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Modular model of TNFα cytotoxicity

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ABSTRACT

Motivation: Tumour Necrosis Factor alpha (TNF) initiates a complex series of biochemical events in the cell upon binding to its type R1 receptor (TNF-R1). Recent experimental work has unravelled the molecular regulation of the signalling complexes that lead either to cell survival or death. Survival signals are activated by direct binding of TNF to TNF-R1 at the cell membrane whereas apoptotic signals by endocytosed TNF/TNF-R1 complexes. Here we describe a reduced, effective model with few free parameters, where we group some intricate mechanisms into effective modules, that successfully describes this complex set of actions. We study the parameter space to show that the model is structurally stable and robust over a broad range of parameter values.

Results: We use state-of-the-art Bayesian methods (a Sequential Monte Carlo sampler) to perform inference of plausible values of the model parameters from experimental data. As a result, we obtain a robust model that can provide a solid basis for further modelling of TNF signalling. The model is also suitable for inclusion in multi-scale simulation programs that are presently under development to study the behaviour of large tumour cell populations.

Availability: We provide supplementary material that includes all mathematical details and all algorithms (Matlab code) and models (SBML descriptions).

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Tumour Necrosis Factor alpha (TNF) is a cytokine that acts as a key regulator of immune functions (Ashkenazi and Dixit, 1998; Tracey and Cerami, 1994; Wallach *et al.*, 1999). TNF is the prototypical member of a growing family of cytokines (Locksley *et al.*, 2001) but, unlike the other members, it can trigger intracellular signals that lead either to cell survival and proliferation or death (Ashkenazi and Dixit, 1998; Locksley *et al.*, 2001; Tracey and Cerami, 1994; Wallach *et al.*, 1999). This dual role is important in the regulation of immune response, because it provides a molecular basis to cellular homeostasis. These opposing signals might lead to inhibition of tumour growth or, on the contrary, to the promotion of tumour development through direct (see, e.g. Tucker *et al.*, 2004) and indirect mechanisms [e.g. by tissue remodelling and stromal development (Balkwill, 2002)]. This motivates a detailed study of the molecular mechanisms involved in TNF signalling.

Many molecular actors of this complex intracellular machinery have been discovered and studied in a variety of cells (see Wajant *et al.*, 2003, for a comprehensive review). This has attracted the attention of modellers who have attempted to unravel the switching mechanism that leads either to cell survival or death, using standard methods of systems biology (Calzone *et al.*, 2010; Cho *et al.*, 2003; Eißing *et al.*, 2004; 2005; Lipniacki *et al.*, 2004; Rangamani and Sirovich, 2007). They have mostly stressed the interplay among intracellular molecules and the network of reactions stimulated by the binding of TNF to its type 1 receptor (TNF-R1). Recent data show that the path that leads to cell survival is triggered by TNF binding to its receptor at the cell membrane, while the cell-death pathway is triggered by internalized TNF/receptor complexes (Micheau and Tschopp, 2003; Schneider-Brachert *et al.*, 2004). Here, we focus on this important bifurcation and develop a reduced model of TNF action, and we use modern statistical techniques to estimate the model parameters.

2 MODEL OF TNF ACTION

2.1 Binding and internalization

A review of TNF signalling is provided in the Supplementary Material. Here we briefly recall that TNF is a homotrimeric molecule that binds to two different receptors, TNF-R1 and TNF-R2, and that TNF-R1 appears to be the key molecule in both normal and tumour cells. Current biochemical data show that the TNF-R1 receptors rapidly self-trimerize at the cell membrane because of the Pre-Ligand Assembly Domain, and interact with TNF homotrimers (Chan *et al.*, 2000). The process of receptor trimerization is much faster than the binding kinetics, and the trimerized receptor behaves as an effective monomer, therefore TNF binding to TNF-R1 can be viewed as the result of a monomeric interaction between one molecule of TNF and one molecule of receptor. With this simplification we can drop 3 equations and 6 parameters from the model, and obtain a better identification of the remaining parameters.

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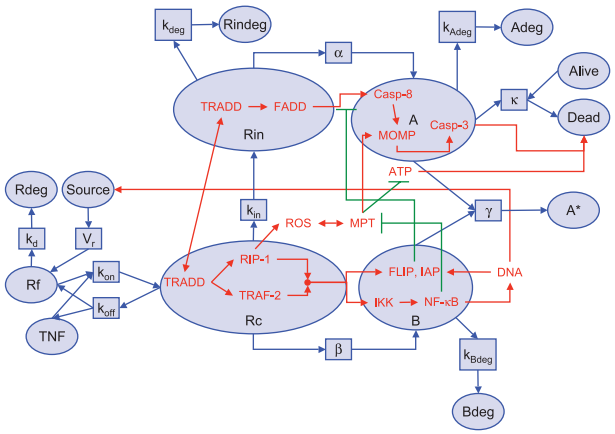

Fig. 1. Map of the modules that describe the main known TNF intracellular signalling pathways. The ovals show the modules that pack several key mechanisms—including the complex biomechanical process of internalization into endocytic vesicles—and replace them with effective actions, and the squares indicate the kinetic parameters. Lines terminating with a bar indicate known inhibitory pathways. The model does not differentiate between death mechanisms and this is because we fit model equations to experimental cytotoxicity data that discriminate between live and dead cells only. This figure is reproduced at a larger scale in the Supplementary Material (Supplementary Fig. SF1).

