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To cite this article: Quentin Marcou *et al* 2018 *Phys. Biol.* **15** 056001

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Physical Biology



PAPER

A model for the integration of conflicting exogenous and endogenous signals by dendritic cells

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Keywords: signaling, immunology, modeling

Supplementary material for this article is available [online](#)

RECEIVED
18 August 2017

REVISED
13 January 2018

ACCEPTED FOR PUBLICATION
23 January 2018

PUBLISHED
16 May 2018

Abstract

Cells of the immune system are confronted with opposing pro- and anti-inflammatory signals. Dendritic cells (DC) integrate these cues to make informed decisions whether to initiate an immune response. Confronted with exogenous microbial stimuli, DC endogenously produce both anti- (IL-10) and pro-inflammatory ($\text{TNF}\alpha$) cues whose joint integration controls the cell's final decision. Backed by experimental measurements we present a theoretical model to quantitatively describe the integration mode of these opposing signals. We propose a two step integration model that modulates the effect of the two types of signals: an initial bottleneck integrates both signals (IL-10 and $\text{TNF}\alpha$), the output of which is later modulated by the anti-inflammatory signal. We show that the anti-inflammatory IL-10 signaling is long ranged, as opposed to the short-ranged pro-inflammatory $\text{TNF}\alpha$ signaling. The model suggests that the population averaging and modulation of the pro-inflammatory response by the anti-inflammatory signal is a safety guard against excessive immune responses.

1. Introduction

Cells constantly integrate signals to adapt to their environment. In the immune system, activating signals are critical to initiate and sustain an efficient immune response, and co-exist with inhibitory signals in order to avoid excessive and uncontrolled immune responses [1, 2]. Immune cells must often integrate such opposing signals, the outcome being key to decision making between immunity versus tolerance [3–5]. This signal integration process in immune cells involves many check points that can involve kinetic proofreading [6–8] or multiple feedback loops [9, 10]. In general, feedback allows the system to adjust its output in response to monitoring itself. Both positive and negative feedback loops have been found crucial to control the strength and duration of the system's activation in order to achieve optimal responses. Such loops represents a fundamental feature in cell development and differentiation [11],

hormonal homeostasis [12], intracellular signalling [13] and in the immune response [1]. Cells can receive feedback through paracrine signals coming from their neighbours or from their own autocrine signals [14, 15]. Since the adaptation to the environment occurs at the population level, autocrine and paracrine feedback may play a different role in a cell population responding to opposing signals, notably as a function of cell density.

Dendritic cells (DC) are an essential component of the innate immune system. Acting as the body's sentinels, they are equipped with a diversity of innate receptors, including pattern recognition receptors such as toll like receptors (TLRs). Engagement of TLRs by TLR ligands leads to DC maturation, a complex process which includes migration to draining lymph nodes, secretion of a diversity of chemokines and cytokines, as well as up-regulation of major histocompatibility class II (MHC-II) and co-stimulatory molecules, such as CD80 and CD86 [16]. The latter represent crucial

molecular checkpoints for orchestrating DC-T cell communication, playing a key role in the activation and expansion of CD4 T cells [17].

A critical question is how the diversity of signals sensed by DC controls the outcome of the DC maturation program. In this process, we can discriminate exogenous signals, i.e the nature and dose of microbial stimuli, and endogenous signals, such as autocrine factors induced by exogenous stimulation. When DC are activated by the bacterial component LPS (exogenous signal), they respond with an increased secretion of TNF- α (TNF α) and interleukin (IL)-10, generally considered as prototypical pro- and anti-inflammatory signals, respectively [18, 19]. As DC are equipped with the corresponding receptors, both TNF α and IL-10 act as endogenous auto-regulatory feedback signals that control the output response of the cell, and influence the final decision to initiate an immune response or not. Current and past studies have mostly studied each of these signals separately. LPS effect on DC has been extensively studied, including at various concentrations revealing dose-dependent effects [20, 21]. Few studies have addressed the role of the IL-10 negative feedback loop, showing that it dampens LPS-induced maturation [22]. TNF α is a DC-activating pro-inflammatory cytokine [19], but its role as a putative positive feedback factor on DC remains elusive. Studies of these DC-targeting regulatory signals suggest strong dependencies and cross-regulatory mechanisms between LPS, IL-10 and TNF α , but the underlying rules remain unexplored. Here we study MoDC (dendritic cells matured from monocytes) that do not express IL-12 receptors and are not strongly affected by TGF- β signaling [23]. In these cells IL-10 has a strong signaling impact, so we consider its role in DC maturation. We also note that the pro- and anti-inflammatory nature of the signals can depend on the cellular context. In the MoDC maturation system TNF α and IL-10 do behave as pro- and anti-inflammatory signals, respectively [18, 19]. Mechanistic understanding requires the integrated analysis of variations in the three signals level, and their consequences on the behaviour of the system.

In this study, motivated by measurements, we propose a minimal theoretical model that explains the experimentally observed effects of LPS, IL-10, and TNF α effects on human DC. Our original model describes the interplay between contradictory exogenous and endogenous signals in the control of DC maturation.

2. Results

2.1. LPS-induced TNF α and IL-10 differentially control DC maturation

Upon activation by the TLR4 ligand LPS, DC undergo a maturation process leading to an upregulation of costimulatory molecules, such as CD86, but also production of pro- and anti-inflammatory cytokines.

