

## 1. Title page

### **A model for the optimization of anti-inflammatory treatment with Chemerin**

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Abbreviated title: A model of anti-inflammatory treatment

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## **2. Abstract and key terms**

### **2.1. Abstract**

Routine treatment of mild to moderate pain with a combination of non-steroidal anti-inflammatory drugs such as paracetamol in combination with corticoid opioids can lead to severe complications including death from gastrointestinal injury or to drug dependence. There is a need for the development of new safer drugs. Chemerin is a mediator promoting resolution of inflammation and it is then a promising candidate for a new treatment. A pilot experimental work using the zymosan induced peritonitis model has found that injecting extra chemerin resulted in an approximately 1% reduction of the total number of inflammatory cells recruited. This paper combines and adapts existing mathematical models of inflammation to reproduce these results and to explore the therapeutic potential of chemerin through simulations. Analysis of the model predicts that the injection of chemerin with a concentration of 2,000 ng ml<sup>-1</sup> within the first 5 minutes of inflammation onset leads to maximal inflammation inhibition. The degree of inhibition is shown to be sensitive to data used for the fit with a mean inhibition of  $22 \pm 3.7\%$  for a series of remove-one bootstrap tests whereas optimal chemerin injection parameters were not. Overall sensitivity analysis identifies parameters of the model that need to be measured more accurately or with increased sampling rate to improve model robustness and confirm chemerin's therapeutic potential.

### **3. Introduction**

Chronic pain, a key feature of inflammation, is a leading cause of morbidity worldwide with an estimated prevalence as high as 50% in Europe (1). Based on a survey of over 46000 respondents, chronic pain is reported to affect 19% of Europeans (2). 7 out of 28 prevalence studies included in a recent report on pain in the EU showed a strong link between chronic pain and ageing (3). Hence, the prevalence of pain is expected to increase in ageing populations.

Typical treatment of mild to moderate pain involves the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as paracetamol (1). However, the use of NSAIDs may lead to complications including gastrointestinal (GI) symptoms (especially for patients with a high cardiovascular risk) which can result in fatal peptic ulceration and bleeding – about 2,500 people die in the UK each year due to NSAID related GI (4). Hence, there is a need for new and safe drugs.

To achieve this goal, there has been interest in characterizing the role of mediators in the successful termination of the inflammatory response. In particular, the role of resolvins and chemerin-derived peptides, which mediate their effects through the ChemR23 G protein-coupled receptors (GPCRs) has been examined (5).

To test the inhibitory effect potential of chemerin an experimental pilot study was performed with a zymosan induced peritonitis mouse model of inflammation (6) (detailed

in the Materials and Methods section). The study showed that recruitment of inflammatory cells is indeed reduced when chemerin with a concentration of  $500 \mu\text{g ml}^{-1}$  is injected in the peritoneum two hours after inflammation onset. There is a need now to characterize both the drug concentration and timing of injection that optimize inflammation inhibition to evaluate chemerin's therapeutic potential. As assessing the time course of just one treatment approach requires the sacrifice of approximately 50 mice for statistical significance it would be time consuming and ethically questionable to do an exhaustive experimental search for the optimum. Hence, we introduce a mathematical model of zymosan-induced peritonitis and its modulation by chemerin to identify chemerin delivery optimization hypotheses for further experimental validation. This modelling study constitutes a step towards developing generic methods for following inflammation kinetics in pre-clinical models prior to any translational studies in human volunteers.

## **4. Materials and Methods**

### **4.1 Ethics Statement**

Animal experiments were performed in accordance with the UK Home Office regulations (Guidance on the Operation of Animals, Scientific Procedures Act, 1986) with ethical approval from the Dunn School of Pathology Local Ethical Review Committee. Animals were euthanised with a rising concentration of carbon dioxide and death confirmed by cervical dislocation.

### **4.2 Measurement of Chemerin inhibition using a zymosan induced peritonitis model**

For acquiring pilot data the experimental procedure described by Cash *et al.* (6) has been used. The method consists in inducing peritonitis by injecting zymosan into a mouse

peritoneum. At regular intervals from onset the animals are sacrificed by exposing them to increasing concentrations of CO<sub>2</sub> and with death confirmed with cervical dislocation. The peritoneum of the sacrificed mice is lavaged to count inflammatory cells and measure the concentration of inflammatory mediators.

Cash *et al.* (6) have that this procedure has several advantages compared to other models of inflammation: 1. it allows to modulate the severity of the inflammatory insult dependent on the concentration of zymosan injected; 2. it allows for the collection of reasonable quantity of exudate for the analysis of multiple inflammatory mediators; 3. compared to artificial cavities the peritoneum provides a more realistic experimental environment as it has lymphatic clearance of inflammatory cells and 4. it is a simple procedure and it is reproducible.

This procedure was used to measure the concentration of inflammatory chemokines such as CXCL1, CCL2 and chemerin as well as the number of neutrophils and monocytes. The steps consisted in inducing peritonitis and killing a variable number of animals at intervals up to 96 hours after onset

To evaluate the inhibitory effect of chemerin the procedure was modified by injecting 500 µg/ml of chemerin 2 hours after inflammation was induced. At 4 and 16 hours post onset the animals were sacrificed and the number of inflammatory cells were measured. Through this procedure it was found that the number of neutrophils and monocytes is reduced by up to 7% 2 hours after onset. At 16 hours the numbers are reduced by 12% and 20% for neutrophils and monocytes respectively. Therefore, a significant inhibitory effect was identified. The remainder of the paper details the design of a mathematical model of the progress of inflammation in the zymosan mouse model to identify likely optimal

chemerin injection parameters and hence reduce the extent of experimental work required to find this result. All collected data is provided in the supplementary material.

#### **4.3 Overview of the mechanisms of acute inflammation modulation by chemerin**

Chemerin is a plasmacytoid dendritic cell, a natural killer cell, and a macrophage chemoattractant naturally found in circulation (7). Chemerin is present in the circulation and is secreted as an inactive precursor. It needs to undergo C-terminal proteolytic cleavage by neutrophil proteases to become active (Fig. 1 A). An increased concentration of neutrophils, aimed at killing infectious agents, is characteristic for inflamed tissue and hence chemerin becomes active as inflammation develops. Activated chemerin becomes a monocyte chemoattractant. Diffusing through the tissue and into blood vessels it binds to ChemR23 receptors of monocytes and recruits them, from the circulation into the tissue and towards the inflammation site, where their role is to clear apoptotic neutrophils and inflammation by-products (Fig. 1 B/C). At the site of inflammation, macrophages produced from monocytes release proteases (Fig. 1 D), which break down chemerin into peptides (Fig. 1 E). In particular, the resulting peptide C15 binds to the receptor ChemR23 of macrophages, which has pro-resolving effects i.e. it inhibits the release of pro-inflammatory chemokines (Fig. 1 F) and it promotes apoptotic neutrophil phagocytosis (Fig. 1 G). The full Chem/ChemR23 system is summarised in Fig. 1 (7).

