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ARTICLE

A Dynamic Quantitative Systems Pharmacology Model of Inflammatory Bowel Disease: Part 1 – Model Framework

Katharine V. Rogers[1], Steven W. Martin[2], Indranil Bhattacharya[3], Ravi Shankar Prasad Singh[4] and Satyaprakash Nayak[2,*]

A mechanistic, multistate, mathematical model of inflammatory bowel disease (IBD) was developed by including key biological mechanisms in blood and gut, including cell differentiation, cytokine production, and clinical biomarkers. The model structure is consistent between healthy volunteers and IBD disease phenotype, with 24 parameters changed between diseases. Modular nature of the model allows for easy incorporation of new mechanisms or modification of existing interactions. Model simulations for steady-state levels of proteins and cells in the blood and gut using a population approach are consistent with published data. By simulating the response of two clinical biomarkers, C-reactive protein and fecal calprotectin, to parameter perturbations, the model explores hypotheses for possible treatment mechanisms. With additional experimental validation and addition of drug treatments, the model provides a platform to test hypothesis on treatment effects in IBD.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ There is a wealth of knowledge on different biomarkers in inflammatory bowel disease (IBD). Literature results show that these biomarkers are highly variable, but we do not have a good unified understanding of the underlying mechanisms of the immune system mechanisms at play in Crohn's disease (CD) and ulcerative colitis (UC).

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study addresses the question of understanding complex diseases, such as IBD, by developing a mechanism-based quantitative systems pharmacology model with parameters estimated from literature and in-house data and application of a single mechanistic framework to model both CD and UC.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study provides us with a single, mechanism-based, modular framework to understand the complex immune interactions involving multiple cell types and cytokines to understand behavior of key biomarkers in IBD.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ This study will help us gain a deep understanding of the multiple entities in the immune system involved in progression and manifestation of IBD. The model developed in this study can be readily applied to understand behavior of key biomarkers, add or modify biological mechanisms and simulate therapeutic effect on populations of IBD subjects.

Inflammatory bowel disease (IBD) is a chronic autoimmune disease associated with intestinal inflammation. IBD has two distinct subtypes; Crohn's disease (CD) is a transmural disease affecting any part of the gastrointestinal tract from mouth to anus, whereas ulcerative colitis (UC) is predominantly limited to the rectum and colon. CD often presents with complications, such as strictures and fistulas, and many patients require surgery that is not curative.[1] Surgery can be curative in patients with UC, but may affect quality of life.[2] Both CD and UC are heterogeneous diseases making treatment complicated. Treatments for IBD include classic anti-inflammatory drugs (5-aminosalicylates), immunosuppressive drugs (azathioprine and methotrexate), anti-TNFα agents (infliximab, adalimumab, golimumab, and certolizumab pegol), integrin blockers (vedolizumab and etrolizumab), and anti-IL12p40 inhibitors (ustekinumab in CD).[3] A better understanding of the disease biology, mechanism of action of various treatments, and their effect on key biomarkers will be instrumental in the development of more effective therapies. Modeling and simulation could have a significant impact in integration of biological and clinical information, providing a thorough understanding and creating a decision-making framework to aid drug development in IBD.

The use of systems modeling for immunology research is a growing field. The ability to generate large data sets to characterize various immune cell types and their products, such as cytokines, chemokines, and interleukins, combined with increased computational and modeling capacity with methodologies, such as quantitative systems pharmacology

[1]Biologics Development Sciences, Janssen Biotherapeutics, Janssen Research & Development, LLC, Raritan, New Jersey, USA; [2]Pharmacometrics, Global Clinical Pharmacology, Pfizer Inc., Cambridge, Massachusetts, USA; [3]Quantitative Clinical Pharmacology, Takeda, Cambridge, Massachusetts, USA; [4]Clinical Pharmacology, Early Clinical Development, Pfizer Inc., Cambridge, Massachusetts, USA. *Correspondence: Satyaprakash Nayak (satyaprakash.nayak@pfizer.com)
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(QSP), has made it feasible to construct and simulate large scale models of the human immune response.[4] The effort toward biomarker-guided clinical trial design also makes the idea of complex mathematical models of the immune system based on human data more realistic.[5] Different approaches of mathematical modeling, including deterministic ordinary differential equations, agent-based, and stochastic, as well as examples of immunology models at the molecular level, cellular scale, and multiscale are extensively reviewed elsewhere.[6] There has been considerable progress in understanding and modeling acute immune response in conditions such as sepsis, inflammatory diseases, trauma, and wound healing, which has added to our understanding of the system.[7–10] Many challenges remain in modeling the human immune system and immunological diseases due to the variability observed in cell and cytokine measurements,[11] the multitude of different analytical methods, heterogeneity of the patient population in many auto-immune diseases, and, most importantly, because of our incomplete understanding of how various components of the immune system interplay with each other in a complex disease, such as IBD. However, in spite of these challenges, a mechanistic model of IBD incorporating detailed information of the immune system can provide very valuable insights regarding the behavior of key biomarkers, mechanism of action of different therapeutic options, and key differences in various subtypes of the IBD population.

Modeling IBD is highly complex as the intestinal system involves the interplay among various cell types, cytokines, the epithelial barrier function, and the microbiome. The location and extent of inflammation differs between patients and the upregulation of inflammatory markers is highly variable. Typical end points in IBD clinical trials are composite scores, such as Crohn's Disease Activity Index (CDAI), Crohn's Disease Endoscopic Index (CDEIS), Simple Endoscopic Score (SES-CD) for CD, and Full/Partial Mayo Score for UC, which give little insight into the mechanism of the disease. A recent shift in the CD space is toward endoscopic end points relating to mucosal healing, which may be more reflective of disease condition but may not be comparable to scores reported in previous trials.[12] Also reported are C-reactive protein (CRP), a general marker of inflammation, as well as fecal calprotectin (FCP) which is upregulated in patients with IBD. Published dynamic models in the IBD field involve the complex intracellular signaling pathways in T helper (Th) cells,[13] a cell-based model where activating and deactivating cytokines were lumped,[14] and a model specific to interleukin-6 (IL-6) signaling.[15] A recent publication by Balbas-Martinez *et al*. of a logic network for IBD includes a comprehensive network of cytokines, cells, bacterial antigens, and recognition receptors, and explores multiple treatment mechanisms by knockout of specific nodes on Metalloproteinases levels (i.e., IL-17 knockout for anti-IL-17 therapy).[16] For a thorough understanding of treatments in IBD, there is a need for a comprehensive model that is both dynamic and quantitative enabling the ability to test dosing schedules, dosing quantities, and predict quantitative changes in clinical biomarkers.

