Received: 26 September 2022 | Revised: 20 December 2022 | Accepted: 20 January 2023

DOI: 10.1002/psp4.12932

ARTICLE

Cell-level systems biology model to study inflammatory bowel diseases and their treatment options

Sabine Stübler[1,2] | **Charlotte Kloft**[3] | **Wilhelm Huisinga**[1,4]

[1]Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

[2]Graduate Research Training Program PharMetrX: Pharmacometrics & Computational Disease Modelling, Freie Universität Berlin & University of Potsdam, Berlin, Germany

[3]Department of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

[4]Institute of Mathematics, University of Potsdam, Potsdam/Golm, Germany

Correspondence
Wilhelm Huisinga, Institute of Mathematics, University of Potsdam, Potsdam/Golm, Germany.
Email: huisinga@uni-potsdam.de

Present address
Sabine Stübler, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riß, Germany

Abstract

To help understand the complex and therapeutically challenging inflammatory bowel diseases (IBDs), we developed a systems biology model of the intestinal immune system that is able to describe main aspects of IBD and different treatment modalities thereof. The model, including key cell types and processes of the mucosal immune response, compiles a large amount of isolated experimental findings from literature into a larger context and allows for simulations of different inflammation scenarios based on the underlying data and assumptions. In the context of a large and diverse virtual IBD population, we characterized the patients based on their phenotype (in contrast to healthy individuals, they developed persistent inflammation after a trigger event) rather than on a priori assumptions on parameter differences to a healthy individual. This allowed to reproduce the enormous diversity of predispositions known to lead to IBD. Analyzing different treatment effects, the model provides insight into characteristics of individual drug therapy. We illustrate for anti-TNF-α therapy, how the model can be used (i) to decide for alternative treatments with best prospects in the case of nonresponse, and (ii) to identify promising combination therapies with other available treatment options.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Patients with inflammatory bowel disease (IBD) are very heterogeneous regarding the IBD dispositions, course of disease, and responsiveness to treatment. Therefore, the choice of treatment for the individual patients is challenging. Several published systems biology models[1–4] have been applied to this problem, with different approaches, focusing on different aspects, and giving different results.

WHAT QUESTION DID THIS STUDY ADDRESS?

How can mathematical modeling be used to help analyzing IBD predispositions and responsiveness to treatment?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We provide a mathematical model of the mucosal immune response that can describe a heterogeneous population of patients with IBD, linking the onset of

disease and responsiveness to different treatments to individual predispositions (model parameters).

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The presented novel systems biology model may serve as a tool to help pre-evaluate potential drug targets, drug combinations, and alternative treatments in nonresponders to a first treatment choice.

INTRODUCTION

Inflammatory bowel diseases (IBDs) are chronic diseases characterized by chronic inflammation of various parts of the gastrointestinal tract.[5] They affect ~0.3% of Western populations,[6] severely decreasing life quality, and can result in life-threatening complications. The main types are Crohn's disease (CD) and ulcerative colitis (UC). The disease results from an over-reaction of the mucosal immune response to commensal bacteria of the intestine, involving destruction of the intestinal barrier.[7] Although many different genetic and environmental predispositions have been found, the pathogenesis is still not fully known.[8] Treatment options involve anti-inflammatory small-molecule drugs as well as biologics—most prominent are monoclonal antibodies (mAbs) targeting the cytokine TNF-α[9]; but also other processes are targeted with the aim to reduce the inflammation. Patients show high interindividual differences, regarding predispositions and disease characteristics, but also responsiveness to the different treatments, which poses large challenges in finding the optimal treatment and dosing regimen for the individual patient.[10–12]

Our objective is to develop a systems biology model of IBD to help understand and analyze these large interindividual differences in the patients' mucosal immune response, and to subsequently better inform target identification and choice of individual treatments. To this end, we screened the large body of literature on the mucosal immune response for relevant data, key molecular/cellular players, and processes. The model was evaluated against responses to mucosal injury and to a salmonella infection, and used to generate a virtual population of healthy individuals and patients with IBD for further analysis. Compared to the few existing systems biology/pharmacology models of IBD,[1–4, 13] we characterized disease based on the phenotype (persistent inflammation after a trigger event) rather than on a priori assumptions on parameter differences to the healthy state, and focused on the cell-level of the immune system, rather than on the cytokine level. Importantly and in contrast to existing approaches,[1–4, 13] the model reproduces (rather than a priori assumes) the known large diversity in disease-causing predispositions and response to different treatments. In addition, we evaluated options for alternative treatments in case of nonresponse and promising combinations of treatment effects.

METHODS

The structure and parameterization of our systems biology model—the main result of this work—was based on qualitative and quantitative literature data. Qualitative data were used to decide for relevant aspects to be included in the model and the model structure. Quantitative data were used to parameterize the model: Parameter values were either taken directly from literature (e.g., half-lives or steady-state concentrations of cell types), estimated from literature data (e.g., transition rates estimated from time-resolved data on concentrations of different states), taken or modified from previously published mathematical models, or set to a value in a reasonable range that led to desired qualitative behavior of the model, when more quantitative data were not available. We used preferably data from human studies, as far as available (e.g., steady-state concentrations of cell types), but also from in vivo (mouse or rat), in vitro, or in silico studies. An in-depth description of the model is given in Appendix S1, including the biological background of the processes included in the model, the mathematical equations constituting the model (Chapter 4.1 in Appendix S1) and a detailed derivation and explanation of these equations (Chapter 1 in Appendix S1).

We aimed for a model describing the mucosal immune system not only at the level of the overall immune response (e.g., successful elimination of pathogens), but also at the cellular level (i.e., detailed dynamics of different immune cell types). To guide model development, we simulated the response to mucosal injury and to a salmonella infection (as a widely used model for gut infection). A healthy mucosal immune response was expected to comprise the elimination of bacteria from the tissue, a chronological sequence of triggered events (e.g., very

fast increase of neutrophils with an early peak, followed by slower increase in the macrophages, and, with a short delay, in T cells) and the return to healthy steady-state (resolution). During model development, the model structure and included cell types were continuously evaluated for their ability to predict the above outlined mucosal immune response and plausibility of the derived parameter values.