CD86 is a classical marker for DC maturation [24], and we will use its expression as a surrogate for it. LPS is not toxic when incorporated into the bacterial outer membrane, but is toxic in solution [25]. We designed an *in vitro* setup to study how it regulates the expression of the downstream cytokines in solution. First, we measured the production of TNF α and IL-10 in response to a standard LPS concentration of 100 ng ml⁻¹ [26, 27]. The secretion of TNF α was more rapid and was significant already after 2 h, while IL-10 was detected only after 4 h following LPS stimulation (figure 1(A)), as previously reported [28]. After 4 h, both cytokines were detected concomitantly in the cellular supernatant (figure 1(A)). TNF α and IL-10 reached concentrations of 3.3 ng ml⁻¹ and 0.18 ng ml⁻¹, respectively after 6 h figure 1(A). In order to address the contribution of these two endogenous cytokines on DC maturation, we monitored CD86 using flow cytometry, in the presence and absence of blocking antibodies (Ab) to TNF α or IL-10 (figure 1(B)). LPS induced significant upregulation of CD86, consistent with an increase in DC maturation (figure 1(B)). Blocking the IL-10 loop induced a significant increase in CD86 expression. This suggested that IL-10 had a dominant negative effect in controlling LPS-induced DC maturation.

2.2. LPS dose determines the endogenous IL-10 and TNF α control of DC maturation

Microbial-derived signals occur at various concentrations in infected tissue, in relationship to the *in situ* microbial load. This process is also linked to microbial clearance, which induces a local decrease in microbial signals. First, we addressed the impact of various LPS doses on endogenous TNF α and IL-10 production (figure 2(A)). Both cytokines exhibited a similar LPS dose-dependent pattern, reaching maximum levels at a LPS concentration of 100 ng ml⁻¹ (figure 2(A)).

Given that TNF α and IL-10 co-exist at variable LPS concentrations, we asked whether LPS levels impact the way these endogenous signals are being integrated by DC. To address this question, we cultured DC in the presence or absence of blocking Abs to the TNF α and IL-10 receptors (TNFR and IL10R) while stimulating them with different concentrations of LPS achieved by serial dilutions (figure 2(B)). As for the standard LPS dose, DC maturation was quantified by CD86 expression 24 h following LPS activation. When none of the loops were altered (no blocking or IgG control), the level of activation increased with LPS concentration and reached a plateau for sufficiently high LPS doses (~ 100 ng ml⁻¹) (blue curve in figure 2(B)). Blocking the pro-inflammatory TNF α loop led to a decreased expression of CD86 (red curve in figure 2(B)), while blocking the anti-inflammatory IL-10 loop led to an increased expression of CD86 (green curve in figure 2(B)). However, TNF α loop-blocking decreased CD86 levels mostly at LPS concentrations lower than

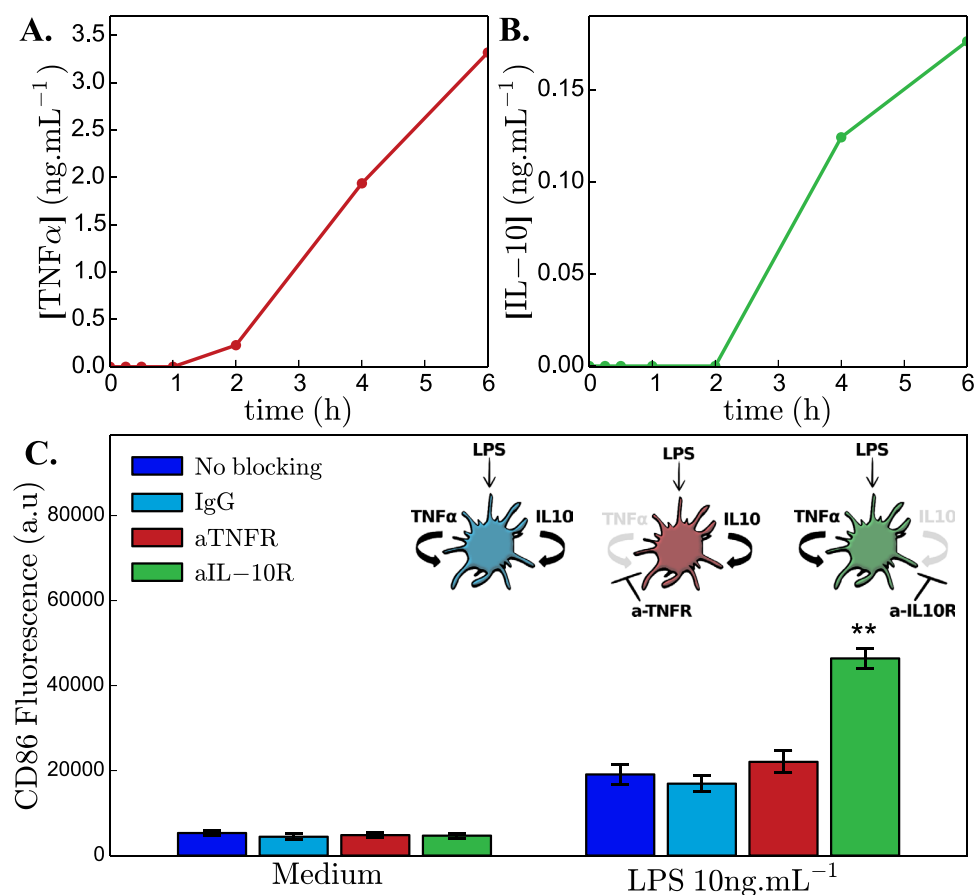


Figure 1. LPS-induced TNF α and IL-10 differentially control DC maturation. Secretion of the cytokines TNF α (A.) and IL-10 (B.) is monitored through time under 100 ng.mL⁻¹ LPS stimulation (shown here is the result of an experiment on a single donor). (C.) CD86 fluorescence of cellular populations is increased by the presence of LPS in the medium after 24 h. Blocking the regulatory loops has no effect when cells are not stimulated when blocking IL-10 pathway increases DC activation. As a control for the non blocking condition, culture with an isotypic antibody does not alter CD86 levels. Bars show the expectation of the log normal distribution, error bars the standard error of the mean of the log normal distributions. Statistical significance of the results is assessed using Welch's t-test in logarithmic space.