With this goal of mechanistic understanding in mind, we have developed a detailed mechanistic, QSP model of IBD with parameters optimized to match literature and in-house data. We have applied the model to gain valuable understanding of different treatment options and have provided model predictions for novel combination treatments, which could be effective in various subgroups of IBD subjects. Part 1 of our two-paper series provides a description including the biology incorporated, model parameterization using steady-state data, and sensitivity analysis. Part 2 of the series focuses on the mechanism of action of specific treatment modalities (e.g., anti-TNFs, anti-IL-12p40, etc.) and provides insight into their effect on key biomarkers in monotherapy and combination therapy in different patient subtypes of IBD. The novelty of this approach is that it provides insights and incorporates knowledge into the overall understanding of the key components of IBD following perturbation with the various treatments, such as anti-cytokines (e.g., anti-TNFα) that have been shown to provide clinical benefit to patients with IBD.

METHODS

Model formulation

The model is structured as a series of ordinary differential equations: $\frac{d\mathbf{x}}{dt} = \mathbf{S}.\mathbf{v}(\mathbf{x};\mathbf{k})$. The vector of state variables, **x**, includes cell types, cytokine concentrations, CRP, and FCP in all compartments, and **v** represents the vector of fluxes (including reactions rates, transport rates, production rates, degradation rates, etc.). **S** is the stoichiometric matrix, which encodes the signaling architecture in the model. The rows of **S** represent a state variable and each column represents a flux. The vector **k** is a list of the kinetic parameters. The network is provided in the supplementary (GutInflammation.sbproj). The mechanism of action of IL-17 inhibition was explored by including a step-function describing the protective effect of IL-17 on epithelial barrier. As an example, the barrier damage leading to activation of macrophages and dendritic cells was active in the model only when IL-17 inhibition reached a predetermined high level.

Estimating model parameters and virtual population generation

The model was built using MATLAB's Simbiology toolbox. The network connections were taken from a combination of literature sources and previous models.[13,17,18] Model parameters were estimated using a population approach in which a population of best parameter sets was selected. The parameters were estimated by solving for baseline levels of species (cell types, cytokines, etc.) in healthy volunteers (HVs), patients with CD, and patients with UC. The base model for HV, CD, and UC was kept the same with only 24 parameters allowed to change between healthy condition and CD, UC diseases (**Supplementary Table – Model Information, Sheet – 05**). The approach for parameter estimation utilized a method developed by Allen, *et al*.[19] that involved creating a plausible population to fit physiological values of cytokines, cell numbers, and biomarkers for HVs, CD, and UC (see **Supplementary Information** for further details). The sources and rationale for these values can be found in **Supplementary Table – Model Information, Sheet – 02**). From the plausible population, a virtual population was chosen for each clinical study to match published clinical population biomarkers reported (i.e., CRP and FCP). The use of a population approach can aid in issues with variability in

the biology as well as variability in the data generation (i.e., from different laboratories and methods). We have provided the model file and MATLAB code to simulate steady-state in **Supplementary Information**.

Model evaluation

The suitability of the model was evaluated by comparing the steady-state results obtained from the model (without any treatment) with the steady-state levels seen in patient population. This is shown in the visual comparison of median levels and variability seen in key biomarkers and cell-types obtained from the model and observed in the literature. The ability of the model to match baseline levels obtained in different studies for CD and UC by varying only the key parameters also adds to our confidence in the model.

Sensitivity analysis

Local sensitivity analysis was performed on the IBD model parameters for all three model states (HVs, CD, and UC) using overall fully normalized sensitivity. The time integrated sensitivity coefficient for each species is calculated by the following equation:

$$S_{ij} = \int_0^t \left(\frac{k_i}{x_j(t)}\right)\left(\frac{\partial x_j(t)}{\partial k_i}\right)$$

where x_j represents the vector of species outputs with respect to which sensitivity is being calculated, k_j is the vector of parameter inputs, and t is the time point for the simulation.