As the parameterization of the model was based on many different publications (including different model species and different measurement methods), the results are not intended strictly quantitative, but rather qualitative, as shown in the presented example of an analysis of IBD predispositions and responsiveness to treatment using a virtual population.

All simulations were performed in Matlab R2018b. The model code is published under https://zenodo.org/record/7574219#.Y9LLJOLMKwo.

RESULTS

Systems biology model of the mucosal immune response

The model is based on ordinary differential equations (ODEs) and comprises 47 model species, 133 reactions, and 79 parameters. The full model scheme of the novel systems biology model describing the healthy mucosal immune response to pathogenic and commensal bacteria can be found in Appendix S1: Figure S25. For better understandability, Figure 1 shows a simplified scheme, including the main cell types and reactions, but neglecting that some cell types are subdivided into more subtypes. In the following, we summarize the considered processes and cell types. We only give the main references (important sources for quantitative data as basis of parameterization or parts of the model structure); a full derivation of the model structure and parameterization, including all references that we based the model development on, can be found in Appendix S1.

Spatial compartments and units

The model accounts for three spatial compartments: intestinal lumen, lamina propria (LP; the uppermost layer of the intestinal wall beneath the epithelium), and mesenteric lymph nodes (LNs). Species in lumen are unitless, and species in LP and LN are modeled as concentrations (number of molecules per ml). Because of their short half-lives,[14] we accounted for cytokines only implicitly with concentrations depending on the producing cell types.

Epithelial barrier and bacteria in lumen

The intestinal epithelium and a mucus layer that is secreted by the epithelium prevent bacteria from invasion into the underlying LP. We described the functionality of the tissue, epithelium, and mucus layer by unitless state variables; a value of 1 represents full barrier functionality, a value of 0 represents complete barrier destruction, and values in between describe the relative residual functionality. Inflow of commensal bacteria into LP depends on the functionality of the epithelium and mucus layer, with higher inflow for lower values (i.e., more destruction) and no inflow for values of 1 (i.e., complete functionality). Pathogenic bacteria (in simulations of pathogenic infection) are also able to penetrate the intact barrier.

Tissue is constantly renewed, with production influenced by anti-inflammatory cytokines and death influenced by pro-inflammatory cytokines. Epithelium production is dependent on the underlying tissue, its death rate is influenced by pro-inflammatory cytokines and inflowing pathogenic bacteria. Mucus production is dependent on the epithelium.

Elimination of bacteria

Bacteria in LP are eliminated by neutrophils, macrophages, and dendritic cells, mainly via phagocytosis (i.e., ingestion and elimination[15]), preventing a systemic infection. This was implemented using a modified model from ref. 16 describing the phagocytosis of bacteria as a saturable process with a maximal elimination rate per phagocytic cell.

Neutrophils

Neutrophils are scarcely present in healthy tissue, but are quickly recruited in case of infections, eliminating bacteria, and recruiting and activating further innate immune cells via pro-inflammatory cytokines. Neutrophils are also important in the resolution of inflammation, as they produce specialized pro-resolving mediators (SPMs), which inhibit further innate immune cell recruitment.[17] We implemented saturable neutrophil recruitment by bacteria and pro-inflammatory cytokines and apoptosis induced by SPM and phagocytosis of bacteria. Apoptotic neutrophils are eliminated by macrophages via phagocytosis (see section "Macrophages" below).

Macrophages

Macrophages, which are important in the elimination of bacteria and production of pro- and anti-inflammatory