10 ng mL⁻¹ (red curve in figure 2(B)). By contrast, the impact of IL-10 loop-blocking on CD86 expression was constant along a wide spectrum of medium to high LPS doses, but absent at low LPS doses (green curve in figure 2(B)). The impact of the two opposite/contradictory loops differed not only in the directionality of the effect but also in mode of the effect: blocking the TNF α loop shifted the onset of the response towards higher LPS dose, while blocking the IL-10 loop affected mainly the amplitude of the response, which significantly increased in the presence of IL-10 blocking compared to its value in the absence of any blocking.

DC maturation with or without blocking the loops in the different LPS doses was quantified using the expression of a second maturation marker CD83. The expression of this marker also increased with increasing LPS dose (figure S1 (stacks.iop.org/Phys-Bio/15/056001/mmedia)). Blocking the TNF α loop led to a similar trend as with CD86, with a strong effect at low LPS doses, and weaker effect at high LPS doses (figure S1). Although both maturation markers were significantly upregulated by LPS, their distribution across the DC population was different. While

CD86 demonstrated a unimodal distribution, CD83 demonstrated a bi-modal one (figure S2). In addition to surface markers, blocking the loops also had a significant effect on cytokine secretion (figures 2(C) and (D)). Importantly, the TNF α and IL-10 loops reciprocally affected each other, as blocking the IL-10 loop increased TNF α secretion (green curve in figure 2(C) compared to the other curves), and blocking of the TNF α loop strongly decreased IL-10 production (red curve in figure 2(D)). This suggests potential cross-regulation of TNF α and IL-10 through DC.

2.3. Modulated bottleneck model explains DC maturation control by opposing endogenous and exogenous signals

In order to qualitatively understand the mechanism behind microbial-induced signal integration in DC, we used the above experimental observations to build a minimal phenomenological steady state mathematical model of CD86 response to LPS stimulation. Our phenomenological model aims at reproducing all the experimental observations (summarized in this paragraph) and previously known facts about the interactions between the three signaling molecules in

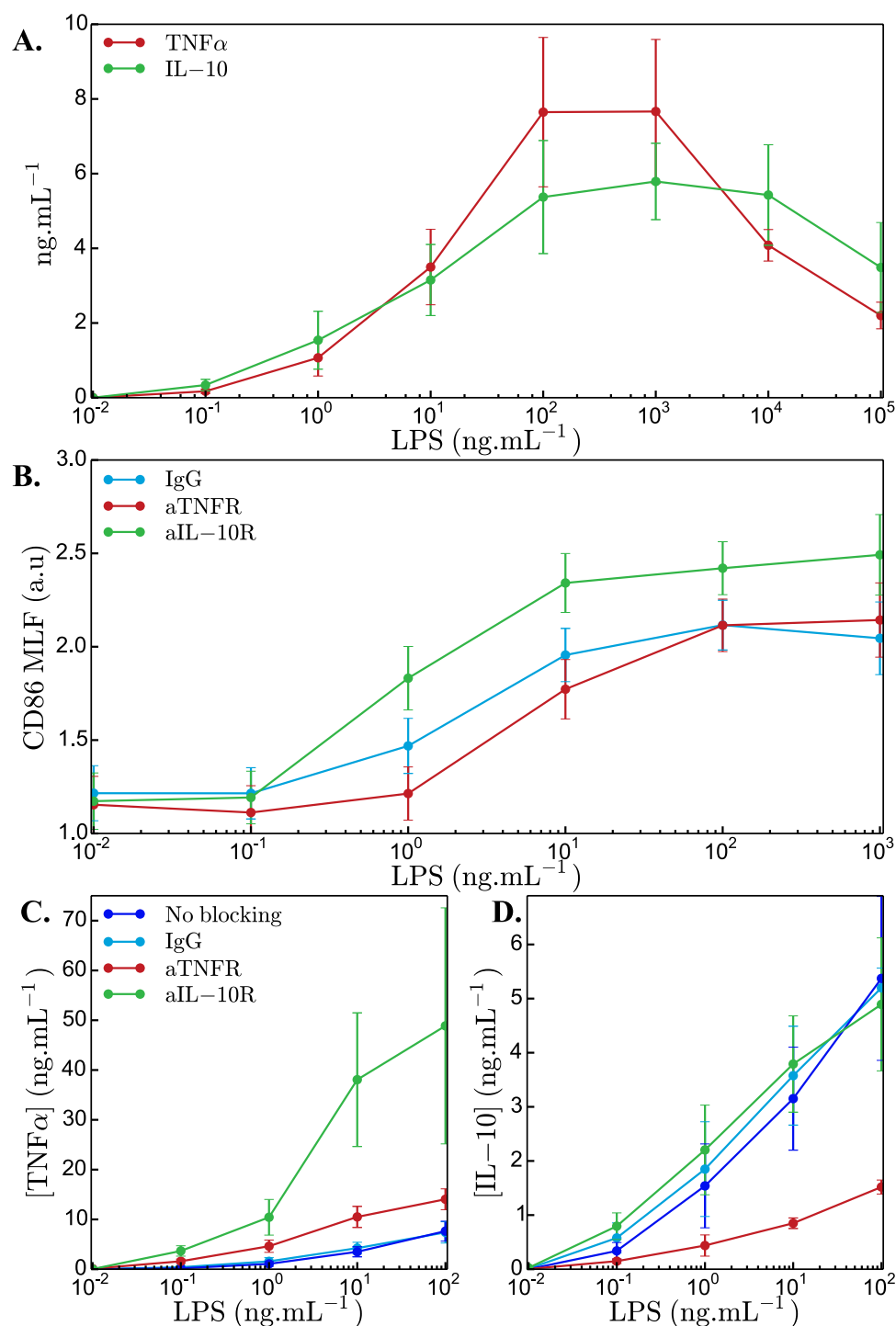
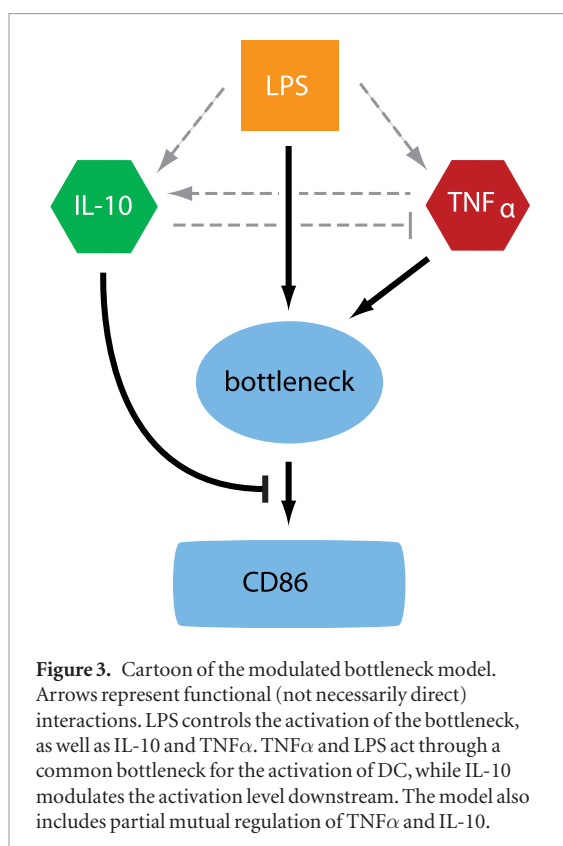