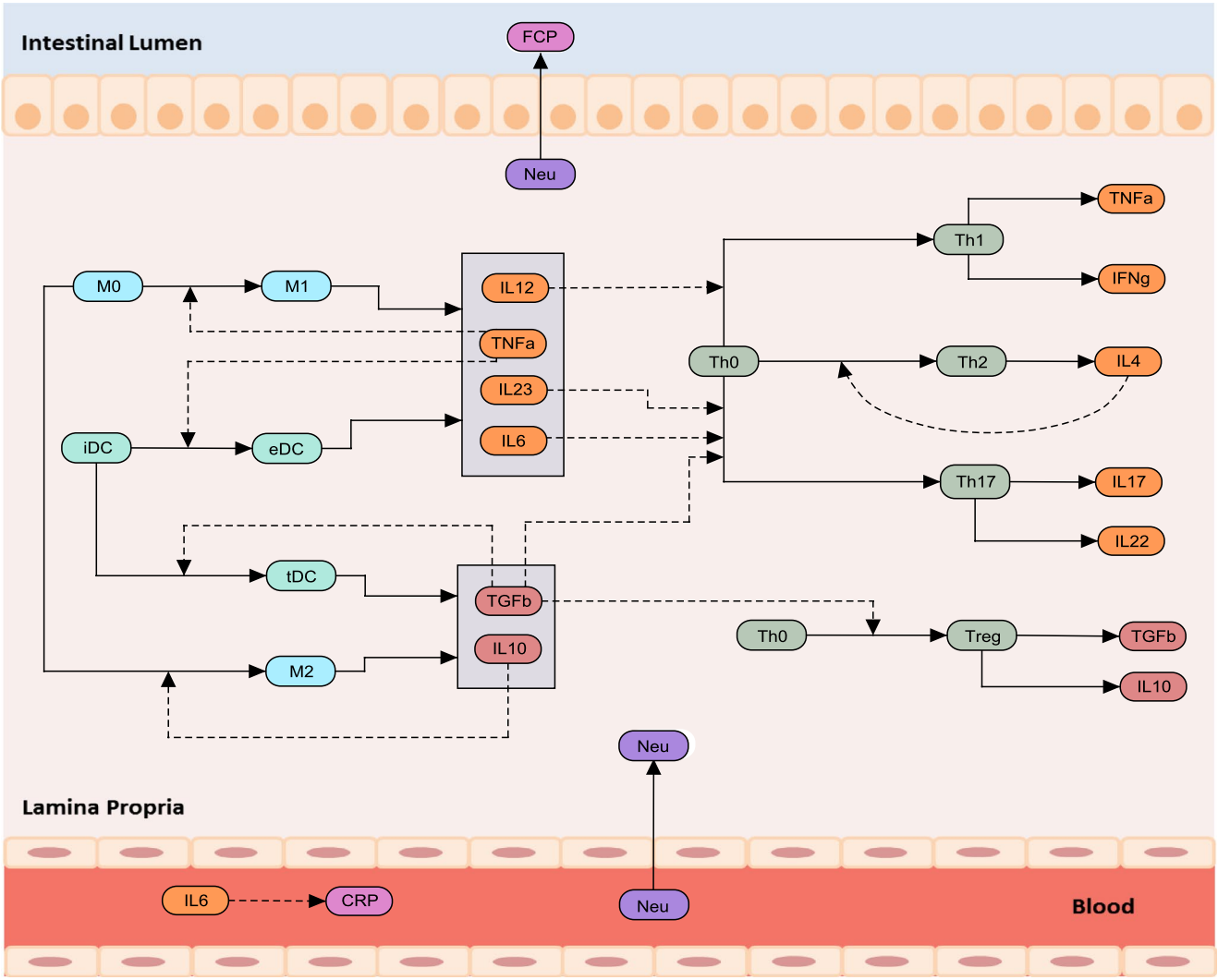

Figure 1 Model structure overview, including major cells, cytokines, biomarkers, and connections. The model consists of 2 major compartments (gut and blood), immune cells, cytokines, and biomarkers (CRP and FCP). Dotted lines indicate that the species in the model impacts the reaction, while solid lines indicate either differentiation of cells, production of cytokines, or transport into the gut or lumen. The boxes indicate multiple cell lines produced by the cells. eDC, effector dendritic cells; FCP, fecal calprotectin; iDC, immature dendritic cells; IFNγ, interferon gamma; IL-4, interleukin 4; IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; IL-22, interleukin 22; IL-23, interleukin 23; M0, resting macrophages; M1, classically activated macrophages; M2, alternatively activated macrophages; Neu, neutrophils; tDC, tolerogenic dendritic cells; TGFβ, transforming growth factor beta; Th0, naïve T helper cells; Th1, T helper 1 cells; Th2, T helper 2 cells; Th17, T helper 17 cells; TNFα, tumor necrosis factor alpha; Treg, T regulatory cells.

RESULTS

Mathematical model of IBD – Formulation

A brief description of the primary biological interactions incorporated in the model is as follows. The basic model structure has two main compartments – the gut (lamina propria) and blood. The model describes interactions between the different immune cells and cytokines in the gut, and the downstream production of the key clinical biomarkers CRP and FCP (**Figure 1**). These interactions were derived from literature sources (**Supplementary Table – Model Information, Sheet – 03**) and the rate constants for these interactions were estimated using both literature and in-house *in vitro* data. The multiscale structure can be represented at the organ level (**Figure 1**) and the cellular (**Figure 2**) and protein levels (**Figure 3**).

Figure 1 illustrates the major interactions of macrophages, dendritic cells (DCs), Th cells, and cytokines, as well as CRP and FCP in the model. In the model, most cytokines and cells are considered to be located in the gut (lamina propria) and some (e.g., IL-6), are in the blood (full list can be found in **Supplementary Table – Model Information, Sheet – 01**). The model includes both adaptive immune cells (Th1, Th2, Th17, and regulatory T cells (Tregs)) and innate immune cells (DCs, macrophages, natural killer cells, and neutrophils) as both these components are thought to play an important role in IBD. In addition, both pro-inflammatory (IL-6, IL-8, and tumor necrosis factor alpha (TNFα)) and anti-inflammatory cytokines (IL-10 and transforming growth factor beta (TGFβ1)), which act as a link between the innate and adaptive immune system are included. Although shown in the figure, the current model does not include the epithelial barrier. A detailed listing of all 334 parameters and 116 reactions in the model can be found in **Supplementary Table – Model Information, Sheet – 03 and 04**).

Cell differentiation involves the interplay of multiple cytokines and a more comprehensive diagram of key interactions of various CD4+ T cells, also known as Th cells, is shown in **Figure 2**. Multiple types of Th cells, including Th17, Th1, Th2, and Tregs, play a key role in IBD and many drugs have been developed to control their activity by targeting cytokines that activate them. Panels a, b, c, and d show the key interactions of Th1, Th2, Th17, and Tregs, respectively. As seen from the figure, differentiation of T-cell subtypes is cytokine dependent (e.g., Th1 (is dependent on IL-12), Th2 (IL-4), Th17 (TGFβ1, IL-6, and IL-23), and Treg (TGFβ1 and IL-2)), and these subtypes then produce signature cytokines particular to them. For example, Th1 cells produce, interferon gamma (IFNγ), Th2 cells produce