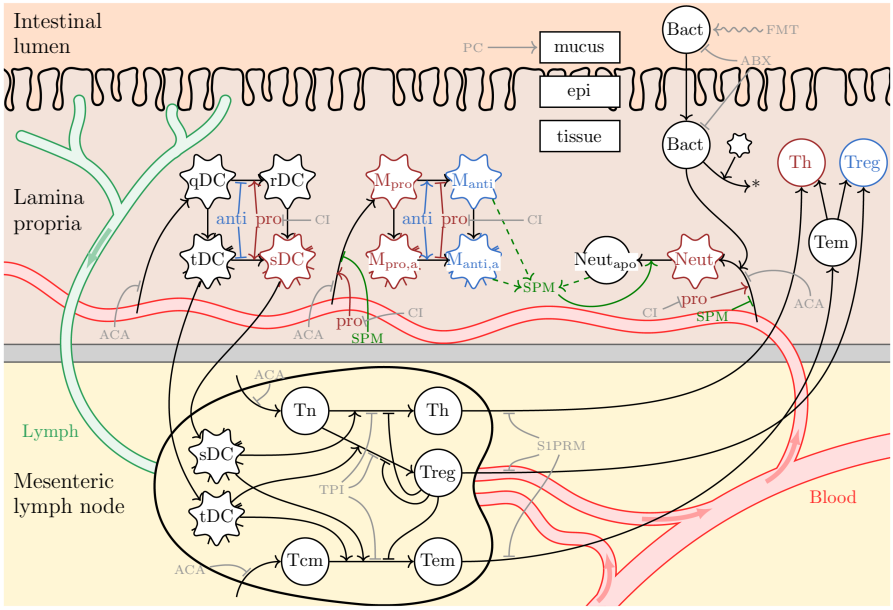

FIGURE 1 Simplified model scheme. Cell types included in the model and their interactions. White circles/shapes represent cell types (model species). Arrows between model species represent transition or migration. Arrows targeting on arrows indicate a stimulation (→) or inhibition (⊣). Pro- and anti-inflammatory cytokines are produced by the respective color-coded cell types. Dashed arrows show production of cytokines by a model species. Gray arrows (stimulating or inhibiting) show the implementation of different treatment effects (CI, cytokine inhibitor; ACA, anti-cell adhesion; S1PRM, S1PR modulator; PC, orally administered phosphatidylcholine; FMT, fecal microbial transplant; TPI, T cell proliferation inhibitor; ABX antibiotics). The full model scheme can be found in Figure S25 in Appendix S1. Compared to the full model scheme, not all state variables and reactions are shown here, some state variables are summarized (e.g., T helper cells). Note that figures describing subprocesses in full detail can be found in Chapter 1 in Appendix S1. pro, pro-inflammatory cytokines; anti, anti-inflammatory cytokines; SPM, specialized pro-resolving mediators; Bact, bacteria; qDC, rDC, tDC, sDC, quiescent, responsive, tolerogenic, and stimulatory dendritic cells; M_{pro}, M_{anti}, pro−/anti-inflammatory macrophages (M1-M3/ M4 & efferocytosis-type); $M_{pro,a}$, $M_{anti,a}$, antigen-experienced pro−/anti-inflammatory macrophages; Neut, $Neut_{apo}$, neutrophils, apoptotic neutrophils; Tn, naive CD4+ T cells; Tcm, Tem, central/effector memory T cells; Th, T helper cells; Treg, regulatory T cells.

cytokines, are recruited from blood in a pro-inflammatory state and turn into an anti-inflammatory state in the LP.[18,19] Based on data from ref. 19, we implemented four different macrophage subpopulations M1–M4. Only subpopulation M1 is recruited from blood, positively influenced by pro-inflammatory cytokines. Macrophages transition through the states depending on anti-inflammatory cytokines, inhibited by pro-inflammatory cytokines. In addition, we implemented a population of macrophages after phagocytosis of apoptotic neutrophils (called efferocytosis). All subpopulations are further divided into (bacterial) antigen-unexperienced and antigen-experienced species, where antigen uptake equals the phagocytosis rate of bacteria. Subpopulations M1 to M3 (in Figure 1: $M_{pro}/M_{pro,a}$) produce pro-inflammatory cytokines; subpopulation M4 and macrophages after efferocytosis ($M_{anti}/M_{anti,a}$) produce anti-inflammatory cytokines; and macrophages after efferocytosis produce SPMs.

Dendritic cells

Dendritic cells take up bacterial antigen in the LP and present it on their surface to activate T cells mainly in LNs, but also in the LP. We implemented four different species of dendritic cells in the LP dependent on activation status and antigen experience: quiescent dendritic cells (recruited from blood; not activated, antigen-unexperienced), responsive dendritic cells (activated, antigen-unexperienced), tolerogenic dendritic cells (not activated, antigen-experienced), and stimulatory dendritic cells (activated, antigen-experienced, producing pro-inflammatory cytokines). Antigen-experienced dendritic cells are able to migrate to LNs.

T cells

T cells, part of the adaptive immune response, shape the innate immune response by providing pro-inflammatory signals via T helper cells, but also by preventing overshooting immune responses via regulatory T cells. We implemented different types of T cells: naive, central memory, effector memory, and effector (helper and regulatory) T cells. The implementation of T cell activation through antigen presentation by dendritic cells (and also in the LP macrophages) is based on contact rates, contact duration, available binding

sites on T cells, and antigen-presenting cells and the probability to encounter the cognate antigen. Once activated, T cell proliferation is implemented using transit compartments based on published T cell growth models.[20,21] Dependent on the activating dendritic cell, the proliferating T cells differentiate into regulatory T cells or T helper cells, where the fractions of T helper cell types 1, 2, and 17 depend on the bacterial cell phagocytosed by the dendritic cell. In contrast to naive T cells, memory T cells' differentiation was implemented in two steps (first into effector memory T cells, then into effector T cells), with a higher activation rate due to a higher probability to encounter their cognate antigen (representing the smaller T cell receptor [TCR] repertoire), and shorter proliferation.[22,23] We implemented production of pro-inflammatory cytokines by T helper cells, with T helper cell type-dependent effects on the targeted processes, and production of anti-inflammatory cytokines by regulatory T cells, with inhibiting effects on innate immune cells. In addition, regulatory T cells inhibited the ability of dendritic cells to stimulate T cells, and inhibited T cell proliferation.[24,25]

Simulation of infection and inflammation

During model development, we used simulations of infection with salmonellae (*S. Typhimurium*) and inflammation due to mucosal injury to evaluate if the model predicts the expected healthy mucosal immune response. We implemented salmonella infection by accounting for their ability to penetrate the epithelial barrier and to survive and proliferate inside macrophages, with data on infection dynamics from refs. 26,27. For the simulation, we considered salmonellae in lumen at $t = 0$. For the mucosal injury scenario, we simulated maximal destruction of the epithelium and tissue by setting their functionality values to zero at $t = 0$. Figure 2 shows the resulting time courses of the mucosal immune response to salmonella infection and mucosal injury. For salmonella infection, we observed an increase of the bacterial concentration in the LP through salmonellae, but also inflowing commensal bacteria, because the salmonellae and cytokines of the resulting immune response degrade the epithelial barrier. For mucosal injury, we observed a massive inflow of commensal bacteria due to the destruction of the epithelial barrier. In both cases, the model predicted a repair of the epithelial barrier and the elimination of inflowing pathogenic and commensal bacteria within ≈ 3–4 weeks, as we would expect from a healthy mucosal immune system. The chronological sequence of the inflow of immune cells, which is quite similar for both scenarios, is in line with literature reports.[28–30] Neutrophils are recruited very quickly, peak early, and subsequently decrease, already