Figure 2. LPS dose determines the endogenous IL-10 and TNF α control of DC maturation. (A.) Titration of TNF α and IL-10 concentrations (ng.mL $^{-1}$) for a wide range of LPS doses after 24 h. Increasing LPS doses increase both TNF α (red) and IL-10 (green) secretion levels. (B.) Activation of DC is monitored by flow-cytometry labeling the co-stimulatory molecule CD86. CD86 mean log-fluorescence (MLF) is shown for a range of LPS concentration incubated for 24 h with isotypic control (blue), anti-TNFR (red) or anti-IL-10R (green) antibodies. CD86 has a sigmoidal dependence on LPS doses. Blocking IL-10 increases the maximal activation level while blocking TNF α decreases the sensitivity. Cytokine response of DC in different conditions, medium (dark blue), isotypic control (blue), anti-TNFR (red), anti-IL-10R antibodies, is measured for different doses of LPS. (C.) Blocking IL-10 increases TNF α secretion (D.) Blocking TNF α decreases IL-10 secretion.

the simplest way, without assuming additional modes of regulation. From figure 2(B) we see that the CD86 response follows a sigmoidal dependence on LPS concentration, which we denote as L and saturates at high LPS level. Additionally, both IL-10 (denoted as I) and TNF α (T) expressions are sigmoidal functions of LPS (figures 2(C) and (D) and see supplementary

material equations (1)–(4)). As we noted above, TNF α upregulates IL-10 expression [29], while IL-10 downregulates TNF α secretion [30, 31] (figures 2(C) and (D)). To avoid behavior that is not observed in the data, we assume there is a basal expression level of both TNF α and IL-10, even in the absence (presence) of the regulator. Results of blocking IL-10 show that



additionally to repressing TNF α , IL-10 also decreases the amplitude of the response (figure 2(B)). Lastly, it has previously been shown that TNF α alone, in the absence of LPS, activates and induces DC [19]. This observation suggested that TNF α does not just act downstream of LPS, but that TNF α and LPS act through a common intermediate in an additive way creating a bottleneck. This last assumption is the main idea behind our model: LPS and TNF α signals are integrated in the expression of one regulatory molecule. The expression of CD86 itself is not regulated directly by TNF α and LPS, but by the concentration and status of this central integrator (figure 3). Since there is no experimental evidence of direct interactions between TNF α and IL-10, and as we will see below we do not need to invoke these interactions to explain the data, we will not consider models with direct regulation.

A schematic representation of the effective regulatory pathway described above is shown in figure 3. A central signal integrator combines the two pro-inflammatory signals, TNF α and LPS, in a single common pathway making this integrator the key regulator of DC decision. The integrator acts as a molecular bottleneck for the pro-inflammatory signals (see figure 3): it responds to increases in the pro-inflammatory signal concentrations only until a certain total concentration. This concentration can be reached either purely by TNF α or purely by LPS, or by their combination (see figure 4(B)). Above this total concentration, set by the effective EC50 (dose at which the response is half of the maximum), the response is saturated and increasing pro-inflammatory signals has no effect on the output. Without the bottleneck effect of the central integra-

tor, the TNF α and LPS pathways would independently control the CD86 response. In this case blocking the TNF α loop would not change the EC50 of the response to LPS. Adding more LPS while the TNF α loop was blocked would lead to a lower saturation level at infinite LPS dose than without blocking. LPS and TNF α are known to control common downstream pathways [32], giving for example NF- κ B as a possible candidate for the bottleneck, which we discuss below. In turn IL-10 has been shown to inhibit NF- κ B activation in human monocytes [33, 34].

The concentration of the integrator molecule controls the amplitude of the response, which is further modulated downstream by the IL-10 anti-inflammatory signal (figures 3 and 4(B)). A plumbing analogy helps illustrate the role of the bottleneck and downstream anti-inflammatory regulation: there is a very high source of water distributed to each house, but the amount of available water is limited by the throughput capacity of the main pipeline (this is the bottleneck that regulates the amount of pro-inflammatory signals—see figure 4(B)). However when you take a shower, you can regulate the waterflow directly at the faucet (this is the inhibitory action of IL-10). In the absence of IL-10, the bottleneck still limits the scale of the inflammatory response. IL-10 can further downregulate it.