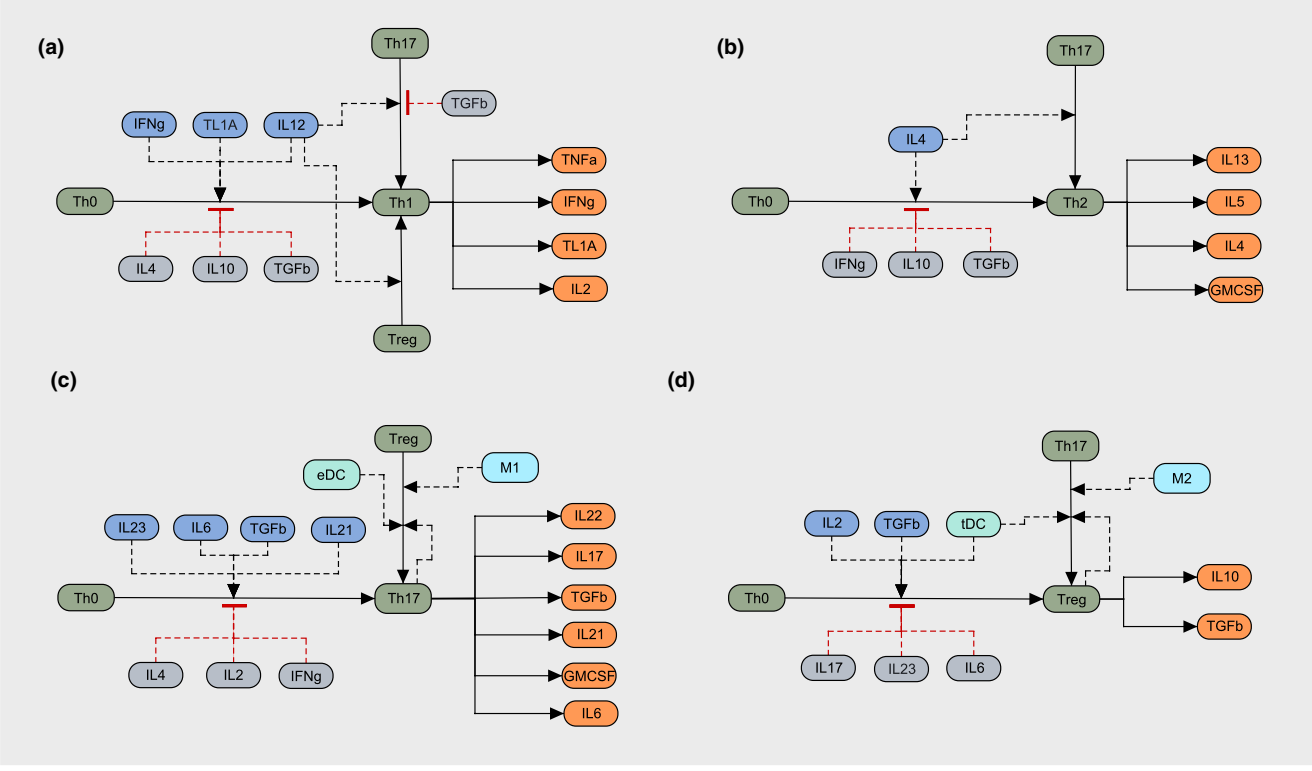

Figure 2 Detailed T cell differentiation in model. Naïve T cells (Th0) in the model can differentiate into (**a**) Th1, (**b**) Th2, (**c**) Th17, and (**d**) Treg cells. T cell differentiation is dependent on the cytokines available and the cytokines produced are dependent on the T cell type. Dotted lines indicate that the species in the model impacts the reaction (T cell differentiation), whereas solid lines indicate either differentiation of T cells or cytokine production by T cells. Red indicates inhibition. eDC, effector dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFNγ, interferon gamma; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; IL-21, interleukin 21; IL-22, interleukin 22; IL-23, interleukin 23; M1, classically activated macrophages; M2, alternatively activated macrophages; tDC, tolerogenic dendritic cells; TGFβ, transforming growth factor beta; Th0, naïve T helper cells; Th1, T helper 1 cells; Th2, T helper 2 cells; Th17, T helper 17 cells; TL1A, TNF like ligand 1A; TNFα, tumor necrosis factor alpha; Treg, T regulatory cells.

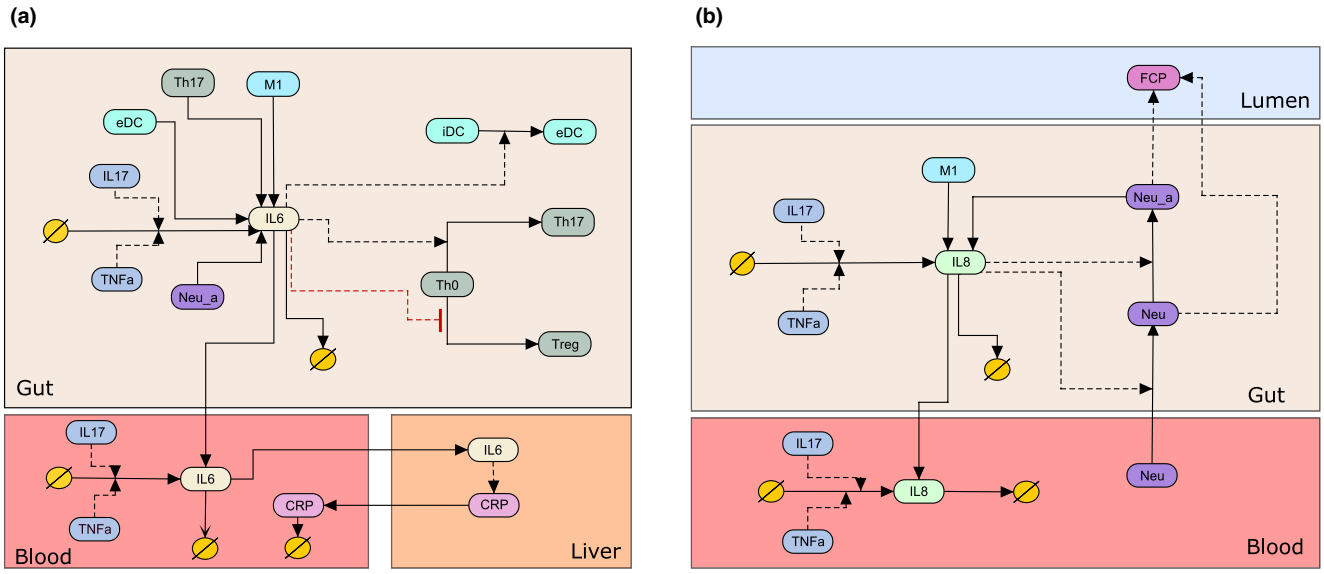

Figure 3 Detailed IL-6 and IL-8 network in the model. (**a**) IL-6 is produced by four cell types in the model and also produced through mechanisms not included (basal rate). IL-6 upregulates Th17 cells, eDC, and CRP production in the liver. (**b**) IL-8 is produced by two cell types in the model and also produced through mechanisms not included (basal rate). IL-8 levels in the tissue cause neutrophil recruitment into the gut and activation of neutrophils. Dotted lines indicate that the species in the model impacts the reaction, while solid lines indicate either differentiation or production, in the case of cells and cytokines, respectively. Red indicates inhibition and yellow circles indicate either a source or a sink for, production and degradation, respectively. eDC, effector dendritic cells; FCP, fecal calprotectin; iDC, immature dendritic cells; IL-6, interleukin 6; IL-8, interleukin 8; IL-17, interleukin 17; M1, classically activated macrophages; Neu, neutrophils; Neu_a, activated neutrophils; Th0, naïve T helper cells; Th17, T helper 17 cells; TNFα, tumor necrosis factor alpha; Treg, T regulatory cells.

IL-4, IL-5, and IL-13, Th17 cells upon differentiation produce IL-17, IL-21, and IL-22, and Treg cells are thought to be responsible for producing TGFβ1 and IL-10.[20] A specific cytokine signature is important as traditionally CD was considered a Th1 driven disease and UC was Th2, but this is an oversimplification of the two diseases and in UC many drugs targeting Th2 mechanisms (anti-IL-13) have failed clinically.[3] In addition, recently, it has been noted that Th cell differentiation is not unidirectional (i.e., naïve T cell to Th1 permanently), but Th cell plasticity exists and Th cells can further differentiate to other lineages.[21] We have included these complexities in the model to accurately describe CD and UC subtypes.