Here, we use an updated version of a model of Bajzer *et al.* (Bajzer *et al.*, 1989; Vuk-Pavlović and Kovach, 1989) to describe the early events of TNF interaction with cells. Bajzer *et al.* assumed that internalized ligand/receptor complexes could be recycled back to the cell surface, however recent data show that the final fate of the endosomes containing TNF complexes is to maturate to lysosomes by progressive fusion with vesicles from the trans-Golgi network loaded with lysosomal enzymes (Schneider-Brachert *et al.*, 2004), and it is very likely that TNF/TNF-R1 complexes do not recycle at all but are finally degraded into lysosomes. Therefore, we modify the model as follows (see Fig. 1, and the Supplementary Material). We assume a steady flow of TNF receptors, so that their number in the cell membrane is held constant: this is described by the zero-order rate constant V_r. The rate k_d regulates the internalization of ligand-free TNF-R1 receptors. A receptor complex R_c forms at the cell membrane upon binding of TNF to TNF-R1, with rate constants k_{on} and k_{off}. This complex is internalized into cells (R_{in}) with rate constant k_{in}, and the internalized ligand/receptor complexes can finally be degraded (R_{indeg}) with rate constant k_{deg}.

2.2 Life and death pathways

Figure 1 shows the modules that represent the main mechanisms triggered by TNF binding to its receptors. This is suggested by the recent work of Schneider-Brachert *et al.* (2004) that elegantly demonstrates that the pathway leading to NF-κB activation and cell survival is initiated at the cell membrane upon formation of TNF/TNF-R1 complexes, while the one that leads to apoptosis and cell death is initiated by complexes that are internalized into endocytic vescicles. In addition to the basic observations of Schneider-Brachert *et al.*, we include the NF-κB-mediated transcription of genes coding for caspase-8 inhibitors such as FLIP. In this way, the intracellular pathways interact dynamically, because the cell survival pathway—that starts earlier since it does not require internalization of TNF/TNF-R1 complexes—can inhibit the apoptotic path. Here, we model both biochemical circuits by means of only two modules, A and B, that denote the paths leading either to death or to cell survival, respectively. We assume that after the initial trigger both pathways proceed irreversibly to their endpoint. The production of B depends on the number R_c of TNF–TNF-R1 complexes on the cell membrane, with rate constant β. Likewise, the production of A depends on the number of internalized ligand/receptor complexes R_{in}, with rate constant α. The cell survival pathway inhibits the apoptotic reactions in A with rate $\gamma[B]$. Finally, molecules in both A and B can be degraded by means of ubiquitination and proteasome cleavage and/or irreversibly inhibited by other molecular species, and these processes are described by the rate constants $k_{A\text{deg}}$ and $k_{B\text{deg}}$, respectively. Eventually, we merge the necrotic and apoptotic paths introducing a single surviving fraction $f(t)$. The complete model is:

$$\frac{d[R_f]}{dt} = V_r - k_d[R_f] - k_{\text{on}}[\text{TNF}][R_f] + k_{\text{off}}[R_c] \quad (1a)$$

$$\frac{d[\text{TNF}]}{dt} = -k_{\text{on}}[\text{TNF}][R_f] + k_{\text{off}}[R_c] \quad (1b)$$

$$\frac{d[R_c]}{dt} = k_{\text{on}}[\text{TNF}][R_f] - (k_{\text{off}} + k_{\text{in}})[R_c] \quad (1c)$$

$$\frac{d[R_{\text{in}}]}{dt} = k_{\text{in}}[R_c] - k_{\text{deg}}[R_{\text{in}}] \quad (1d)$$

$$\frac{d[B]}{dt} = \beta[R_c] - k_{B\text{deg}}[B] \quad (1e)$$

$$\frac{d[A]}{dt} = \alpha[R_{\text{in}}] - \gamma[B][A] - k_{A\text{deg}}[A] \quad (1f)$$

$$\frac{df(t)}{dt} = -\kappa[A]f(t) \quad (1g)$$

where we have introduced the concentrations of free receptors (R_f) and free ligands (TNF) in addition to those defined above. At the population level, Equation (1g) represents the dynamics of the surviving fraction $f(t)$, while at the single-cell level $f(t)$ can be interpreted as the probability that a cell is still alive at time t and $\kappa[A]$ is the rate for a single-hit death mechanism: this notion is supported by a number of different cytotoxicity experiments, see, e.g. Carmichael *et al.* (1993); Chapman (2003); Lefkovits and Waldmann (1979); Neville and Hudson (1986); Tubiana *et al.* (1990). Notice also that if $[A]$ is approximately constant in the time range $(t, t+\Delta t)$, we recover a familiar formula for the surviving fraction: $f(t+\Delta t) = f(t)\exp(-\kappa[A]\Delta t)$. The complete list of parameters that we infer from experimental data is given in Supplementary Table ST1.