The bottleneck model reproduces all the experimentally observed features in figure 2(B). It further predicts the combined effect of blocking both the IL-10 and TNF α loops (figure 4(A)). We graphically represent the predictions of the model for the four blocking conditions at low and high LPS concentrations in figure 4(B). At high LPS dose the bottleneck limits the signaling of the master integrator, regardless of whether both TNF α or LPS are sensed or only LPS, and the IL-10 further reduces the strength of the response. At low LPS concentrations the effect of the bottleneck is reduced but IL-10 further modulates the output. We experimentally validated the bottleneck model by blocking both loops simultaneously in LPS stimulated DC. In agreement with the prediction, the condition in which both loops were blocked affected the CD86 EC50 expression similarly to blocking the TNF α loop (figure 4(C)) (the data from figure 2(B) is replotted in figure 4(C) adding the yellow curve that describes the simultaneous blocking of the two signaling channels). At higher LPS doses the CD86 amplitude increased similarly to blocking IL-10 alone, also in agreement with the model predictions. We also note that the secondary interactions of TNF α activating IL-10 and IL-10 repressing TNF α explain the experimentally observed results presented in figures 2(C) and (D). Since TNF α effectively represses itself (figure 3), blocking the TNF α receptor (red line in figure 2(C)) decreases the response of TNF α directly from LPS signaling, decreasing IL-10 concentrations, and effectively alleviating the IL-10s repression of TNF α , which results in the observed increase of TNF α concentrations in figure 2(C). Similarly, blocking the

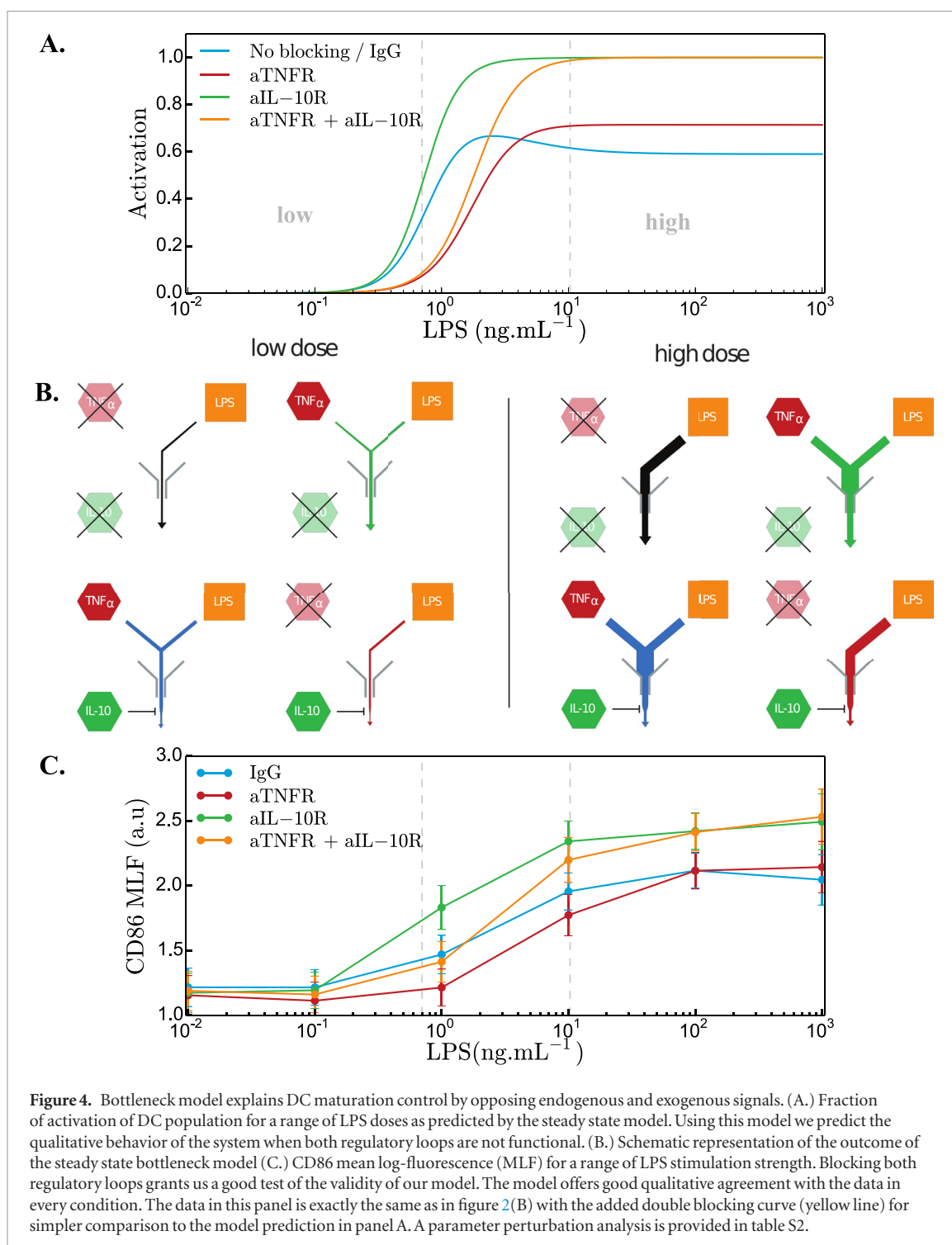


Figure 4. Bottleneck model explains DC maturation control by opposing endogenous and exogenous signals. (A.) Fraction of activation of DC population for a range of LPS doses as predicted by the steady state model. Using this model we predict the qualitative behavior of the system when both regulatory loops are not functional. (B.) Schematic representation of the outcome of the steady state bottleneck model (C.) CD86 mean log-fluorescence (MLF) for a range of LPS stimulation strength. Blocking both regulatory loops grants us a good test of the validity of our model. The model offers good qualitative agreement with the data in every condition. The data in this panel is exactly the same as in figure 2(B) with the added double blocking curve (yellow line) for simpler comparison to the model prediction in panel A. A parameter perturbation analysis is provided in table S2.