Cytokines secreted by activated T-cells also play an extensive role in inflammation in further stimulating or inhibiting key parts of the immune system. **Figure 3** shows the role of two of the cytokines, IL-6 (panel a) and IL-8 (panel b), included in the model. IL-6 is introduced in the model as it is a well-studied pro-inflammatory cytokine that is produced by Th17, DCs, and macrophages and is known to be upregulated in IBD.[22] TNFα and IL-17 signaling lead to increased production of IL-6 through cells not currently included in the model but included as factors influencing the basal rate of IL-6 production.[23] Neutrophils can lead to cleavage of soluble IL-6R and production of IL-6, although the latter is still under debate.[24] IL-6 regulates the balance of Th17 and Treg cells by inducing Th17 differentiation and inhibiting Treg differentiation.[25] The commonly measured biomarker CRP is produced through an IL-6 stimulus mainly in hepatocytes and is often upregulated in inflammation.[26] IL-8 (CXCL8) is a chemoattractant leading to migration of neutrophils from the blood to tissue, and is also an activator of neutrophils.[24] Macrophages and neutrophils produce IL-8.[2427] In addition, TNFα and IL-17 signaling lead to increased production of IL-8 through cells not currently included in the model and, therefore, they are included as mediators on the basal rate of IL-8 production.[23] Calprotectin is an abundant neutrophil protein and has been included in the model as FCP production through neutrophil apoptosis and measured in feces.[28]

To take into account the complex biology of IL-17, a protective effect of IL-17 on epithelial barrier function was included in the model structure as a step function. When free IL-17 levels fall below the basal levels (i.e., non-IL-23 signaling), epithelial damage occurs, bacteria enters the mucosa, and macrophages and DCs become activated (see Methods).[29]

The base model was parameterized for HVs, patients with CD, and patients with UC. In order to simulate a CD or an UC subject, only 24 parameters involving all basal production of cytokines and cells in the gut were allowed to vary between diseases (**Supplementary Table – Model Information, Sheet – 05**).

Parameter estimation with steady-state results in HV and IBD populations

The model parameters were estimated using published and Pfizer internal data to fit steady-state protein concentrations over a population of parameter sets. **Figure 4** shows model results for steady-state levels of six key proteins and biomarkers in the gut and blood after parameter optimization for HVs, and CD and UC subjects without any treatment effect at steady-state. A population of 40,000 plausible patient parameter sets was generated for HV, CD, and UC models (**Figure**

S1). We used a method developed by Allen *et al*., which selects a population subset from a large number of plausible patients to best fit observed physiological outcomes data in order to generate the baseline virtual population.[19] Using this procedure, a subset of 357 parameter sets for the HV, CD, and UC models were selected to match baseline levels and variability in CRP and FCP data from published literature (**Figure 4a,b**). Using the selected parameter sets, baseline HV levels of key cytokines, such as IFNγ (**Figure 4c**) and IL-6 (**Figure 4e**), were simulated and compared against published literature values. Biomarkers representing the differences between HV and IBD are shown in the case of IL-21 (**Figure 4d**) and normalized TNFα (**Figure 4d**). As seen from the figure, model predictions of different cytokines with parameter sets selected to match CRP and FCP matches well with published data for HVs as well as patients with CD and patients with UC. Our model also accurately predicted the increased fold change levels of cytokines in HVs, CD, and UC in the gut vs. blood (internal data not shown).

Simulation of response to IL-17 inhibition

To test the IL-17 protective effect included in the model, multiple sustained levels of IL-17 inhibition were simulated utilizing the CD model and the baseline population determined for the steady-state concentrations (n = 357). **Figure 5a,b** shows the median fold changes from baseline of different inflammation markers with different levels of IL-17 inhibition ranging from 0 to 100% at weeks 6 and 12, respectively. Model predictions show that, although there is an improvement (decrease) in CRP and FCP concentrations as IL-17 inhibition increases up to 90%, inhibition of IL-17 > 90% results in increased levels of mucosal IFNγ and TNFα; both of which negatively impact the epithelial barrier and exacerbate mucosal inflammation.[30] The figure shows the prediction of increased mucosal IFNγ and TNFα due to anti-IL-17, which is supported by a preclinical study in which anti-IL-17 increased mucosal real-time polymerase chain reaction expression of these cytokines in dextran sulfate sodium treated mice.[31] Our model predicts that for cases when steady-state inhibition of IL-17 is above 90%, there is an initial decline in CRP due to the initial rapid decrease in IL-6 concentration from IL-17 signaling, but as IL-17 inhibition is continued, CRP concentrations start increasing and eventually surpass the baseline values in some cases (**Figure 5c**). A similar response is seen for FCP (**Figure 5d**). It is important to note