3 METHODS AND RESULTS

3.1 Bayesian inference

We split the analysis of the TNF signalling model in two stages. First we used the data of Grell *et al.* (1998) to infer suitable kinetics for TNF receptor binding and internalization. A model containing only the receptor internalization module, Equations (1a–1d) is provided in the Supplementary Material as a separate SBML file. At the second stage of our analysis, we used the complete model (1a–1g) to perform model parameter inference from the cytotoxicity data of Scherf *et al.* (1996). We used the Bayesian inference framework to perform knowledge updating based on experimental

evidence. This framework employs probability distributions to express one's confidence in values of quantitative model parameters. It requires the *a priori* choice of suitable distributions of parameter values. Afterwards, a consistent mathematical procedure is used to combine these prior distributions with experimental evidence and produce corresponding *a posteriori* parameter distributions. Our choice of priors is based on the existing knowledge of biochemical kinetics involved in similar signalling networks. We use wide log-normal distributions in ranges of model parameters which would be considered reasonable by biochemists working in this field; for example, no *a priori* support is assigned to negative parameter values, as we require non-negative parameter values. We have selected reasonable parameter ranges based on the existing literature: detailed justifications are given in the Supplementary Material. We employ a Sequential Monte Carlo sampler proposed by Del Moral *et al.* (2006) to produce parameter posteriors. A detailed description of this method as well as our complete MATLAB code of the sampler is given in the Supplementary Material.

3.2 Binding and internalization kinetics

The first stage of our analysis requires the accurate knowledge of several experimental conditions, selecting the optimal experimental data is not trivial and requires careful inspection of an extensive scientific literature. Finally we chose the data of Grell *et al.* (1998) for the reasons given in the Supplementary Material. Figure 2 shows the data in Grell *et al.*, along with the predictions drawn from our model, given the parameter posterior identified using this dataset. Column 2 of Supplementary Table ST5 lists the maximum a posteriori (MAP) estimates of the relevant model parameters. It must be noted that existing data did not allow a unique parameter identification, and the resulting posterior has high variance. This result, however, is still useful, as it is used as a prior for the next stage of our analysis, where it is updated with more experimental data. A detailed description of the obtained posterior can be found in the Supplementary Material.

3.3 Dose/response cytotoxicity assays

Since Grell *et al.* (1998) did not report background values in their spectrophotometry measurements of cell viability, these data cannot be used for cytotoxicity estimates, and we take the data in Scherf *et al.* (1996) to estimate the remaining parameters. Scherf *et al.* measured TNF cytotoxicity against MCF7 (human breast carcinoma cells) and Colo205 (human colon carcinoma cells) by the 3[H]-leucine incorporation assay, a method with very low background. We employed the posterior obtained at the first stage of our analysis as the prior for receptor binding parameters at the second stage. The rest of the parameters were assigned weakly informative priors based on existing biochemical literature. MAP estimates of the model parameters are given in Supplementary Tables ST7 and ST8, and the corresponding model predictions are shown in Figure 3, as well as in Supplementary Figures SF6 and SF7. We wish to stress that the inference procedures have uncovered important correlations between the parameters, that are properly expressed by the covariance and correlation matrices reported in the Supplementary Material. Marginalized posterior distributions of individual model parameters are also shown in Supplementary Figures SF8–SF11.

3.4 Model validation and robustness

Independent biochemical data on the expression kinetics of proteins in modules *A* and *B* were used to validate the prediction capabilities of the model (Supplementary Figs SF15 and SF16). Using the estimated parameters, we find that the surviving fraction after TNF administration is a nearly sigmoid function of the initial TNF concentration for all times (see Fig. 4). Thus the model provides a very specific prediction using the set of parameters from the Bayesian estimate, and we have investigated its robustness with respect to parameter changes, using the parameter values in Supplementary Tables ST7 and ST8 as starting points for the numerical study. There are 5 parameters that regulate the interplay of the *A* and *B* pathways, α, β, γ, k_{Adeg} and k_{Bdeg}, while κ cannot influence the stability properties of the model.