IL-10 receptor effectively increases IL-10 concentrations, although the effect is much smaller in magnitude (figure 2(D)).

The model proposes a possible mechanism for signal integration. To test whether the features of the model are constrained by the data, and whether even simpler model assumptions would still be compatible with the experimental observations, we fitted all the parameters of the model to the data of figures 2(B) and (C) using Maximum Likelihood. We assumed Gaussian experimental errors, which we estimated from the pooled error over all donors. We compared models

with various assumptions and levels of simplicity (see supplementary material): a bottleneck model such as described above; a model without a bottleneck, where the LPS, TNF α and IL-10 signals are all integrated into a single regulation function; a model with just LPS and TNF α activation; and a linear model of activation. To compare the models of different complexities, we used the Akaike Information Criterion, which penalizes the likelihood score of a model with the number of its parameters. The bottleneck model was the best fitting model (table S1), indicating that the bottleneck is a necessary ingredient to explain the data. The model

prediction for the levels of cytokines as a function of dose (figure 2(A)) resulting from the fitting procedure are shown in figure S6.

We evaluated the robustness of the parameter fit by sampling from the posterior distribution obtained by Bayes' rule with a flat prior, using a Monte-Carlo algorithm (see supplementary material). The standard errors and confidence intervals for each parameter, reported in table S2, show that all the parameters are constrained by the data within an order of magnitude of their optimal value. This analysis also estimates the effect of varying the parameters on the accuracy of the model: for instance, changing K_C by 50% would make the prediction fall significantly outside the error bars, while changing K_I or K_T by the same amount would have little effect on the output, as these parameters are less constrained by the data.

2.4. Paracrine signalling predominantly controls DC maturation

DC in our experiment, as in the organism, are not isolated and signal integration depends on the diffusion of cytokines: a cytokine produced by a given cell could be picked up by a receptor on the surface of this same cell (autocrine loop) or by a neighboring cell (paracrine loop). Since we cannot directly measure inter-cellular communication with single molecule resolution, we designed and performed cell dilution experiments to get insight into DC communication at a larger spatial scale. At high cellular concentrations, cells can sense signals from nearby cells (figure 5(A)), and at large dilutions, only from themselves (figure 5(C)). Large dilution conditions correspond to pure autocrine signaling. This experiment is based on the assumption that the effect of a purely paracrine loop will decrease as cells are diluted, while a purely autocrine loop will not be sensitive to dilution of the population density. Since the effect of the $\text{TNF}\alpha$ feedback loop was observed at low LPS concentrations, whereas the IL-10 feedback was active at high LPS concentrations, we performed dilution experiments at two distinct LPS doses.

To predict the behavior of the DC response in the dilution experiments we combined our phenomenological bottleneck model (figure 3) with diffusion-based estimates for the probabilities of autocrine and paracrine absorption in an effective heterogeneous medium [35] (see supplementary material for details). The model ignores cross-talk across cytokines. Using previously measured kinetic and geometric parameters (see table S3), the theoretical calculation predicts that a large fraction of the signaling is paracrine in nature. In figures 5(E) and (G) we plot the predictions for the mean log CD86 expression at a low and high LPS concentration as a function of the cell concentration. If most of the signaling is paracrine in nature, as we see that at high LPS concentrations (figure 5(G)), with increasing cell dilutions all the blocking conditions converge to nearly the same activation levels,

equal to the levels predicted in the case when all the loops are non-functional (orange curve in figures 5(B) and (G)). For very low cell density we expect the paracrine feedback loops to have no effect on CD86 expression and all feedback takes place by autocrine loops. Measurements of ligand affinity of $\text{TNF}\alpha$ and IL-10 to their respective membrane receptors [36–38] show that $\text{TNF}\alpha$ has a greater affinity for its receptor than IL-10 does. We thus predict that $\text{TNF}\alpha$ autocrine fraction should be greater than IL-10. Since the effect of the $\text{TNF}\alpha$ feedback is observed at low LPS concentrations, and the IL-10 feedback at high LPS concentrations, we expect the convergence of the curves corresponding to different conditions at low LPS concentrations to be less pronounced than at high concentrations. Our model predicts (see figure 5(E)) that the curves corresponding to blocking the $\text{TNF}\alpha$ loop do not converge to those where the $\text{TNF}\alpha$ is active at high dilutions for low LPS concentration.

To experimentally assess the effect of dilutions on the loops we activated DC with either low (1 ng ml^{-1}) or high (100 ng ml^{-1}) LPS in different cell dilutions with the initial culture concentration being $10^6 \text{ cells ml}^{-1}$ (figures 5(F) and (H)). In agreement with our model we could observe that at both low and high LPS doses all conditions were converging to the same amount of activation. Because of the saturation effect we could not observe a slower convergence for the case of a blocked $\text{TNF}\alpha$ loop for high LPS dose, however it was observable for low LPS dose (figure 5(F)). In the case of the lower LPS dose, in which the $\text{TNF}\alpha$ loop plays a more specific role, we observed that despite serial dilution, the effect of blocking the loop was maintained, at least to some extent, suggesting the existence of an autocrine signaling. Interestingly, in the higher dose of LPS, the effect of IL-10 loop was rather sensitive to dilutions, suggesting that in a context of high microbial load IL-10 acts in a paracrine manner.