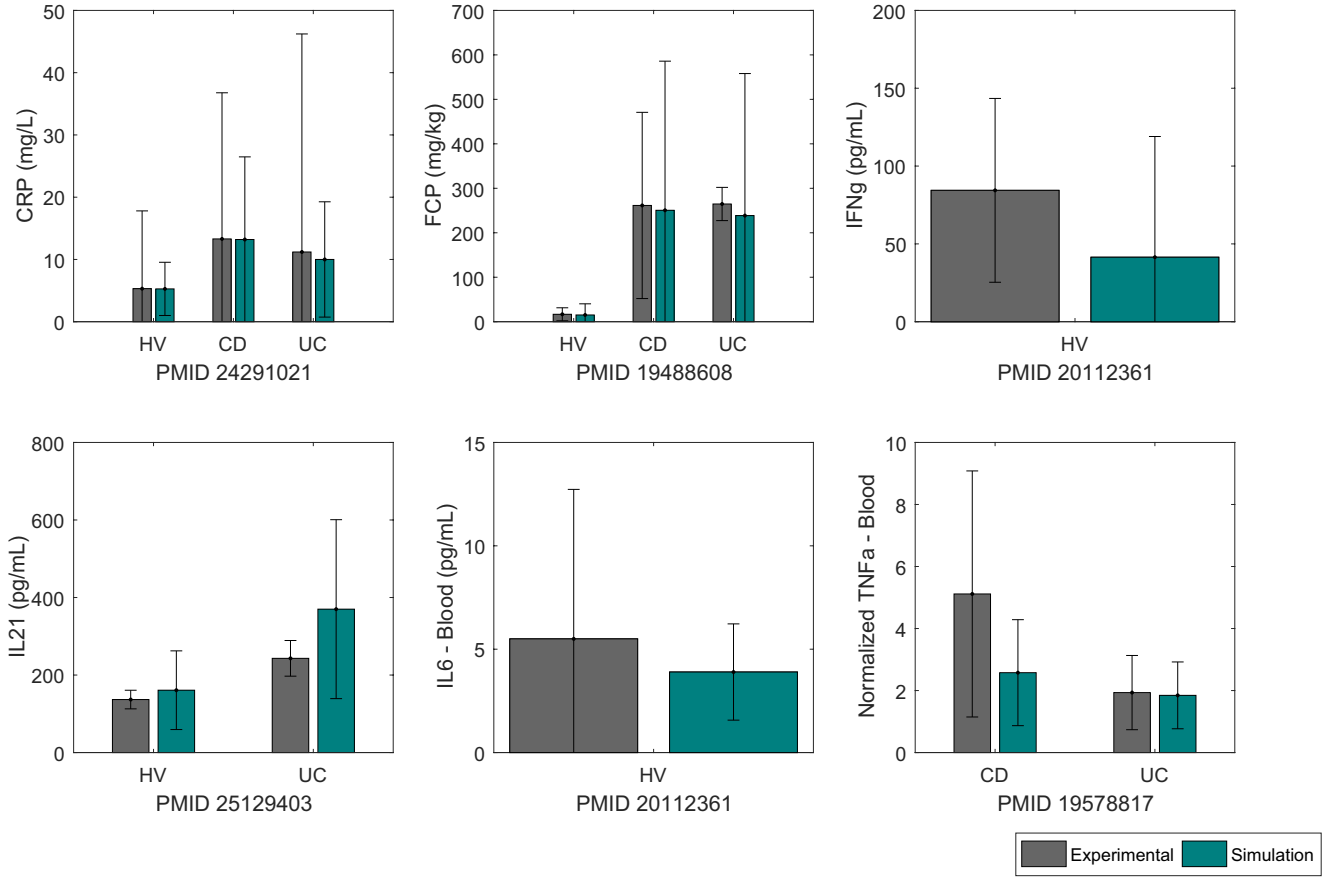

Figure 4 Steady-state results for selected baseline population in the absence of treatment. Simulation for baseline population (n = 357) vs. experimental data for absolute values of CRP (blood), FCP (feces), IFNγ (gut), IL-21 (gut), and IL-6 (blood). CD and UC TNFα (blood) values for experiment and model data have been normalized to the HV experimental and model mean, respectively. Bars indicate the mean value of the population and error bars indicate standard deviation. CD, Crohn's disease; CRP, C-reactive protein; FCP, fecal calprotectin; HV, healthy volunteer; IFNγ, interferon gamma; IL-6, interleukin 6; IL-21, interleukin 21; TNFα, tumor necrosis factor alpha; UC, ulcerative colitis. *For the plot, all values were converted to mean using formula from PubMed ID (PMID) 25524443.

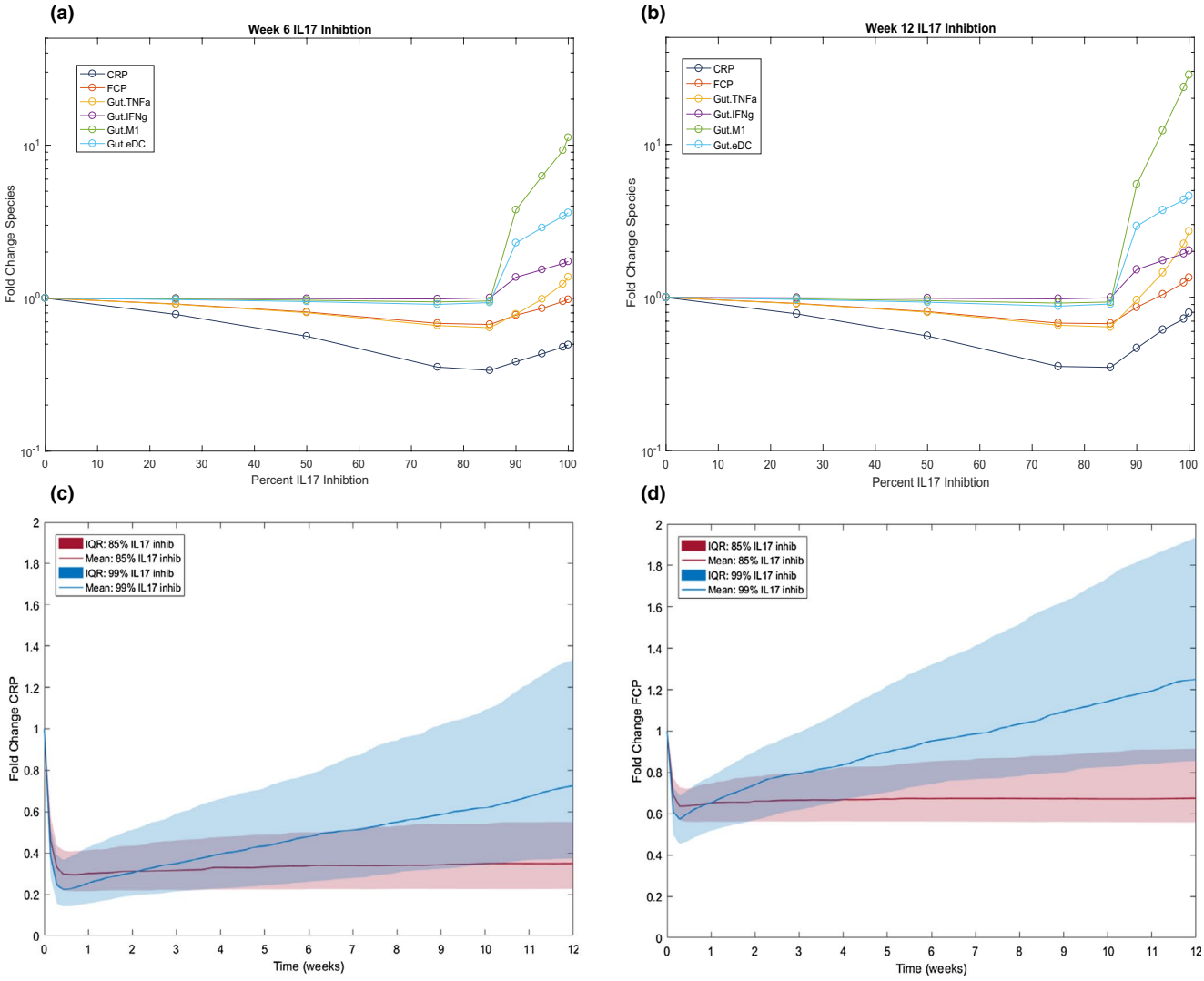

Figure 5 Simulation results of a theoretical IL-17 inhibitor. The theoretical median fold change in cells and cytokines due to different levels of IL-17 inhibition at week 6 (**a**) and week 12 (**b**). Dots indicate the inhibition levels that data was calculated for. The population fold change over time for CRP (**c**) and FCP (**d**) for 85% and 99% inhibition of IL-17. The baseline population (n = 357) was used for the simulation. CRP, C-reactive protein; FCP, fecal calprotectin; IL-17, interleukin 17; IQR, interquartile range.

that the increase in CRP and FCP seen in model predictions is dependent on the extent of IL-17 inhibition (e.g., CRP did not increase above baseline within 12 weeks for a majority of simulated population), but in all 357 cases a 99% inhibition of IL-17 resulted in an increase in CRP and in a few parameter sets (24/357) increases in CRP and FCP occurred as low as 75% IL-17 inhibition.