Therefore, the key characteristic affecting the resolution of the inflammatory response that needs to be modelled is the competing action of pro- and anti-inflammatory mediators that affect the recruitment of neutrophils and monocytes after their sequential arrival to the inflammatory site.

**Fig 1:** Schematic representation of the key seven stages of the modulation of inflammation by chemerin (7). A: neutrophils are recruited to the inflammation site and secrete pro-chemerin proteases. B and C: proteases activate chemerin precursors. The activated chemerin is a chemoattractant for monocytes, which are recruited to the inflammation site from blood vessels and are transported through chemotaxis. D: once at the inflammation site monocytes differentiate into macrophages, which clear apoptotic neutrophils and inflammation debris. This activity leads to the release of macrophage proteases which breakdown chemerin into peptides, including the C15 peptide. E: the C15 peptide binds to the macrophages, which (F) inhibits their release of pro-inflammatory cytokines and chemokines and (G) promotes phagocytosis of apoptotic neutrophils.

#### 4.4 Mathematical models of acute inflammation

Several mathematical models of acute inflammatory response were developed. Lauffenburger and Kennedy (8,9) characterized leukocyte recruitment to a bacterial infection site. The role of cytokines and anti-inflammatory mediators was later characterized by Rudnev and Romanyukha (10) and Reynolds *et al.* (11) respectively.

The recruitment and function of different populations of inflammatory cells is, however, only found in the work by Dunster *et al.* (12) and Smith *et al.* (13). Additionally, the work by Smith *et al.* (13) is the only work that models the sequential arrival of neutrophils followed by monocytes. Furthermore, the work by Dunster *et al.* (12) equates pro-inflammatory and anti-inflammatory mechanisms.

The model proposed here aims at predicting a generic inflammatory response in the presence of an intervention. It consists in the combination of the relevant mechanisms from other inflammatory response models, as detailed above, and the further inclusion of the pro- and anti-inflammatory effects of chemerin based on the work by Cash *et al.* (7). The model is described in the sections below.

#### 4.5 Model of the modulation of zymosan induced peritonitis by chemerin

The combined and adapted model is composed of eight variables including: the inflamogen zymosan ( $Z$ ), the number of neutrophils ( $N$ ), the number of apoptotic neutrophils ( $A$ ), the number of monocytes ( $M$ ), cytokines CCL2 ( $C_2$ ) and CXCL1 ( $C_1$ ), chemerin ( $C_h$ ), and anti-inflammatory mediators ( $G$ ). The model is summarized in Fig. 2. Parts of the model are explained in the following subsections.

**Fig2:** Schematic representation of the model of inflammation modulation by chemerin. (J<sub>10</sub>) Zymosan [ $Z$ ] is injected into the peritoneum and triggers the production of CXCL1 [ $C_1$ ] as well as the production of chemerin (J<sub>1</sub>). (J<sub>4</sub>)  $C_1$  then acts as a chemoattractant for neutrophils [ $N$ ]. (J<sub>2</sub>) At the inflammatory site neutrophils start to degenerate and to become apoptotic [ $A$ ]. (J<sub>11</sub>) Both  $N$  and  $A$  promote the production of  $C_1$  that recruit  $N$  further. (J<sub>3</sub>) Additionally,  $N$  and  $A$  promote monocyte [ $M$ ] chemokines such as CCL2 [ $C_2$ ] and chemerin [ $C_h$ ]. (J<sub>5</sub>)  $C_2$  and  $C_h$  recruit  $M$ . (J<sub>6</sub>) both  $N$  and  $M$  phagocytose  $Z$ . (J<sub>9</sub>) Enzymes produced by monocytes break down chemerin, which leads to the production of anti-inflammatory peptides such as  $C_{15}$ . (J<sub>7</sub>)  $M$  also performs the phagocytosis of  $A$  which is promoted by  $C_{15}$ . (J<sub>8</sub>) as  $A$  is being phagocytosed the production of other anti-inflammatory chemokines [ $G$ ] is promoted. (J<sub>10</sub>) Both  $G$  and  $C_{15}$  inhibit the recruitment of further neutrophils. (J<sub>13</sub>) Finally, through diffusion all the inflammatory intervenient considered are extracted to the circulation.

##### 4.5.1 Pro and anti-inflammatory chemokine production

Attractants for inflammatory cells expressed in the zymosan peritonitis model include IL-6, CCL4, CXCL10, CXCL1 and CCL2. Here, only CXCL1 and CCL2 chemokines have been considered as they are the main chemokines for the recruitment of neutrophils and monocytes respectively (14).

Following Smith *et al.* (13) the recruitment of the neutrophils and monocytes is sequential. However, the authors assumed a constant delay between the arrival of the two species, which would not be appropriate here, as the model is intended to capture the



injection of chemerin at different times during inflammation. Instead, neutrophil and monocyte specific chemokines were defined.

As neutrophils are first recruited cells, it was assumed that the tissue cells produce  $C_1$  as zymosan is injected. Additionally, the data shows that chemerin starts being produced by the tissue as soon as zymosan is inject. Therefore, chemerin production was assumed to be triggered by the injection of zymosan. The mechanisms responsible for the production of cytokines involve enzymatic reactions (12). Therefore, the production of  $C_1$  and  $C_h$  in response to  $Z$  is assumed to have Michaelis-Menten kinetics as:

$$J_1(\mu_Z) = V_{\mu_Z} \left( \frac{Z}{k_{\mu_Z} + Z} \right), \quad (1)$$

where  $\mu_Z$  stands for  $C_1$  or  $C_h$  and  $k_{\mu_Z}$  and  $V_{\mu_Z}$  are the Michaelis-Menten parameters related to each chemokine.

At the inflammation site the neutrophils that are not cleared by the circulation become apoptotic. As described in Smith *et al.* (13), the rate of apoptosis,  $k_{an}$ , is proportional to the number of neutrophils as:

$$J_2 = k_{an}N, \quad (2)$$

Both neutrophils and apoptotic neutrophils contribute to the promotion of inflammation by stimulating the production of CXCL1. Additionally, neutrophils intervene in mechanisms that result in the recruitment of monocytes (15). It is assumed that all those mechanisms can be captured by making the production of CCL2 and chemerin dependent on  $N$  and  $A$ . Neutrophil production of chemokines was modelled based on the work by Dunster *et al.* (12) as:

$$J_3(I, \mu) = V_{I, \mu} \left( \frac{I^{w_{I, \mu}}}{k_{I, \mu}^{w_{I, \mu}} + I^{w_{I, \mu}}} \right), \quad (3)$$

where  $I$  stands for either  $N$  or  $A$ ,  $\mu$  for the type of cytokine produced ( $C_1$ ,  $C_2$  or  $C_h$ ) and  $k_{I,\mu}$  is a parameter specific to each cytokine and cell pair and  $w_{I,\mu}$  is the order of the chemokine function rate.