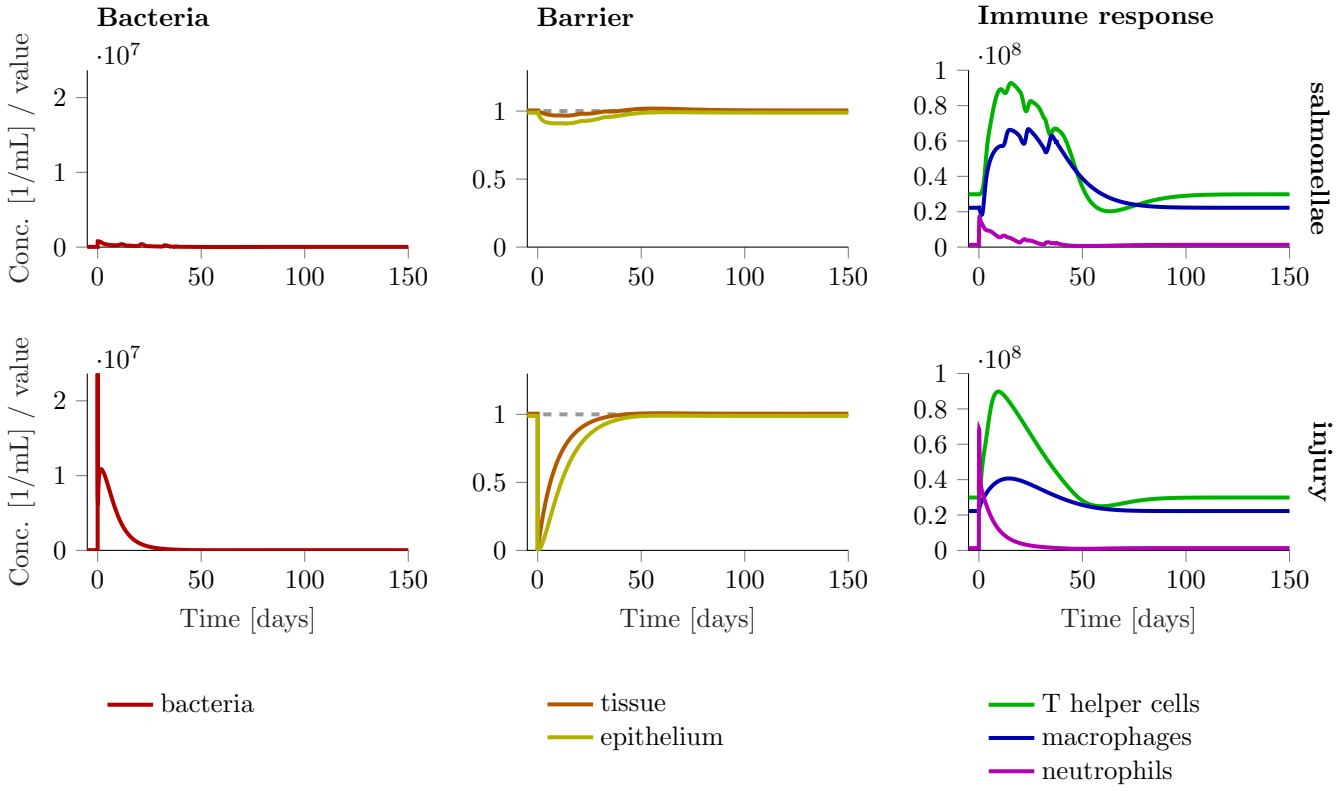

FIGURE 2 Reference time course of salmonella infection (top) and mucosal injury (bottom). Concentrations of bacteria in LP (sum of commensal and, if present, pathogenic), barrier state variables (unitless, describing the relative functional intactness), and selected immune cells in LP over time in response to salmonella infection or mucosal injury at time $t = 0$. LP, lamina propria.

starting the resolution of the inflammation. Following the neutrophils, macrophages are recruited into the LP, and with short delay (≈ 1 day; not well visible as shown on the time scale), T cell concentrations in the LP increase.

In summary, we conclude that the implemented processes and cell types are sufficient to adequately describe the healthy mucosal immune response to salmonella infection and mucosal injury, as our novel systems biology model is able to qualitatively, and, to a certain extent, quantitatively, describe the healthy mucosal immune response to different triggers. In the sequel, we use the term "reference individual" to denote the systems biology model with the above described "reference parameters."

Virtual population of healthy individuals and patients with IBD

Patients with IBD have accumulated several risk factors (genetic predispositions and environmental stimuli) so that an additional trigger, probably a perturbation of the mucosal barrier, results in the outbreak of the disease.[31,32] In contrast to many other systems biology approaches (e.g., refs. 1,3), we did not define which parameter changes elicit the disease, but rather defined the disease by its phenotype of chronic inflammation, characterized by a high immune activity as a consequence of failed resolution of inflammation.

More precisely, we defined virtual patients with IBD as individuals that (i) were asymptomatic prior to the trigger (i.e., in a steady-state comparable to the healthy steady-state of the reference individual), and (ii) developed chronic inflammation in response to the mucosal injury trigger described above (in the previous section, see Figure 2, bottom row). (See also Chapter 2.1 in Appendix S1 for more details on the definition of virtual patients with IBD.) Consequently, patients with IBD developed a new steady-state with higher immune cell concentrations—in contrast to healthy individuals, who were able to resolve the inflammation after a mucosal injury trigger and returned to the pre-trigger healthy steady-state. In the sequel, we use the term "IBD individual" to denote both patients with IBD and individuals with IBD dispositions before the outbreak of disease.

We generated healthy and IBD individuals as follows: for an individual, we sampled a complete set of parameter values from a log-normal distribution around the set of reference parameters. For that, we assumed for all parameters the same relative standard deviation of $\sigma = 0.4$ of the underlying normal distribution, as the variability of single parameters was not known. The resulting population comprised healthy and IBD individuals, in addition to individuals with permanent chronic inflammation (already before the trigger) or chronic infection (uncontrolled growth of commensal bacteria in the LP). The latter two types of individuals were excluded in the sequel, because they were irrelevant for our subsequent study of IBD. By randomly sampling 1,000,000 individuals, we obtained a virtual population of 69.5% healthy and 2.2% IBD individuals. The fraction of IBD individuals in our virtual population depends on σ and was purposely designed to be higher than the reported prevalence (ref. 6) to not miss out on extreme values for some parameters potentially leading to IBD.