3. Discussion

Innate immune recognition is key to promote an efficient anti-microbial immune response, but also needs to be controlled, in order to avoid immunopathology. It is known that immune activating and immune dampening signals are both rapidly produced and co-exist within any inflamed tissue [2]. However, the interplay between exogenous microbial signals, and endogenous pro- and anti-inflammatory signals has not been formalized in an integrated manner. This is critical to the decision making of the immune response, as it is driven by multiple dynamic signals, conveying different types of information to innate immune cells. By combining experiments with modeling, we showed that the final response of the DC population relies on integrating the initial signal with the induced pro- and anti-inflammatory responses using feedback loops. The integration is based on two steps: first the pro-inflammatory signals are integrated

through a bottleneck and then the amplitude of the result is further modulated by the anti-inflammatory signal. The key element of this integration occurs at the signal bottleneck, which controls the effective concentration range (EC50) of the response to LPS and limits the maximum pro-inflammatory response. The anti-inflammatory regulation that follows is mostly paracrine, as opposed to the bottleneck integration that has an autocrine component, suggesting that the final response is modulated based on the population level response.

Bottleneck signal integration in molecular systems have mostly been proposed for the integration of two positive signals. They were suggested as a means for TNF α activation [39]. Here we propose that a bottleneck is the essential component in making the decision to the response in the presence of two opposing signals: IL-10 and TNF α . Since the negative regulation by IL-10 acts after the bottleneck, it regulates the maximum level of activation, while the positive TNF α acts before the bottleneck thereby affecting the activation threshold. The two opposing signals thus control distinct aspects of the dose response. This feature is independent of the fact that the two signals have opposing effects: the possibility of additional pre- or post-bottleneck regulation would have the same effect on two positive signals.

The modulated bottleneck model is purely phenomenological and aims at describing the observed integration in an effective way, by contrast to more detailed mechanistic models such as proposed for TNF α IL-10 interplay in macrophages [40] and microglia [41]. We do not propose a detailed explanation of how the two cytokines are integrated mechanistically. Further experiments are needed to explore this question in more detail, as well as the possible integration of detailed knowledge about signaling mechanisms. The model proposes an integration mode that seems to be dominant in the experimental system we looked at. Of course, real cells function in many environments and other integration modules with behaviors not predicted by the modulated bottleneck could be present in DC. In general, the modulated bottleneck model is consistent with all experimental observations and known forms of interactions. Simple alternatives to the bottleneck hypothesis cannot explain the data. For example, a model with negative feedback acting on any of the signals would give a plateau that would be either sensitive to the activation signals, or insensitive to IL-10 inhibition. Future experiments that block other DC maturation factors with similar regulatory profiles will give more insight into the role of the bottleneck integrator, or whether other integration modules should be favored.

A natural candidate for this bottleneck integrator is the widely studied [42] nuclear factor NF κ B: several studies demonstrated how LPS and TNF α trigger NF κ B nuclear translocation [32], and how IL-10 inhibits NF κ B or its target genes in certain cell types

[43]. Additionally the saturation effect observed in our data was also seen when looking at NF κ B nuclear translocation due to the limited and constant amount of NF κ B [10]. It is also known that, while IL-10 is known to signal through Jak-STAT pathway, IL-10 also inhibits NF κ B [33, 34] giving experimental support for the modulation interaction. Future experiments that block other DC maturation factors with similar regulatory profiles will give more insight into the role of the bottleneck integrator.

Additionally to the main modes of signal integration based on the bottleneck and IL-10 repression, TNF α activates IL-10 expression, while IL-10 represses TNF α . These secondary interactions do not change the basic flow of signal integration, but are predicted by the model to produce a maximum in the CD86 at intermediate LPS concentrations (figure 4(A)). Since LPS activates both IL-10 and TNF α , repression of TNF α slightly shifts the EC50 of the response to larger LPS concentrations, while activation of IL-10 results in a larger moderation of the response than in the absence of TNF α for high LPS concentrations.

The presented results are population averages over multiple independent measurements. The fluorescence distributions plotted in figure S2 show a large heterogeneity in the population, indicating that particular cells can have very different responses. The error bars indicate the standard error of the mean over multiple experiments. The measurement noise is impossible to distinguish from the natural heterogeneity of the response in the population. Given this heterogeneity, the mean CD86 response in the double blocked mutant is consistent with the theoretical prediction.

Dendritic cells often are surrounded by other dendritic cells and, through secreting signaling molecules, communicate with each other to make a decision at the population level. This collective decision making process can help make the right readout in a noisy environment thus reducing response variability as for wound healing [44]. By sharing their response, cells in a population can confirm initial measurements by sensing the signals that their neighbors secrete. Alternatively, cells could simply use the feedback loops to amplify their own initial signal to accelerate their response.

Previous experiments have highlighted the difference between population and single cell measurements in TNF α responses [32]. The nature of the signal (paracrine or autocrine) controls the spatial range of the responding cells and determines the lengthscale on which the decision is made. Feedback loops are necessary elements for integrating population-level signals. The signalling range controls whether there is population level averaging, or whether each cell only listens to itself. Here, by using a combination of dilution and fluorescence experiments with modelling, we show that the anti-inflammatory IL-10 signal is paracrine and long range, as opposed to the autocrine and short range pro-inflammatory TNF α signal. Cells rely