#### 4.5.2 Neutrophil and monocyte chemotaxis

Neutrophil and monocyte chemotaxis was modelled based on the work by Smith *et al.* (13). Neutrophils are recruited at a rate,  $k_{c_1n}$ , proportional to CXCL1 concentration. Additionally, according to Smith *et al.* (13), a maximum number of neutrophils,  $N_{max}$ , is set to account for the saturation of space available for these cells as inflammation progresses. The resulting flow of neutrophils is represented by

$$J_4 = k_{c_1n}[C_1] \left(1 - \frac{N}{N_{max}}\right). \quad (4)$$

Similarly, monocyte chemotaxis is modelled by setting the rate of recruitment to be proportional to chemerin and CCL2 concentrations and imposing a maximum number of monocytes,  $M_{max}$ , to account for the saturation of space as inflammation progresses. Hence, monocyte recruitment is modelled as

$$J_5 = (k_{c_2m}[C_2] + k_{hm}[C_h]) \left(1 - \frac{M}{M_{max}}\right), \quad (5)$$

where  $k_{cm}$  and  $k_{hm}$  are the rate constants for monocyte recruitment due to CCL2 and chemerin respectively.

#### 4.5.3 Zymosan and apoptotic neutrophil phagocytosis

Following the work of Dunster *et al.* (12) the phagocytosis rate of zymosan is performed by both neutrophils and monocytes at rates  $k_{pn}$  and  $k_{pm}$  proportional to their number respectively

$$J_6 = k_{pn}N + k_{pm}M. \quad (6)$$

The apoptotic neutrophils are phagocytosed by monocytes as in Dunster *et al.* (12) at a rate  $k_{pa}$  as:

$$J_7 = k_{pa}AM. \quad (7)$$

#### 4.5.4 Anti-inflammatory chemokine production and role in neutrophil recruitment and phagocytosis action

Two anti-inflammatory cytokines are considered: a generic chemokine  $G$ , as in Durnster *et al.* (12), and the chemerin peptide  $C_{15}$ . The former is produced proportionally ( $k_g$ ) to the phagocytosis of apoptotic neutrophils by monocytes as:

$$J_8 = k_g J_6. \quad (8)$$

$C_{15}$  is formed by the breakdown of chemerin by macrophage proteases (Fig.1 IV). As it is an enzymatic reaction it is modelled with Michaelis-Menten kinetics with parameters  $V_{H15}$  and  $K_{m,C15}$  as:

$$J_9 = V_{C15} \frac{[C_h]}{K_{m,C15} + [C_h]} M. \quad (9)$$

Following Durnster *et al.* (12) the anti-inflammatory chemokines inhibit neutrophil recruitment. Here, the recruitment of neutrophils is performed by CXCL1. It was then

assumed that the anti-inflammatory chemokines inhibit the production of CXCL1. This was done by modifying Eq. 1 and Eq. 3 following Anderson *et al.* (16) leading to

$$J_{10} = V_{C_1,Z} \left( \frac{Z}{k_{Z,C_1} + Z} \right) \left( \frac{1}{\left( 1 + \left( \frac{[C_{15}]}{k_{Z,C_{15}}} \right)^{w_{Z,C_{15}}} \right) \left( 1 + \left( \frac{[G]}{k_{Z,G}} \right)^{w_{Z,G}} \right)} \right), \quad (10)$$

$$J_{11}(I, C_1) = V_{I,C_1} \left( \frac{I^{w_{I,C_1}}}{k_{I,C_1}^{w_{I,C_1}} + I^{w_{I,C_1}}} \right) \left( \frac{1}{\left( 1 + \left( \frac{[C_{15}]}{k_{I,C_{15}}} \right)^{w_{I,C_{15}}} \right) \left( 1 + \left( \frac{[G]}{k_{I,G}} \right)^{w_{I,G}} \right)} \right), \quad (11)$$

where  $I$  stands for  $N$  and  $A$  and  $k_{Z,C_{15}}$ ,  $k_{I,C_{15}}$ ,  $w_{Z,C_{15}}$ ,  $w_{I,C_{15}}$  and  $k_{Z,G}$ ,  $k_{I,G}$ ,  $w_{Z,G}$ ,  $w_{I,G}$  are the parameters for  $C_{15}$  and  $G$  inhibition respectively.

Additionally,  $C_{15}$  promotes phagocytosis (17). To account for this promotion the rate of phagocytosis defined in Eq. 7 was adapted by including  $C_{15}$  dependence based on the chemokine promotion model by Dunster *et al.* (12) leading to:

$$J_7' = k_{pa}AM \left( 1 + \frac{[C_{15}]}{k_{C_{15}}} \right). \quad (12)$$

#### 4.5.5 Injection of additional chemerin model

The rate of injection of extra chemerin  $[C_h]_i$  was modeled as the derivative of a step function as:

$$J_{12}([C_h]_i, \tau) = [C_h]_i \frac{\delta e^{-\delta(t-\tau)}}{1 + e^{-\delta(t-\tau)}}, \quad (13)$$

where  $\delta$  and  $\tau$  are the parameters that define the speed of the step function and the time when the step occurs respectively.  $\tau$  was chosen to match the experimental procedure

performed where extra chemerin is injected 2 hours after inflammation is induced. The remaining parameter  $\delta$  was defined by trying to ensure that it would make the step function as fast as possible and to ensure solution stability. Taking these two aspects into consideration the maximum speed for the injection was found to be 10 min. This is unrealistic as an injection is on the time scale of a few seconds. However, as the experiments take up to 96 hours it is here assumed that the difference between the injection taking seconds or a few minutes is negligible.

#### 4.5.6 Clearance model

The rate of clearance of free inflammatory agents by the vasculature is assumed to be proportional to their concentration as

$$J_{13}(U) = \gamma_U U, \quad (14)$$

where  $\gamma_U$  is the rate of vascular clearance of species  $U$  ( $N, M, A, [C_1], [C_2], [C_h], [C_{15}]$  or  $[G]$ ).

#### 4.5.7 The System of ordinary differential equations

The rate of change of the different species was defined as presented by equations 15 to 23 in Table 1. Additionally, the Matlab code to generate the model simulations here presented can be found in the complementary material.

