter p, describing the strength of the positive feedback between NF-κB and cytokines. We find that the positive feedback must have an appropriate intermediate strength in order to create oscillations. Too-weak positive feedback leads to almost no response, whereas too-strong positive feedback leads to a sustained strong amplification of cytokine concentration, a situation which can be related to chronic inflammatory response.

A spatial model is highly relevant for understanding possible spatial effects that might appear in nature and which are not captured in most laboratory experiments because of space-averaging or mixing. Our spatial model of the tissue naturally leads to the propagation of traveling waves of high cytokine concentration, because the system behaves as an excitable medium.

Excitable media are also observed in many other biological systems which share the need of sending information over many-cell distances, and the resulting traveling waves are in good agreement with the expected spatial form of a neutrophil directing signal.

We find that spatial effects play a large role in the model and contributes to the model's ability to propagate repetitive waves. By changing the parameters of the model, we observe qualitatively different spatial patterns and we see that a even very strong positive feedback leading to chronic inflammation gives rise to oscillations close to the absorbing

boundaries representing the blood stream. Hence the situation corresponding to chronic inflammation would also recruit neutrophils from the bloodstream, but they would not be able to orient themselves once in the tissue because there is no directed signal to guide them.

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