Figure 3 shows the time course of the mucosal immune response to the mucosal injury trigger in the virtual populations of healthy and IBD individuals. Comparing the average IBD individual to an average healthy individual, we observed no visible difference before the trigger (see Figure S23 in Appendix S1), but a larger increase of T cells in the first days, followed also by a larger increase of macrophages. Although in healthy individuals the inflammation is resolved and the cell concentrations return to pre-trigger steady-state, the IBD individuals reach a post-trigger steady-state with much higher immune cell concentrations than before the trigger and a less intact mucosal barrier. Although not visible in the figure, the neutrophil and bacteria concentrations in the LP are also much higher in patients with IBD (see Figure S23 in Appendix S1). The interindividual variability in cell concentrations (e.g., neutrophils, macrophages, and T cells; see Figure 3, columns 3 and 4) is very high, both in healthy and IBD individuals.

Analysis of IBD predispositions

Individuals of the virtual population are defined by their set of parameter values. We compared the distributions of parameter values among healthy and IBD individuals to identify differences and to search for (combinations of) parameters that allow for a discrimination between healthy and IBD individuals. Figure 4a shows the distributions of parameter values that differ most between healthy and IBD individuals, measured using a Two-Sample Kolmogorov–Smirnov test. In line with literature findings about the complex etiology of IBD,[31,33] we did not find a single parameter that allows to identify IBD individuals. This is a consequence of the large overlap of the two parameter distributions. Using a linear discriminant analysis (LDA), we could correctly classify 87% of individuals to be healthy or diseased (in a population of the same number of healthy individuals and patients with IBD) based on all parameters. The best predictors identified by the LDA were $h_{\text{pro,deact,M}}$, $h_{\text{rec,M}}$, and $h_{\text{pro,act,DC}}$, resulting in 71, 73, or 76% correctly classified if used alone, two together or all three together, respectively. In summary, our model can

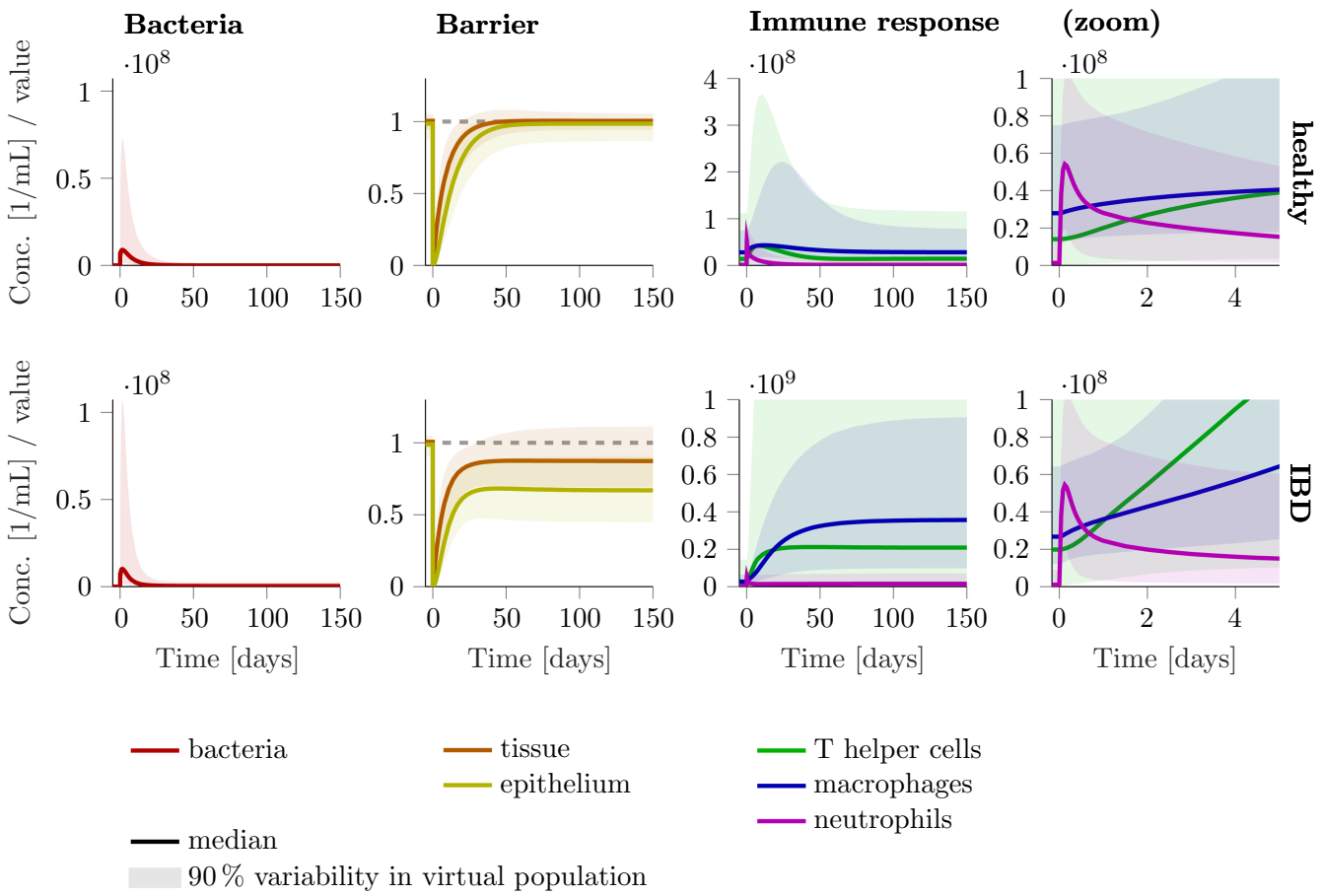

FIGURE 3 Population time course of immune response to mucosal injury in healthy individuals (top) and patients with IBD (bottom). Concentrations of bacteria in LP, barrier state variables (unitless, describing the relative functional intactness), and selected immune cells in LP over time in response to mucosal injury at time $t = 0$. Solid lines show the median, dashed lines show the 5th and 95th percentiles (within the population) of the respective state variable. The fourth column is a zoom-in of the first 5 days of the third column. IBD, inflammatory bowel disease; LP, lamina propria.

reproduce the enormous diversity of predispositions and the complex interplay of different processes in the development of the disease. We considered this an additional confirmation of our model.