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Table 1: Summary of the conservation of mass equations of the chemerin inflammation modulation model

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Equation	#	Initial conditions	Units
$\frac{d[C_1]}{dt} = J_{10} + J_{11}(N, [C_1]) + J_{11}(A, [C_1]) - J_{13}([C_1])$	(15)	0	ng ml <sup>-1</sup>
$\frac{d[Z]}{dt} = -J_6$	(16)	100	ng ml <sup>-1</sup>
$\frac{d[C_2]}{dt} = J_3(N, [C_2]) + J_3(A, [C_2]) - J_{13}([C_2])$	(17)	5.17×10 <sup>-3</sup>	ng ml <sup>-1</sup>
$\frac{dN}{dt} = J_4 - J_7' - J_{13}(N)$	(18)	1.71×10 <sup>-1</sup>	cell×10 <sup>6</sup>
$\frac{d[C_h]}{dt} = J_1(C_h) + J_3(N, [C_h]) + J_3(A, [C_h]) + J_{12} - J_{13}([C_h])$	(19)	1.92	ng ml <sup>-1</sup>
$\frac{dM}{dt} = J_5 - J_7' - J_{13}(M)$	(20)	7.72×10 <sup>-2</sup>	cell×10 <sup>6</sup>
$\frac{dA}{dt} = J_7' - J_8 - J_{13}(A)$	(21)	0	cell×10 <sup>6</sup>
$\frac{d[C_{15}]}{dt} = J_9 - J_{13}([C_{15}])$	(22)	0	ng ml <sup>-1</sup>
$\frac{d[G]}{dt} = J_8 - J_{13}(G)$	(23)	0	ng ml <sup>-1</sup>

#### 4.6 Implementations

The model was developed using Matlab (version R2016a, Mathworks, MA, USA). The IQM SBML package (version V.2.1, Basel, Switzerland) developed by Schmidt and Jirstand (18) was used for fitting the model. Additionally the optimization algorithms provided by the IQM package were used for identifying the optimal injection of chemerin to inhibit recruitment of inflammatory cells. The model simulations were performed using the Matlab provided solver 'ode23s' catered for stiff ODE systems. The solver was used

with default Matlab settings. Sensitivity analysis was carried using the Compolongo-Morris method. The method was implemented based on the work by Saltelli et al. (19).

#### 4.7 Data for model fitting

The procedure described by Cash *et al.* (6) was used to obtain the data used to fit the model. The animal experiment consists in inducing inflammation by injecting 100  $\mu\text{g/ml}$  of zymosan into the mouse peritonitis cavity. 500  $\mu\text{g ml}^{-1}$  of recombinant murine chemerin (R&D systems; aa17-156) were injected two hours later. At different times up to 96 hours after zymosan injection the animals were sacrificed. For each animal the number of neutrophils, monocytes and the concentration of chemokines CCL2, CXL1 and chemerin in the peritoneum cavity was measured. For each data point 5-10 animals were sacrificed.

### 5 Results

#### 5.1 Model fitting

Wherever possible model parameters were defined based on values reported in the literature or directly available from data. Malech (20) reported the rate constant of neutrophil clearance ( $\gamma_N$ ). The clearance rate of all cytokines ( $\gamma_{C1}, \gamma_{C2}, \gamma_H$  and  $\gamma_{C15}$ ) was set to the measured generic value (21). All set parameters are summarized in Table 2. Experimentally, it was possible to define the saturation levels  $N_{max}$  and  $M_{max}$  as  $4 \times 10^6$  and  $9 \times 10^6$  cells respectively based on the measurements performed when 500  $\mu\text{g/ml}$  of zymosan is injected into the peritoneum.

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**Table 2:** List of model parameters for which values are reported in the literature.

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Parameter	Description	Units	Value	Reference
$\gamma_N$	Diffusion rate of neutrophils from the inflammatory site to the capillary	$h^{-1}$	$6.3 \times 10^{-2}$	(20)
$\gamma_{C1}$	CXCL1 extraction rate from the inflammatory site into the capillary.	$h^{-1}$	$8.3 \times 10^{-1}$	(21)
$\gamma_{C2}$	Capillary extraction rate of CCL2	$h^{-1}$	$8.3 \times 10^{-1}$	(21)
$\gamma_{Ch}$	Chemerin extraction rate from the inflammatory site into the capillary	$h^{-1}$	$8.3 \times 10^{-1}$	(21)
$\gamma_{C15}$	Extraction rate of peptide $C_{15}$ by the capillary	$h^{-1}$	$8.3 \times 10^{-1}$	(21)
$\gamma_G$	Rate of removal of inhibitory chemokine G by the capillary	$h^{-1}$	$8.3 \times 10^{-1}$	(21)

The remaining parameters were fitted numerically. The allowed range for all fitted parameters was set empirically so that to reflect the rate at which different variables evolve during the experimental study. Two sets of data were used for the fitting: 1)  $N$ ,  $M$ ,  $[C_1]$ ,  $[C_2]$  and  $[C_h]$  in control data and; 2)  $N$ ,  $M$  at 2 and 14 hours after  $500 \text{ ng ml}^{-1}$  of chemerin are injected.

The model has 47 parameters with a wide parameter space and needs to be fitted to 40 data points spanning 5 variables of which 2 are measured in two experiments. For this reason, since it is hard to envisage how the fit varies as the parameter space is sampled, methods designed to avoid local minima were considered to perform the fit.

The initial selection of the fitting method was performed by running all global optimization methods from the SBML toolbox the method that produced the best fit in a reasonable time was the simplex method (22). The result of the fit is shown in Fig. 3 and the full list of fitted parameters is provided in Table 3.



**Fig3:** Results from fitting of the model to the data. (A) control cytokines CCL2 and CXCL1; (B) control chemerin; (C) control neutrophils and monocytes and; (D) neutrophils and monocyte when extra chemerin is injected after 2 hours from peritonitis onset.

Fitting errors were normalized with respect to the maximum value measured for each variable to ensure equal weighting of each one in the final fit. This was achieved by setting the root mean square (RMS) value to

$$RMS(x) = \sqrt{\sum_{x=1}^7 \frac{(S_x - D_x)^2}{\max(D_x)}}, \quad (24)$$

where  $S_x$  is the simulated value,  $D_x$  is the data point, the index  $x$  stands for the different variables being fitted and  $\max(D_x)$  stands for the maximum of the dataset.

As shown in Fig.3 the fit exhibits all key characteristics observed in measurements: the sequential arrival of neutrophils and monocytes (Fig. 3 C); the removal of neutrophils and monocytes from the inflammatory site (Fig. 3 C); as well as sharp peaks of numbers and concentrations of inflammatory cells and chemokines respectively. Achieved RMS values are predominantly low and approximately 0.05, 0.1, 0.09 and 0.12 for neutrophils, monocytes, chemerin and CXCL1 respectively. The RMS value for CCL2 is 0.47 and is due to a rapidly varying function where a small time delay between the fitted and measured curves leads to a large RMS error.