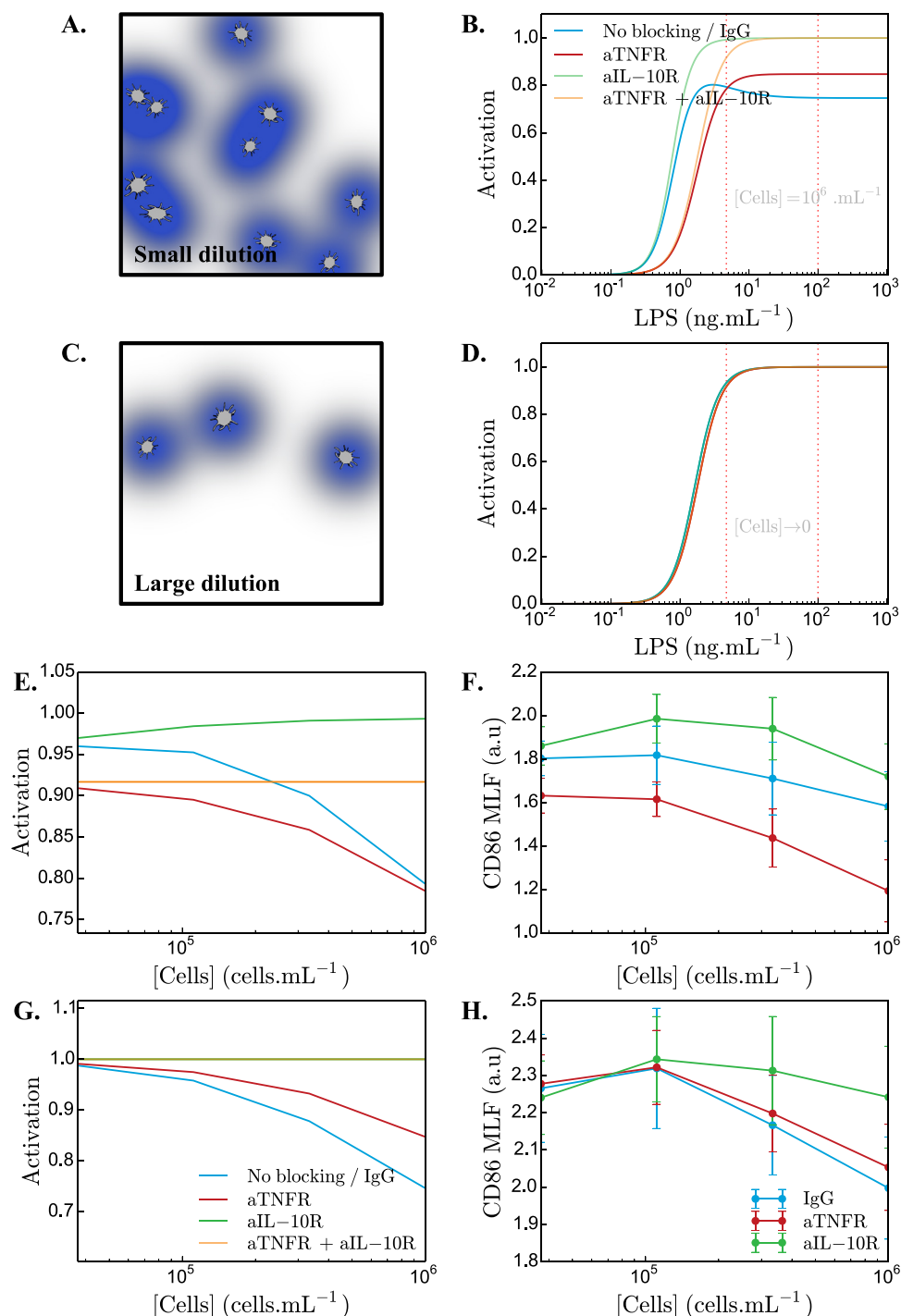


Figure 5. Discriminating autocrine from paracrine loops using dilutions. (A.) and (B.) Cartoon and prediction of our steady state model with diffusion for high cell concentration (the cell concentrations in the cartoons are illustrative and not quantitative). (C.) and (D.) Cartoon and prediction of our steady state model with diffusion for very low cell concentration. (E.) Prediction of our steady state model with diffusion on DC activation for a weak LPS stimuli. Computing the expected activation for a range of cell concentrations gives us a qualitative prediction for serial dilutions experiments. (F.) Corresponding dilution experiment with low dose of LPS (1 ng.mL⁻¹). (G.) Model prediction for high dose of LPS. (H.) Corresponding dilution experiment with high dose of LPS (100 ng.mL⁻¹). LPS concentration values used for the model predictions in (E.) and (G.) are shown with red dashed lines in (B.) and (D.). The parameters of the model are set to the values summarized in table S3.

on local signals to detect bacterial signals, but integrate anti-inflammatory signals from anywhere in the population to modulate their response.

Such a localized pro-inflammatory response can be useful in the case of an infection: cells that are further away from the source of the signal do not need to respond. In view of their signalling ranges, autocrine

or paracrine feedback loops have different roles: autocrine signaling modifies the strength of response to LPS of the cell itself, while paracrine signaling is used to transmit information to neighboring cells that may not have been exposed to LPS directly. Such a combination of local, excitatory feedback with global, inhibitory regulation has been suggested as a general way

to sense differences in spatial concentration profiles, and has been proposed as a mechanism for detecting spatial concentration gradients in the slime mold *Dictyostelium* [45–47], or more recently for wound healing [44] and in the context of morphogenesis of mammary epithelial cells in response to a gradient of the epidermal growth factor [48]. Our results extend this concept to the immune system following innate microbial sensing.

In summary, in this study we quantified how a cell makes decisions about the appropriate response to a given concentration of the bacterial signal LPS in the environment, and as a result whether to initiate an inflammatory response or not. More broadly, the mechanisms described give a way to integrate information and make decisions in the presence of conflicting signals. Furthermore we show how simple biophysical models give us insights into cell-cell communication in cell density regimes that are inaccessible by single-cell microscopy [14, 15].

Acknowledgments

This work was supported by MCCIG grant no. 303561, by the Agence Nationale pour la Recherche (ANR), by the Fondation pour la Recherche Medicale (FRM), by the European Research Council (ERC Starting Grant 306312 and ERC Consolidator Grant 281987), by ANR-10-IDEX-0001-02 PSL, ANR-11-LABX-0043, CIC IGR-Curie 1428, and by EMBO and Institut Curie post-doctoral fellowships to ICL.

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