Three of the four parameters with strongest correlation to the disease (see Figure 4a) are Hill factors (h), influencing the reaction rates of macrophage deactivation ($h_{\text{pro,deact,M}}$), macrophage recruitment ($h_{\text{rec,M}}$), and dendritic cell activation ($h_{\text{pro,act,DC}}$). Those Hill factors determine how sensitive the reaction rate is to a change in the cytokine concentration driving this reaction. They have a large potential to favor the switch between asymptomatic and chronically inflamed steady-states that we see in IBD individuals, as a larger Hill factor results in a larger difference in reaction rates for small changes in the cytokine concentration. Because Hill kinetics are often used as an empirical simplified model for a saturable, cooperative process (as in our case), the physiological interpretation of Hill factors beyond "sensitivity" is difficult. In our model, we used Hill kinetics for all reactions influenced by pro- and/or anti-inflammatory cytokines (i.e., recruitment and activation or deactivation). Due to lack of detailed knowledge on the cooperativity of those reactions, most Hill factors were set to 1 (i.e., no cooperativity), in the reference individual. The Hill factors were, however, still included in the model to allow for cooperativity in individuals of the virtual population, as we could not exclude that they may be important. The presented results of Hill factors being strongly correlated with the disease, show their potential importance for the development of IBD. Among the parameters that are strongly correlated with the disease, several relate to macrophages (e.g., the maximal macrophage recruitment rate $\lambda_{\text{M,max}}$ shown in Figure 4a). This suggests an important role of macrophages for IBD, which again is in line with extensive reports in literature.[18,34]

We next identified those parameter changes between healthy and IBD individuals that are relevant for the development of disease (i.e., the IBD dispositions of our virtual patients). Because the IBD individuals are based

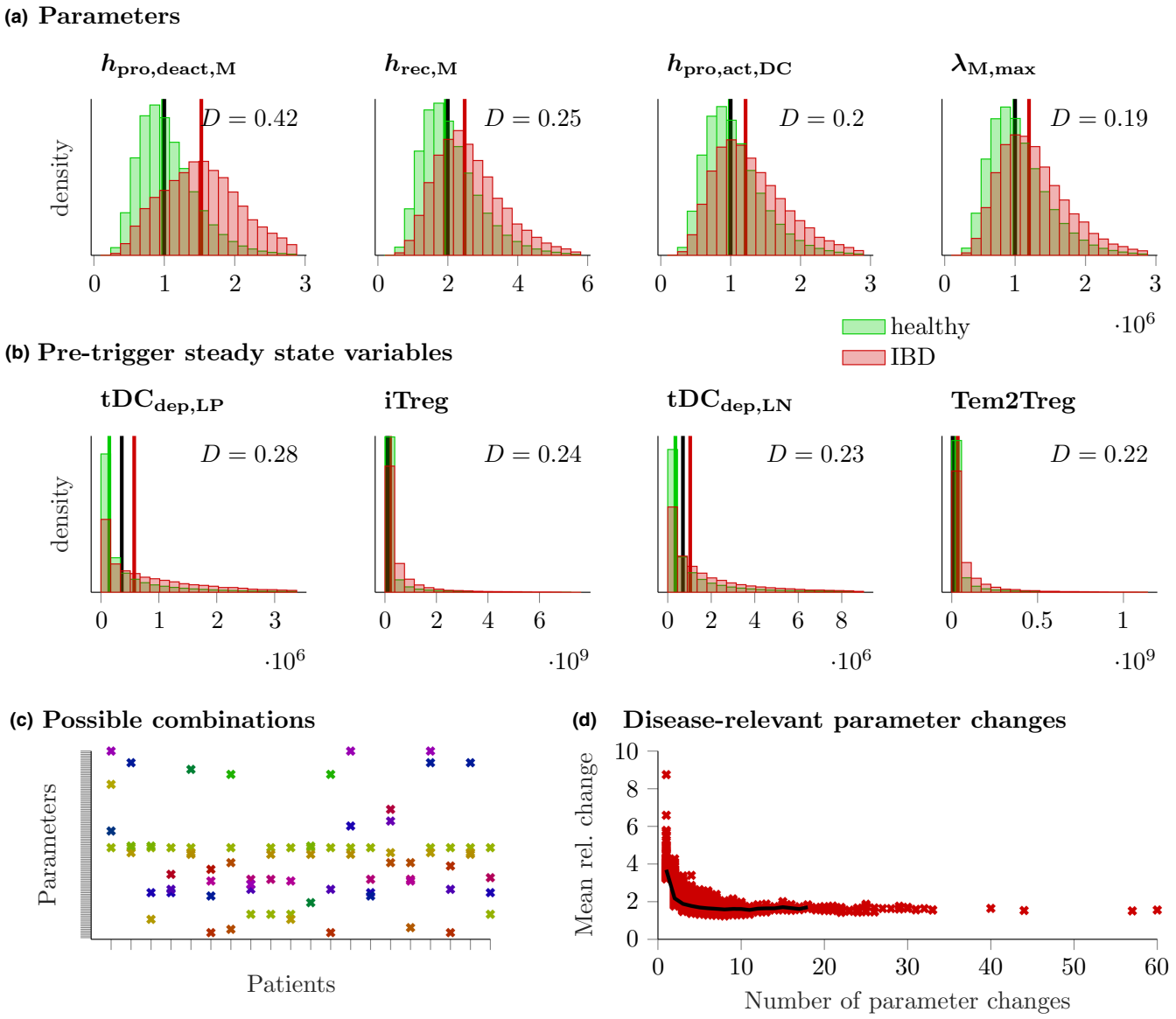

FIGURE 4 Differences between healthy individuals and patients with IBD. (a/b) Histograms of (a) parameter value distributions and (b) pre-trigger steady-state distributions for the four parameters/pre-trigger steady-state variables with highest Kolmogorov–Smirnov test statistic D (testing for difference between healthy and IBD), for healthy (green) and IBD individuals (red). Green and red vertical lines indicate the corresponding population medians; the black line indicates the reference value (for parameters overlaying the healthy median). Outliers (values above 1.5 times the 95th quantile) are not shown to improve readability of the plot. (c) Disease-relevant parameter change combinations for 20 (randomly selected) virtual patients with IBD with exactly four disease-relevant parameter changes. Colors are solely used to discriminate the different parameters. (d) Average disease-relevant parameter changes versus the number of disease-relevant parameter changes. Each cross denotes one virtual patient with IBD. The black line shows the average extent over all patients with the same number of disease-relevant parameter changes. IBD, inflammatory bowel disease.