RMS values for chemerin injection experiments were also low with 0.01 and 0.03 for neutrophils and monocytes respectively.

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**Table 3:** summary of fitted parameter values of the chemerin inflammation modulation model

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Parameter	Description	Units	Value
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$k_{pN}$	Rate of zymosan phagocytosis by neutrophils	$\text{h}^{-1}$	2.013
$k_{pM}$	Rate of zymosan phagocytosis by monocytes	$\text{h}^{-1}$	1.285
$V_{C_1Z}$	Rate of CXCL1 production in response to zymosan injection	$\text{ng ml}^{-1} \text{h}^{-1}$	2.86
$k_{C_1Z}$	Rate of CXCL1 production in response to zymosan injection	$\text{ng ml}^{-1}$	$1 \times 10^{-3}$
$k_{AN}$	Rate of neutrophil apoptosis	$\text{h}^{-1}$	$1.152 \times 10^{-3}$
$k_{NG}$	$G$ rate of inhibition of CXCL1 production by neutrophils	$\text{ng ml}^{-1}$	$6.259 \times 10^{-3}$
$k_{ZG}$	$G$ rate of inhibition of CXCL1 production by zymosan	$\text{ng ml}^{-1}$	$1.218 \times 10^{-3}$
$V_{NC_1}$	Rate of CXCL1 production by neutrophils	$\text{ng ml}^{-1} \text{h}^{-1}$	4.46
$k_{NC_1}$	Rate of CXCL1 production by neutrophils	$\text{cell} \times 10^6$	10.273
$k_{NC_{15}}$	$C_{15}$ rate of inhibition of CXCL1 production by neutrophils	$\text{ng ml}^{-1}$	$1.632 \times 10^{-1}$
$V_{AC_1}$	Rate of CXCL1 production by apoptotic neutrophils	$\text{ng ml}^{-1} \text{h}^{-1}$	$1.307 \times 10^{-3}$
$k_{AC_1}$	Rate of CXCL1 production by apoptotic neutrophils	$\text{cell} \times 10^6$	$3.553 \times 10^{-1}$
$k_{AC_{15}}$	$C_{15}$ rate of inhibition of CXCL1 production by apoptotic neutrophils	$\text{ng ml}^{-1}$	$2.631 \times 10^{-3}$
$k_{AG}$	$G$ rate of inhibition of CXCL1 production by apoptotic neutrophils	$\text{ng ml}^{-1}$	$2.819 \times 10^{-2}$

$V_{C_1N}$	Rate of neutrophil chemotaxis by CXCL1	cell $\times 10^6$ h $^{-1}$	2.65
$k_{pA}$	Rate of apoptotic neutrophil phagocytosis by monocytes	h $^{-1}$	$3.412 \times 10^{-3}$
$k_{C_{15}}$	$C_{15}$ rate of apoptotic neutrophil phagocytosis by monocytes	ng ml $^{-1}$	$1.2 \times 10^{-3}$
$k_{C_2M}$	Chemoattractant rate of monocytes by $C_2$	h $^{-1}$	$5.182 \times 10^{-1}$
$k_{C_hM}$	Chemoattractant rate of monocytes by $C_h$	h $^{-1}$	$1 \times 10^{-3}$
$y_M$	Monocyte clearance rate by the capillary	h $^{-1}$	$1.986 \times 10^{-2}$
$V_{C_{15}}$	Maximum velocity for the enzymatic reaction that produces $C_{15}$ .	ng ml $^{-1}$ h $^{-1}$	$2.687 \times 10^{-1}$
$w_{NC_h}$	Parameter of chemerin production function by neutrophils	dimensionless	2.12
$w_{AC_h}$	Parameter of chemerin production function by apoptotic neutrophils	dimensionless	$2.769 \times 10^{-1}$
$w_{NC_2}$	Parameter of CCL2 production function by neutrophils	dimensionless	$1.621 \times 10^{-3}$
$w_{AC_2}$	Parameter of CCL2 production function by apoptotic neutrophils	dimensionless	5
$w_{NC_1}$	Parameter of CXCL1 production function by neutrophils	dimensionless	5
$w_{AC_1}$	Parameter of CXCL1 production function by apoptotic neutrophils	dimensionless	2.321
$w_{NG}$	Parameter of G inhibition of CXCL1	dimensionless	3.431

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	production function by neutrophils		
$w_{AG}$	Parameter of $G$ inhibition of CXCL1	dimensionless	1.736
	production function by apoptotic neutrophils		
$w_{AC_{15}}$	Parameter of $C_{15}$ inhibition of CXCL1	dimensionless	$1.819 \times 10^{-2}$
	production function by neutrophils		
$w_{NC_{15}}$	Parameter of $C_{15}$ inhibition of CXCL1	dimensionless	$4.395 \times 10^{-3}$
	production function by apoptotic neutrophils		
$w_{ZG}$	Parameter of $G$ inhibition of CXCL1	dimensionless	1.048
	production function in response to zymosan injection		
$w_{ZC_{15}}$	Parameter of $C_{15}$ inhibition of CXCL1	dimensionless	$3.802 \times 10^{-1}$
	production function in response to zymosan injection		
$K_{mC_{15}}$	Michaelis-Menten constant of $C_{15}$ production from monocyte proteases cleavage of chemerin	ng ml <sup>-1</sup>	$1.695 \times 10^{-1}$
$k_g$	Rate of $G$ production in response to apoptotic neutrophil phagocytosis by monocytes	ng ml <sup>-1</sup> h <sup>-1</sup>	$3.553 \times 10^{-3}$
$V_{NC_h}$	Maximum rate of chemerin production by neutrophils	ng ml <sup>-1</sup> h <sup>-1</sup>	2.33
$k_{NC_h}$	Michaelis-Menten constant for the chemerin production by neutrophils	cell $\times 10^6$	$1.932 \times 10^{-2}$