Identification of key mechanisms by sensitivity analysis

To identify the key mechanisms and interactions affecting clinical biomarkers, CRP and FCP, a local sensitivity analysis for CRP and activated gut neutrophils was performed against model parameters. We used activated gut neutrophils as a surrogate for FCP, as FCP is defined as a Michaelis–Menten equation dependent on total neutrophils in the gut. Sensitivity coefficients were calculated for the 357 parameter sets, previously used for the steady-state calculations for the HV, CD, and UC models. The top 15 sensitive parameters (by normalized sensitivity coefficient) in CD for CRP are listed in **Table 1** (left columns). Top parameters include those related to IL-6, CRP production, IL-17, TNFα, and Th0 (naïve T cells) transport to the gut. **Table 1** (right columns) also includes the top 15 sensitive parameters in CD for activated gut neutrophils. In this case, top parameters include neutrophil production, transport, and degradation. Other sensitive parameters involve IL-6, TNFα, IL-17, and the degradation of Th cells, and macrophages. Overall, there was similarity in the top 50 sensitive species in UC and CD, but they did vary slightly among HV, CD, and UC (**Supplementary Information**). Parameters were varied by 0.01, 0.1, 2, and 10 times the baseline value for all 357 parameter sets using the CD model and effect of varying the parameters on CRP and FCP was recorded. **Figure 6** shows the results of perturbing a unique and unrelated subset of the

Table 1 Results of Sensitivity Analysis

	CRP		Activated neutrophils	
Ranking	**Top CD parameters**	**Parameter description**	**Top CD parameters**	**Parameter description**
1	kdeg_IL6	Degradation rate of IL-6	kdeg_Neu	Degradation of Neu
2	KmProtSyn	Half-sat, IL-6 on CRP production	vf_5	Rate of Th0 transport
3	kmax_IL6_b	Maximum IL-6 production rate in the blood	Th0_blood_constant	Amount of Th0 available to move to the gut
4	kIL6_bloodtoliver	Transport rate of IL-6 from blood to liver	kbasal_Neu	Basal production of Neu in blood
5	kCRP_LivertoBlood	Transport rate of CRP from liver to blood	vf_94	Rate of transport of Neu from blood to gut
6	VmProtSynth	Rate of CRP production	kbasal_Th0	Basal production rate of Th0
7	kdeg_IL17	Degradation rate of IL-17	vf_115	Rate of Neu activation
8	kdeg_TNFa	Degradation rate of TNFa	kbasal_TNFa	Basal production rate of TNFa
9	k6_37	Half-sat, IL-17 on IL-6 production	kdeg_TNFa	Degradation rate of TNFa
10	k5_37	Half-sat, TNFa on IL-6 production	kdeg_Th17	Degradation rate of Th17
11	kbasal_IL17_b	Basal rate of IL-17 production in the blood	kdeg_M0	Degradation rate of M0
12	kCRP_BloodtoLiver	Transport rate of CRP from blood to liver	kdeg_IL6	Degradation rate of IL6
13	vf_5	Rate of Th0 transport	kbasal_IL6	Basal production of IL6
14	Th0_blood_constant	Amount of Th0 available to move to gut	kdeg_Th0	Degradation rate of Th0
15	kbasal_TNFa_b	Basal rate of TNFα production in the blood	k1_51	IL-17 production by Th17

The left two columns show the top 15 sensitive rate parameters for CRP and the right two columns show the same for activated neutrophils.
CD, Crohn's disease; CRP, C-reactive protein; IL, interleukin; Neu, neutrophils; Th0, naïve T helper cells.

50 parameters selected on the concentration of CRP and FCP. We used a cutoff value of 5 mg/L for CRP and 250 mg/kg for FCP for significance, in accordance with previously proposed values in the literature.[32] Our robustness analysis revealed that there are a few parameters that affect only one clinical marker and not the other, whereas others affect both the markers in similar or opposite ways. For example, increasing the maximal velocity (V_{max}) rate of IL-6 production increases CRP and has no effect on FCP, whereas increasing V_{max} rate of neutrophil transport to the gut increases FCP and has no effect on CRP. In contrast, modulating the V_{max} of Th0 cell transport into the gut affected both CRP and FCP, with the change in the parameter value and clinical biomarkers moving together in the same direction. Modulating naïve T cell differentiation to either Th17 or Treg also affected both CRP and FCP, but had the opposite effects, with decreasing V_{max} of Th17 differentiation leading to lower CRP and FCP levels.

DISCUSSION

The application of QSP modeling in drug development is rapidly increasing with mechanistic models being utilized to understand biomarker response, simulate specific populations, and aid in dose selection and clinical trial design. IBD is a complex disease with many different mechanisms involved in giving rise to the disease phenotype. In such a scenario, because of its dynamic and mechanistic nature, a detailed QSP model provides an ideal platform to gain critical understanding of behavior of important disease biomarkers, relative importance of key mechanisms, and differences in treatment response. A differentiating factor for this model as compared with others is its ability to simulate both CD and UC from the same underlying biological mechanisms but different initial conditions, which better represent the particular IBD subtype. The model has been developed in a modular fashion with each biological interaction incorporated as a Michaelis–Menten type reaction, thus making it easy to add, remove, or modify interactions based on new biological information. Using a novel method by Allen *et al*. to generate virtual populations, the model accurately predicted steady-state levels of multiple cytokines, as well as two critical clinical biomarkers, CRP and FCP, thus providing confidence in application of the model to study different treatment effects and compare the response of various biomarkers.

In this part (part 1) of a two-part series, we apply the model to understand the impact of IL-17 inhibition and test a possible hypothesis for disease exacerbation, as observed in the clinic. The IL-23/IL-17 axis inhibition (e.g., ustekinumab) has been proven to be effective in CD but IL-17-specific inhibitors have been associated with lack of efficacy. Many therapies targeting IL-23/IL-17 axis are in clinical development for CD (risankizumab and brazikumab). Two anti-IL-17 inhibitors, secukinumab and brodalumab, which target IL-17A and IL-17RA, respectively, failed in clinical trials and may have worsened disease.[33,34] The addition of epithelial damage causing increased macrophage and DC activation, presumably due to bacterial infiltration, was captured in the model at high levels of IL-17 inhibition. With this added mechanism, the model predicted increased TNFα and IFNγ in the gut, similar to the preclinical Ogawa *et al*. study[31] and also predicted increases in FCP and CRP at high levels of IL-17 inhibition (> 90%) compared with lower inhibition levels. This effect is similar to the one expected in the case of epithelial barrier disruption and subsequent restoration during treatment with increased macrophage and DC activation expected in case of barrier disruption.