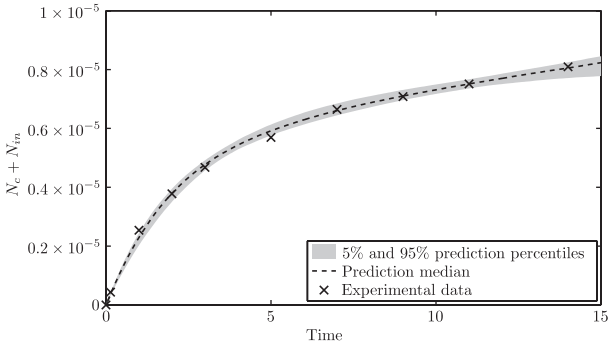

Fig. 2. Prediction of the behaviour of $[R_c](t)+[R_{\mathrm{in}}](t)$ versus t, obtained from the model conditioned on the inferred posterior, using the data of Grell *et al.* (1998). Units follow the specifications of Supplementary Tables ST2 and ST3, i.e. concentration in μM and and time in min. This figure is reproduced at a larger scale in the Supplementary Material (Supplementary Fig. SF2).

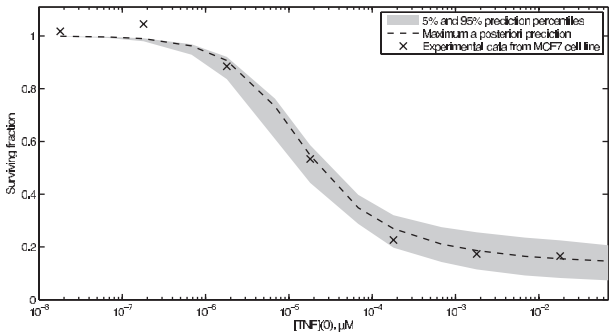

Fig. 3. Predictions drawn from our model with the parameter values from the identified posterior, compared to the original data from the MCF7 dataset (surviving fraction versus initial TNF concentration). A similar, successful prediction is drawn for the Colo205 dataset (Supplementary Fig. SF7). This figure is reproduced at a larger scale in the Supplementary Material (Supplementary Fig. SF6).

We carried out an extensive numerical exploration of this 5-dimensional parameter space, as a function of TNF concentration. To this end, parameters were varied on a regular logarithmic grid in a range corresponding to the extremes of the marginalized posterior distributions shown in Supplementary Figures SF8–SF11, scaled by 1 or 2 orders of magnitude in either direction (see Supplementary Figs SF18 and SF19 and the detailed description in the Supplementary Material). This analysis shows that the model does not change its qualitative behaviour even with very large parameters perturbations, that no unexpected and/or undesired patterns emerge, and that the model describes the balance between cell survival and death for a broad range of parameter values. We conclude that the model is structurally stable and robust (Strogatz, 1994).

4 DISCUSSION

The modular model sketched in Figure 1, and defined by Equations (1a–1g) is a robust, structurally stable description of the dual TNF action. Although it replaces an accurate description of known mechanisms with effective actions, it provides a solid basis for more elaborate models, it establishes kinetic bounds for model

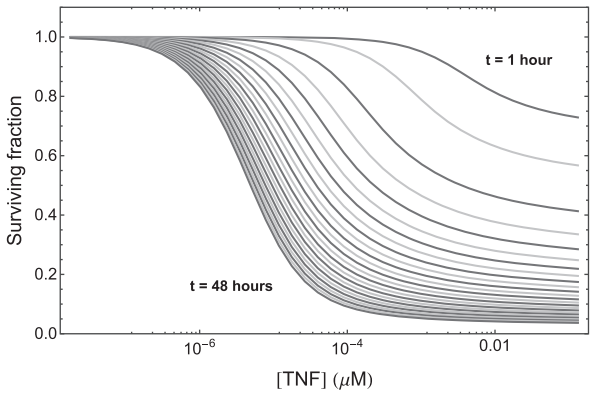

Fig. 4. TNF cytotoxicity as a function of both TNF concentration and time. Using the parameter values estimated for MCF7 cells and listed in Supplementary Table ST7, we have computed the fraction of surviving cells has been computed after 1, 2, 4, 6, 8, …, 48 h of treatment, for different initial TNF concentrations. For all times the response to TNF has a nearly sigmoid shape. This figure is reproduced at a larger scale in the Supplementary Material (Supplementary Fig. SF17).

parameters, and helps understand the differences in sensitivity to TNF of various cell lines. The model is also suitable for integration into complex multi-scale simulation programs of tumour growth such as VBL (Chignola and Milotti, 2004; Milotti and Chignola, 2010), or other computational models (Dionysiou and Stamatakos, 2006; Jiang *et al.*, 2005; Wang and Deisboeck, 2009). We plan to use the model to explore in detail the response of tumour cell clusters to TNF therapy and to investigate tumour/immune system interaction dynamics.

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Conflict of Interest: none declared.

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