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$V_{AC_h}$	Maximum rate of chemerin production by apoptotic neutrophils	ng ml <sup>-1</sup> h <sup>-1</sup>	5.887×10 <sup>-3</sup>
$k_{AC_h}$	Michaelis-Menten constant for the chemerin production by apoptotic neutrophils	cell×10 <sup>6</sup>	5.736×10 <sup>-2</sup>
$V_{NC_2}$	Maximum rate of CCL2 production by neutrophils	ng ml <sup>-1</sup> h <sup>-1</sup>	1.899×10 <sup>-3</sup>
$k_{NC_2}$	Michaelis-Menten constant for the CCL2 production by neutrophils	cell×10 <sup>6</sup>	1.073
$V_{AC_2}$	Maximum rate of CCL2 production by apoptotic neutrophils	ng ml <sup>-1</sup> h <sup>-1</sup>	26.749
$k_{ZC_{15}}$	C <sub>15</sub> inhibition rate of CXCL1 production in response to zymosan injection	ng ml <sup>-1</sup>	1.061×10 <sup>-2</sup>
$k_{AC_2}$	Michaelis-Menten constant for the CCL2 production by apoptotic neutrophils	cell×10 <sup>6</sup>	2.5×10 <sup>-2</sup>
$\gamma_A$	Apoptotic neutrophil clearance rate by the capillary	h <sup>-1</sup>	1.75×10 <sup>-3</sup>
$k_{C_hZ}$	Rate of chemerin production in response to zymosan injection	ng ml <sup>-1</sup>	1.099
$V_{C_hZ}$	Rate of chemerin production in response to zymosan injection	ng ml <sup>-1</sup> h <sup>-1</sup>	3.15

## 5.2 Optimizing the therapeutic potential of chemerin

The aim of this work is to identify the optimal concentration and time of injection of chemerin to inhibit inflammation. These two parameters,  $[C_h]_i$  and  $\tau$  respectively, are the inputs to Eq. 13. The extent of the inhibitory effect  $W$  of chemerin is evaluated by comparing the average number of all inflammatory cells over the course of the experiment, i.e. 96 hours, with and without chemerin injected. This can be expressed as

$$W([C_h]_i, \tau) = \frac{\sum S_{x,o} - \sum S_{x,ch}([C_h]_i, \tau)}{\sum S_{x,o}} \times 100. \quad (25)$$

where  $x$  stands for the type of cell being compared. To optimize Eq. 25 the simplex method (18) was implemented.

With this protocol two conditions were tested. The first consists in optimizing the percentage of chemerin inhibition over a range of  $[C_h]_i$  so that the total number of cells ( $x = N, M, A$ ) is minimized, as shown in Fig. 4 A. It was found that for the whole range of  $[C_h]_i$  the optimal time for injecting chemerin was as soon as possible after inflammation onset. Hence, to simulate immediate chemerin injection the initial concentration of chemerin was set to  $[C_h] = [C_h]_0 + [C_h]_i$  in Eq. 19 (Table 1). The maximum inhibition is approximately 27% when  $2 \times 10^3$  ng ml<sup>-1</sup> of chemerin are injected. The maximum inhibitory effect is reduced by 1% when the injection is delayed by 5 minutes.

After the maximum inhibitory effect is reached, injection of further chemerin will increase the total number of cells. This effect can be explained by the dual role that chemerin exhibits in the inflammatory pathway. On one hand it attracts monocytes and on the other hand it leads to further production of C15 that inhibits neutrophil recruitment and promotes apoptotic neutrophil phagocytosis. The maximum inhibition found is more than double of the effect observed in the pilot experimental study.

When breaking down the 27% reduction of the total of inflammatory cells into the three types of inflammatory cells considered there was an inhibition of approximately 53% and 6% in the number of monocytes and neutrophils respectively. However, there was an increase of apoptotic neutrophils by approximately 160%.

As apoptotic neutrophils have been found to play a crucial role in the degeneration of acute inflammation into chronic inflammation (11) a second set of optimizing tests were made to identify the optimal conditions for injecting chemerin to reduce the number of neutrophils and apoptotic neutrophils ( $x = N, A$ ). As in the case of total cell inhibition optimization, the maximum inhibition of neutrophils for injections of increasing chemerin concentration occurs when chemerin is injected simultaneously with the trigger for inflammation. Furthermore, as seen in Fig.4 B, a maximum inhibition was found of approximately 35% and 88% in neutrophils and apoptotic neutrophils respectively. However, as chemerin concentration increases so does the number of monocytes by approximately 74%.

**Fig4:** Simulations made to minimize Eq. 24 for chemerin injections over a range of chemerin concentrations. (A) Eq. 24 was minimized when  $x = N, M, A$ . It resulted in a maximum reduction of 6% and 53% in  $N$  and  $M$  respectively and a 160% increase in apoptotic neutrophils when  $2 \times 10^3$  ng ml<sup>-1</sup> of chemerin are injected. (B) The number of neutrophils and apoptotic neutrophils was minimized ( $x = N, A$  in Eq. 24) resulting in a 35% reduction in neutrophils, a 88% reduction in apoptotic neutrophils and an increase of monocyte recruitment of 70% when  $\geq 1 \times 10^5$  ng ml<sup>-1</sup> of chemerin are injected.

### 5.3 Sensitivity analysis

To assess the risk of overfitting, a screening algorithm was performed on the fitted model. The model consists of 8 ODE equations (Equations 15-23) with 47 parameters. Due to its large size and the number of parameters it was decided to use a global rather than

local method. As it is anticipated that this complex model will not be stable over the whole parameter space the global Compolongo-Morris (C-M) method was chosen.

Furthermore, to assess the risk of sensitivity to data, a Remove-One Bootstrap (23) analysis was performed. Both studies are reported below.

### 5.3.1 Compolongo-Morris screening analysis

This algorithm consists in exploring the parameter space by varying each parameter from of model (including the parameters in Table 2 and Table 3 and the data derived parameters  $N_{max}$  and  $M_{max}$ ) in turn over a pre-defined range (19). As the range found for parameter definitions in the literature, as in Smith *et al.* (13), is large, the range was chosen to be  $\pm 80\%$  of the value fitted.

Each time a parameter is varied its impact on the output of the model is evaluated, which is termed elementary effect (EE). This analysis aims at identifying which parameters impact the model fit and the model predictions of cell recruitment inhibition by injecting extra chemerin, therefore the EEs were defined as the sum between: 1. the RMS between the model with the new parameter definitions and the data using Eq.24 and 2. the RMS between the fitted model simulations when extra chemerin is injected and the model with the new parameter values, again using Eq. 24. Inclusion of this sum ensures that the analysis takes into consideration the behaviour of the model with and without extra chemerin injected.

The analysis is run over a series of EE calculations. From all the EEs standard deviation ( $\sigma$ ) and absolute mean ( $\mu^*$ ) are calculated for each parameter. The advantage of computing the absolute mean is that it captures the cumulative effect of varying each



parameter in contrast to the mean, which would be affected by the signs of EEs. In Fig. 5 the results are plotted for all the parameters. A large value of  $\mu^*$  establishes that the parameter has a large effect on the output while those with a small value are unimportant. Additionally, the value of  $\sigma$  can be used to distinguish between those parameters that have a linear independent effect (small  $\sigma$ ) and those with a non-linear effect (large  $\sigma$ ).