on random perturbations of the reference parameter values, the question remained whether all perturbations are "necessary" for the IBD disposition. Therefore, we identified for each IBD individual the minimal set of parameter changes (in the following called disease-relevant parameter changes) that still resulted in the outbreak of the disease following the mucosal injury trigger (see Chapter 2.3 in Appendix S1 for more details). While both "types" of virtual IBD individuals, defined by the original and the minimal set of parameter changes, lack the ability of resolution after the mucosal injury trigger, they might differ in the precise time course of their immune response. We found that the sets of disease-relevant parameter changes are very heterogeneous. Figure 4c illustrates the diversity of changed parameters of 20 (randomly selected) IBD individuals with exactly four disease-relevant parameter changes. The parameters we identified earlier to be most correlated with the disease (see Figure 4a), however,

remained most frequently among the disease-relevant parameter changes (e.g., $h_{\text{pro,deact,M}}$ is disease-relevant in 68% of the patients). Figure 4d shows the average extent of the disease-relevant parameter changes over the number of disease-relevant parameter changes for all IBD individuals. We observed a wide variety of numbers of disease-relevant parameter changes (1–60, median 4), and a negative correlation between the average extent of parameter change and the number of simultaneous changes. In conclusion, an IBD disposition can result from both a small number of large parameter changes or a higher number of small parameter changes, with many different possible combinations. In the remaining part of the paper, we always used the original parameter sets when considering IBD individuals.

We finally compared the pre-trigger steady-state variables between healthy and IBD individuals to investigate if we can already identify IBD individuals before the onset of the disease based on phenotype (i.e., cell concentrations). Figure 4b shows the distributions of state variables that most correlate with the disease. Using an LDA, we could correctly classify 67% of individuals to be healthy or diseased (in a population of the same number of healthy individuals and patients with IBD) based on all pre-trigger steady-state concentration values. Given the limited ability to really measure the identified cell concentrations in addition to measurement uncertainty, our analysis suggests that it is unlikely to identify a large fraction of patients with IBD based on cell concentration measurements before disease onset.

Implementation of different treatment effects

To analyze existing and potential new therapeutic interventions, we implemented the effect of several drug and non-drug treatment options and performed virtual clinical studies in our virtual IBD population. Treatment response was analyzed by a virtual analog of the common Crohn's disease endoscopic index of severity (CDEIS), which takes values close to zero in healthy individuals, and higher values for higher levels of inflammation. The CDEIS was determined from the model's state variables (epithelium, tissue, and neutrophils) at different time points (see Table S6 in Appendix S1). For the clinical study simulations, we restricted our virtual IBD population to patients with CDEIS > 6 (as in e.g., ref. 35). Response to treatment was defined as CDEIS < 4 (as in e.g., ref. 35). Instead of implementing specific drugs, we implemented more general treatment effects (i.e., groups of drugs with similar targets). For example, cytokine inhibitors include monoclonal antibodies targeting tumor necrosis factor (TNF)-α, interleukin (IL)-6 or the JAK–STAT pathway, as all of those specific drug mechanisms translate to an inhibition of pro-inflammatory cytokines in our model. Each treatment effect was characterized by a parameter η, describing the extent of modulation of the targeted processes. The involved model parameters or reaction rates were multiplied by $(1-\eta)$ with $1 \geq \eta > 0$ accounting for inhibition and $\eta < 0$ for stimulation. In Figure 1, the gray arrows indicate the targets of the implemented treatment effects: (i) cytokine inhibitors inhibit pro-inflammatory cytokines (implemented by inhibiting production); (ii) anti-cell adhesion treatment inhibits inflow of neutrophils, macrophages, and dendritic cells into the LP and of T cells into LNs; (iii) sphingosine-1-phosphate receptor type 1 (S1PR) modulators inhibit T cell egress out of the LNs; (iv) orally administered phosphatidylcholine increases the mucus layer (implemented by increased production); (v) fecal microbial transplant (FMT) changes the bacterial composition that determines if a Th1, Th2, or Th17 response is elicited (here η describes the fraction of Th2-eliciting bacteria); (vi) T cell proliferation inhibitors inhibit T cell proliferation in LNs and the LP; and (vii) antibiotics inhibit bacterial proliferation in the lumen and LP.[36] For all treatment effects, we simulated a treatment of 24 weeks assuming simplified pharmacokinetics (i.e., a constant treatment effect parameter η over the treatment duration). Figure 5 shows the time course of two exemplary patients with IBD in response to a cytokine inhibitor and an anti-cell adhesion treatment. Patient A (a nonresponder to the cytokine inhibitor, but responder to anti-cell adhesion treatment) and patient B (a responder to both treatments, but with relapse shortly after the end of anti-cell adhesion treatment) nicely exemplify the inter-individual differences in the responsiveness in the virtual IBD population.