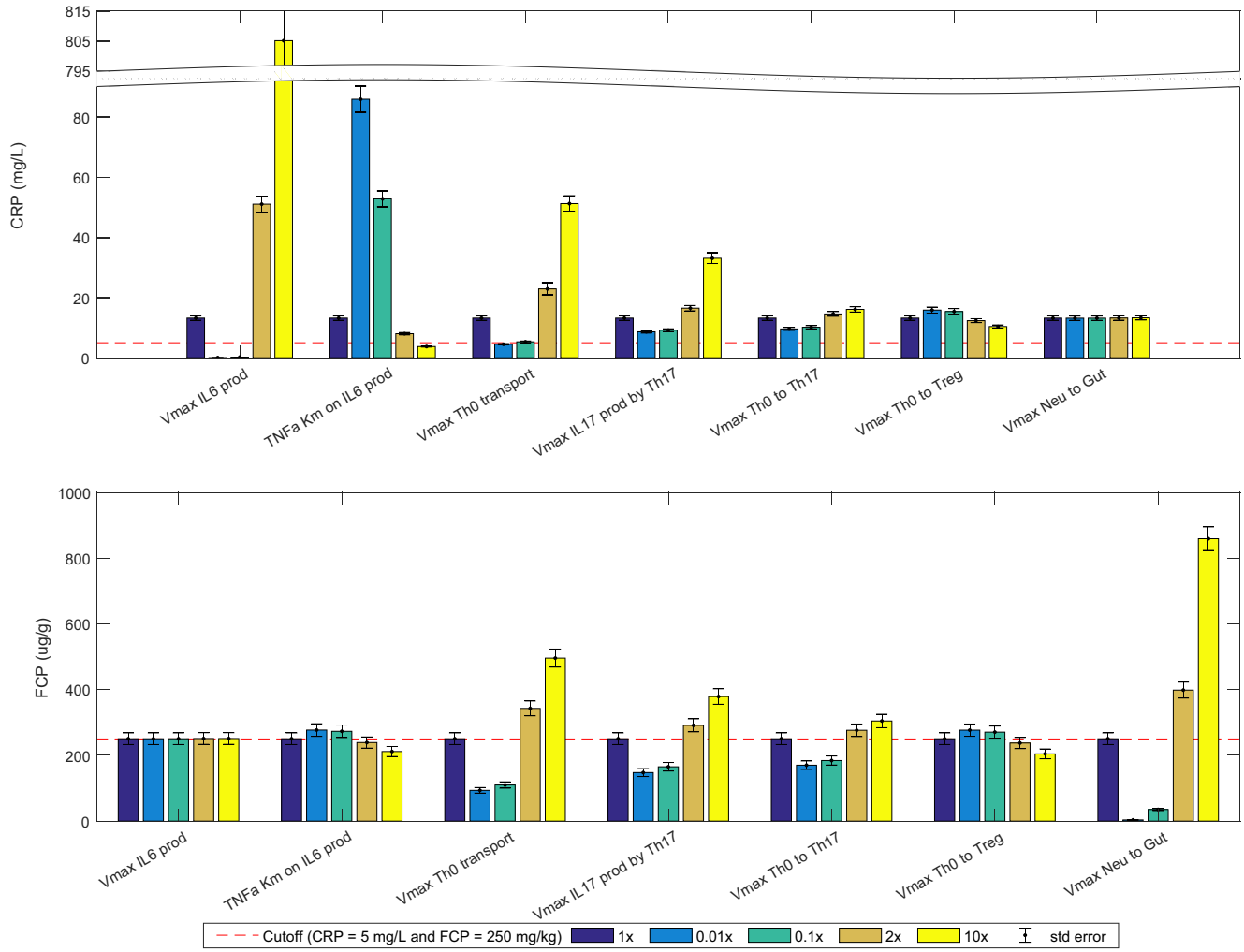

Figure 6 Effect of parameter perturbation on key biomarkers. Parameters were perturbed by 0.01, 0.1, 2, and 10 times the baseline parameter value. Updated CRP and FCP steady-state levels were calculated for the entire baseline population (n = 357) with the perturbations. Bars show mean of the population response and error bars indicate standard error. The cutoff for CRP was chosen as 5 mg/L and FCP was 250 mg/kg. CRP, C-reactive protein; FCP, fecal calprotectin; V_{max}, maximal velocity.

Treatment-induced restoration of the epithelial barrier could have multiple effects not currently considered that will need to be validated with specific data observed with such treatments. Thus, this shows the utility of the model to gain a thorough understanding of seemingly perplexing observations from the clinic.

To identify important parameters, sensitivity analysis was performed on the model for the HV, CD, and UC cases. For CRP, many sensitive parameters as expected involved IL-6 production, IL-6 effect on CRP production, and CRP production rate. Other sensitive parameters involved Th0 cells transport to the gut, Th0 to Th1 or Th17 differentiation, and degradation of Th cells. Many approved drugs and drugs in development target these mechanisms, such as anti-adhesion molecules that target the recruitment of leukocytes to the site of inflammation and others that block cytokines leading to Th cell differentiation, such as ustekinumab or JAK inhibitors.[35] Activated gut neutrophils were sensitive to parameters involving cytokines IL-8, TNFα, IL-6, and granulocyte-macrophage colony-stimulating factor, which are important in either the transport and/or activation of neutrophils in the gut. Macrophage and DC activation also are in the top 50 sensitive parameters for activated neutrophil levels, suggesting targeting the activation of these cells as a possible treatment. Robustness analysis was performed to confirm how modulation of these parameters affected CRP and FCP concentrations. We saw that the parameters highly sensitive in the case of CRP (i.e., V_{max} of IL-6 production), had no effect on FCP. This was seen clinically as anti-IL-6 inhibitor had a dramatic effect on CRP and no significant treatment effect on FCP.[36] This also highlights an important point, that the change in CRP and FCP is highly mechanism dependent and they should not be expected to behave similar to each other in response to a treatment.

Modeling IBD is highly complex and thus this model has limitations that need to be considered. The details of active cell-transport are not included in the model, transfer of cells (e.g., neutrophils) from the blood to the gut is modeled using a simple transfer equation. As the model has been developed in a modular fashion, additional information regarding

cell transport and additional cytokines in the blood can be added once experimental data becomes available. Currently, the model does not include an intestinal epithelial barrier and, therefore, cannot predict decreases in inflammation due to healing of this barrier. In addition, not included explicitly is bacterial activation of cell types or cell binding (i.e., T cells binding to macrophages), instead, basal production rates of different cytokines and cells are modified between healthy and diseased. Another major limitation is the lack of spatial considerations in the model at the tissue level. For example, in CD, location of inflammation can be in the ileal, colorectal, ileocolonic, and upper gastrointestinal tract. However, the current model assumes one compartment for the gut and a homogeneous level of inflammation across the compartment.

In summary, a dynamic, mechanism-based QSP model was developed for IBD by mathematically describing key biological interactions in the gut. The model reasonably replicates steady-state values of cytokines, cells, and biomarkers in HV, CD, and UC. In part 1, we present the model structure, development of a plausible virtual population, test a hypothesis to explain increasing levels of CRP and FCP at high (> 90%) inhibition of IL-17, and determine sensitive parameters for CRP and FCP. In part 2, we present the application of the model to gain a deeper understanding of current therapeutic mechanisms in IBD and explore novel drug combinations. We believe that the current model, because of its mechanistic and modular nature, can be explored to aid in clinical drug development in IBD by exploring new target mechanisms, drug combinations, relevant biomarkers, and specific subpopulations.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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