EE statistics are plotted in Fig5. Fig. 5 B shows that the model is most sensitive to the parameters pertaining to the production and removal of the chemokines CXCL1 and CCL2. Additionally, the rate of apoptotic neutrophils by monocytes ( $k_{pa}$ ) has a marked impact on the behaviour of the model. The data derived parameters that describe the maximum number of neutrophils ( $N_{max}$ ) and monocytes ( $M_{max}$ ) have also been found to impact the fit and the models predictions. Related to cellular number kinetics the rate of monocyte extraction rate ( $\gamma_m$ ) was also found to substantially impact model behaviour.

To quantify how the most sensitive parameters (Fig 5 B) impact model predictions in more detail, the Compolongo-Morris method was reapplied. The remaining parameters of the model were kept constant while the sensitive parameters were varied by  $\pm 5\%$ . EEs were redefined to assess the change in predicted maximum inhibition, optimal time of injection and optimal chemerin concentration as in Eq. 24. It was found that the optimal concentration was constant for all simulations, the percentage of total cell inhibition was found to vary between 20% and 34% and the maximum neutrophil inhibitory effect varied between 30% and 40%.

Fig. 5 A presents the parameters found to perturb the summed RMS by less than 1%. These parameters are related to the production of cytokines by apoptotic neutrophils and to the inhibitory effect of the generic pro-resolving cytokine ( $G$ ). As the parameters presented in Fig. 5 A ( $k_{A,G}$ ,  $k_{A,C_{15}}$ ,  $k_{N,C_2}$ ,  $V_{N,C_2}$ ,  $V_{A,C_2}$ ,  $V_{A,C_1}$ ,  $k_{A,C_1}$ ,  $w_{A,C_1}$ ,  $k_{A,C_h}$ ,  $w_{A,G}$ ,  $V_{N,C_h}$

$k_{C_h,Z}, k_{Z,G}, k_{m,C_{15}}, w_{A,C_2}$ ) have negligible effect on the output of the model they were removed. Starting with the parameters  $V_{A,C_h}$  and  $k_{A,C_h}$  their removal leads to

$$J_3(A, C_h) = 0. \quad (26)$$

Second, as  $k_{Z,G}$  has negligible effect on the model response, it was assumed that  $w_{Z,G}$  also has minimal impact on the model output and therefore they were removed leading to

$$J_{10}(C_1) = V_{N,C_1} \left( \frac{Z}{k_{Z,G} + Z} \right) \left( \frac{1}{\left( 1 + \left( \frac{[C_{15}]}{k_{Z,C_{15}}} \right)^{w_{Z,C_{15}}} \right)} \right). \quad (27)$$

Similarly for apoptotic neutrophils the parameters  $V_{A,C_1}, k_{A,C_1}, w_{A,G}, k_{A,G}$  were removed, leading to

$$J_{11}(A, C_1) = 0. \quad (28)$$

Simulations were performed to test the impact of removing the remaining parameters on the model fit and predictions. These simulations illustrate the limitations of the Compolongo-Morris which does not take into account the non-linear relationship between parameters. From these simplifications it was possible to reduce the free parameters from 47 down to 35 and an increase of summed RMS by less than  $1 \times 10^{-3}\%$ .

**Fig5:** Resulting Compolongo-Morris parameter standard deviation ( $\sigma$ ) and absolute mean ( $\mu^*$ ) (19). (A) the parameters that influence the output the least and (B) the parameters that lead to largest increase of RMS difference between the data and the model.

### 5.3.2 Remove-One Bootstrap analysis

In the fitting process 7 variables tracked during the Zymosan induced peritonitis experiment were used. To check how the fit and key predictions are affected by measurements, 1 data point from each 7 datasets before fitting was removed at random i.e. it was possible to have up to 7 data points removed at the same time for a simulation

instance. The experiment was performed 100 times and changes in predicted optimal chemerin dosing and maximal expected inhibition were recorded. A histogram presenting the distribution of results was included in the supplementary materials.

Inflammation inhibition varied between 10-35% (mean of 22% and standard deviation of 3.7%) of the control value (i.e. no chemerin injected). The optimal injection time and the optimal chemerin concentration did not change.

A further set of experiments was performed where only one data point at a time was removed per simulation instance. For measurements with a low standard derivation the removal of a single data point did not affect inhibition significantly. In case of high standard deviation measurements i.e. in the dynamic range of the curves presented inhibition varied between 10% and 60%.

## **6. Discussion**

This work has led to the development of the first mathematical model of chemokine mediated leukocyte recruitment and clearance during zymosan induced peritonitis. As evidenced by results in Section 5.1, it was possible to fit the model to achieve good agreement with data from a pilot experimental study. RMS values were predominantly low, however for more confidence in the quality of the fit more data points should be acquired in the future to sample the studied functions with more frequency as they vary rapidly. Furthermore, individual point estimates in the dynamic range are based on measurements with a high standard deviation. More measurements per point or more precise timing for data collection will be necessary in the future to improve fit quality. Indeed, a Remove-One bootstrap analysis showed that removing a single measurement from a signal point

estimate in this dynamic range could lead to the inhibition changing by up to 25 percentage points (i.e. from 10% to 35% with mean 22% and standard deviation 3.7%).

Sensitivity analysis identified 12 out of 47 parameters that had little impact on the results of the model simulation. Furthermore, the analysis highlighted those parameters with high impact on the output of the model pointing to model assumptions that should be verified experimentally.

The fitted model was used to maximize theoretically the degree of inflammation inhibition by chemerin. In brief, a maximum inhibition of 27% of the average of total number of inflammatory cells over the course of the experiment was predicted for an immediate chemerin injection with concentration of about  $2 \times 10^3$  ng ml<sup>-1</sup>. Optimization aimed at minimizing neutrophil recruitment found an optimal chemerin concentration of  $10^5$  ng ml<sup>-1</sup> to be injected immediately after inflammation onset. These results are discussed in more detail below.

Furthermore, individual point estimates in the dynamic range are based on measurements with a high standard deviation. More measurements per point or more precise timing for data collection will be necessary in the future to improve fit quality. Indeed, a Remove-One bootstrap analysis showed that removing a single measurement from a signal point estimate in this dynamic range could lead to the inhibition changing by up to 50 percentage points (i.e. from 10% to 60%).

### **6.1 Sensitivity analysis**

In the model here presented production of CXCL1 depends on zymosan concentration while the production of CCL2 depends on the number of neutrophils and apoptotic neutrophils attracted by CXCL1. Hence, the output of the model is sensitive to any

variation of the rate of production of these chemokines as was correctly identified by sensitivity analysis. As the range set for these parameters during the fitting process was set somewhat arbitrarily, future experimental work is needed to confirm the values found and to provide more theoretical insight into the mechanisms behind the observed delay in recruitment of leukocytes.

The parameters that define apoptotic neutrophil phagocytosis (rate of monocyte phagocytosis and the rate of C15 promotion of phagocytosis) exert a noticeable effect on the output of the model. This is due to the assumption that the inflammatory process can only be resolved after total clearance of apoptotic neutrophils. In addition, the rate of circulatory monocyte clearance is also relevant for the behaviour of the model for the same reasons.

Conversely, the C-M method also identified the parameters that least affect the output. These are related to chemerin production by apoptotic neutrophils and to the inhibitory effect of  $G$  on chemokine production. In both cases this lack of importance might be explained by a lack of data to inform the fitting.

The fitting process led to a solution where the number of apoptotic neutrophils is much lower than that of neutrophils (order of magnitude of  $10^7$  cells). Checking whether this is a realistic distribution should form part of future validation and development of the model.

The model assumed that there is a generic chemokine  $G$  inhibiting neutrophil recruitment further experimental work would be needed to identify prominent individual chemokines involved in this process.

## **6.2 Treatment perspectives involving Chemerin/ChemR23 pro-resolving pathway**

Chemerin has been associated with a variety of conditions that have as one of its pathological mechanisms chronic inflammation. These include, obesity, metabolic syndrome and diabetes (24). Additionally, chemerin has been proposed as a biomarker for chronic inflammation, as its increased expression is found to be unique to the inflammatory region (25).

Assuming two therapeutic strategies consisting in either the reduction of the total number of inflammatory cells during the course of inflammation or focus on the reduction of the number of apoptotic neutrophils the model predicted theoretical optimal solutions for both. In the first case, the model predicts that injecting chemerin up to 7 min after injecting zymosan at a concentration of  $2 \times 10^3 \text{ ng ml}^{-1}$  will have the maximum overall inhibitory effect of 27%. Individual cell recruitment in that case was reduced by 35% for monocytes and by 6% for neutrophils with a 160% increase of the number of apoptotic neutrophils.

This result suggests that it is impossible to reduce the number of apoptotic neutrophils and monocytes simultaneously. This is a concern since, as suggested by Kumar *et al.*(14), both types of cells are involved in the pathological mechanisms that lead to the degeneration of acute inflammation into chronic inflammation. This behaviour can be explained by the fact that the model assumes that inflammation can only be resolved if apoptotic neutrophils are cleared. Additionally, it is assumed that the mechanisms responsible for the clearance of these cells are diffusion of apoptotic cells into the circulation and monocyte phagocytosis. Therefore, a reduction in the number of monocytes will result in a larger presence of apoptotic neutrophils. Therefore, it should be investigated if there are other mechanisms of removal of apoptotic neutrophils. If a more dominant mechanism exists besides monocyte phagocytosis, perhaps there could be a

strategy by which the injection of chemerin could reduce the number of monocytes and apoptotic neutrophils simultaneously.

In the second case, the model predicts that a large concentration of chemerin ( $1 \times 10^5 \text{ ng ml}^{-1}$ ) needs to be injected up to 7 min after zymosan injection. Such a treatment would lead to approximately an 35% reduction of neutrophils as well as a 88% reduction is the number of apoptotic neutrophils. However, it also leads to a 70% increase in the number of monocytes. Above  $1 \times 10^5 \text{ ng ml}^{-1}$  there is no increase in neutrophil inhibition and there is a linear increase in the number of monocytes.

The inhibitory potential of chemerin identified by the model is informed by the efficiency of the fit. The model captures well the behaviour of variables in the control environment and when chemerin is injected until 16 hours after peritonitis onset. Although, there are data for longer periods of time in the control environment there are no measurements when chemerin is injected. Consequently, when fitting the model to both types of datasets the fitting algorithm assumes that the profile of neutrophil and monocyte behaviour resembles that of the control data. When computing the inhibitory effect of chemerin using Eq. 24 the full 96 hours of simulation are used. Therefore, although the results show that chemerin is able to inhibit the number of cells recruited it does not show if injecting chemerin aids in a faster resolution of inflammation. To overcome this limitation it is proposed that using the zymosan induced peritonitis procedure (6) measurements of cellular species should be made at around 40 hours after zymosan is injected. With the new data it would be possible to confirm if the clearance of neutrophils and monocytes is faster in the presence of extra chemerin.

The results of this study contribute to the on-going debate on whether chemerin has a generic anti-inflammatory function or whether it applies only to specific conditions (24). Animal models have demonstrated a requirement of chemerin signalling in the development of inflammation and leukocyte infiltration (26). Conversely, animal studies have shown that inhibition of endogenous chemerin activity exacerbates inflammation (24). Additionally, the chemerin inhibitory pathway might require the involvement of other mediators e.g. Resolvin E1, which shares some of the receptors to which chemerin attaches (27).

Using the models referred in this work it was possible to capture the broad dynamics of the inhibitory role of chemerin during zymosan-induced peritonitis and to identify a high potential for inhibition with a specific selection of chemerin concentration and time of injection. These results encourage further experimental work to confirm simulation outcomes and allow for a detailed planning of future experimental work. The Chemerin/ChemR23 pro-resolving pathway could be involved in many conditions and it is hoped that this model will serve as basis for the study of the role of chemerin in pathophysiology.

#### **Author Contribution:**

Dr. Daniel Regan-Komito, Dr. Asif J. Iqbal and Professor David R. Greaves were responsible for the experimental study that identified the inhibitory effect of chemerin on inflammation during Zymosan induced peritonitis.

Additionally, Simão Laranjeira under the supervision of Dr. Piotr Orlowski and Professor Stephen J. Payne developed the mathematical model of the modulation of inflammation by chemerin.



Furthermore, Dr. Daniel Regan-Komito, Dr. Asif J. Iqbal and Professor David R. Greaves reviewed and edited the description of the clinical and biological motivation for the work and the description of the experimental work. Dr. Piotr Orłowski and Professor Stephen J. Payne reviewed and edited sections describing the mathematical model and its analysis as well as the discussion section.

**Competing interests:**

We declare that there are no competing interests.

**Funding statement:**

The work presented was part of the Centre for Doctoral Training at Oxford funded by the Research Council (RCUK- EP/G036861/1). Additionally, the work was supported by the British Hear Foundation (FS/11/82/29332 and RG/15/10/23915).

**Ethics statement:**

Animal experiments were performed in accordance with the UK Home Office regulations (Guidance on the Operation of Animals, Scientific Procedures Act, 1986) with ethical approval from the Dunn School of Pathology Local Ethical Review Committee. Animals were euthanised with a rising concentration of carbon dioxide and death confirmed by cervical dislocation.

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