Figure 6a shows how the response rates of the treatment effects in the virtual IBD population depend on the treatment effect parameter η. Interestingly, we observed that the maximal response rate is limited for most of the treatment effects. In particular, antibiotic treatment was not able to achieve significant response rates in our virtual population. For S1PR modulators, we observed a decrease in response rate for treatment effect parameters η close to 1, which can be explained by the two counteracting effects of inhibition of egress of both T helper cells and regulatory T cells from the LNs. In addition, we analyzed the relapse rate (immediate relapse shortly after end of treatment, without any further stimulus; shown by the difference of the two lines in Figure 6a). We found that it was lowest for FMT, phosphatidylcholine, and cytokine inhibitors, and highest for anti-cell adhesion treatment and S1PR modulators. Clinical response rates to drugs using the different treatment effects are highly variable, typically not

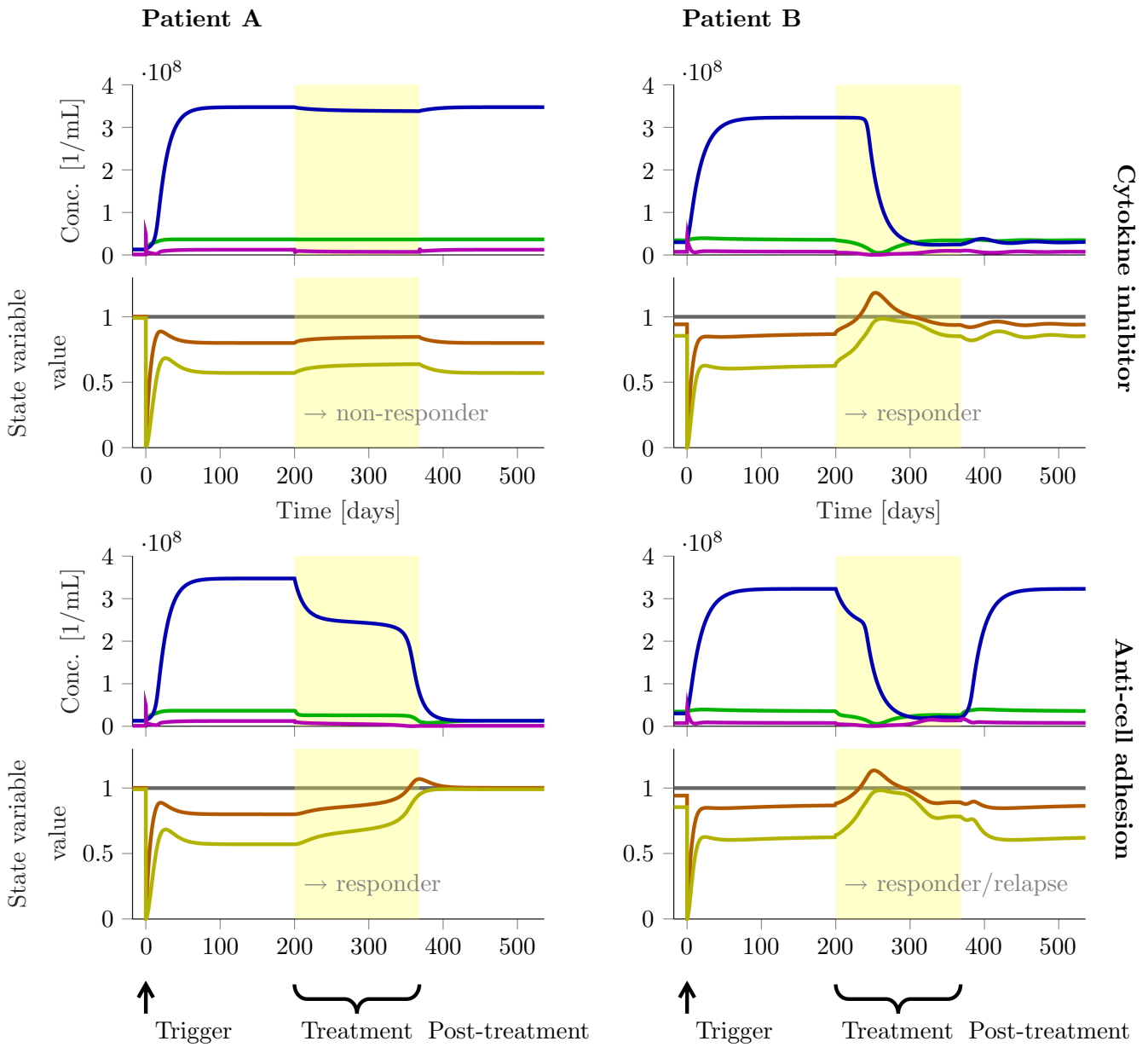

FIGURE 5 Illustration of responders and nonresponders to different treatments. Selected immune cells in the LP (T helper cells [green], macrophages [blue], and neutrophils [violet]) and barrier state variables (tissue [orange] and epithelium [yellow]) over time in two exemplary patients (patient A and patient B). Mucosal injury (trigger) at $t = 0$ leads to chronic inflammation. Treatment started at $t = 200$ d for 24 weeks (yellow background), using a cytokine inhibitor (top; treatment effect extent parameter $\eta = 0.1456$ corresponding to a response rate of 30%) or anti-cell adhesion (bottom; $\eta = 0.261$, corresponding to a response rate of 30%). After the end of treatment, the levels reach a post-treatment steady-state. Patient A is a nonresponder to the cytokine inhibitor, but responder to anti-cell adhesion therapy. Patient B is responder to both treatments, but shows relapse after the end of treatment with anti-cell adhesion. Colors of cell concentrations/state variables correspond to Figures 2 and 3. LP, lamina propria.

exceeding 70% (e.g., cytokine inhibitors anti-TNF-α monoclonal antibodies: up to ~70%,[37] anti-cell adhesion treatment vedolizumab: ~50%,[38] S1PR modulator ozaminod: 41%,[39] orally administered phosphatidylcholine: ~50% in phase II, although phase III recently stopped,[36,40] FMT: ~50%,[36] antibiotics: limited data and controversial discussion[36]; where the reported response rates naturally also depend on the clinical trial and the score used to measure response). For sake of simplicity, in the following analyses, we set η for each treatment effect to the value resulting in a response rate of 30% in our virtual IBD population. Through this, we could compare the treatment effects in general without having to account for specific response rates of specific drugs. We also performed the analyses assuming a response rate of 50%, and the main results presented below continue to hold.

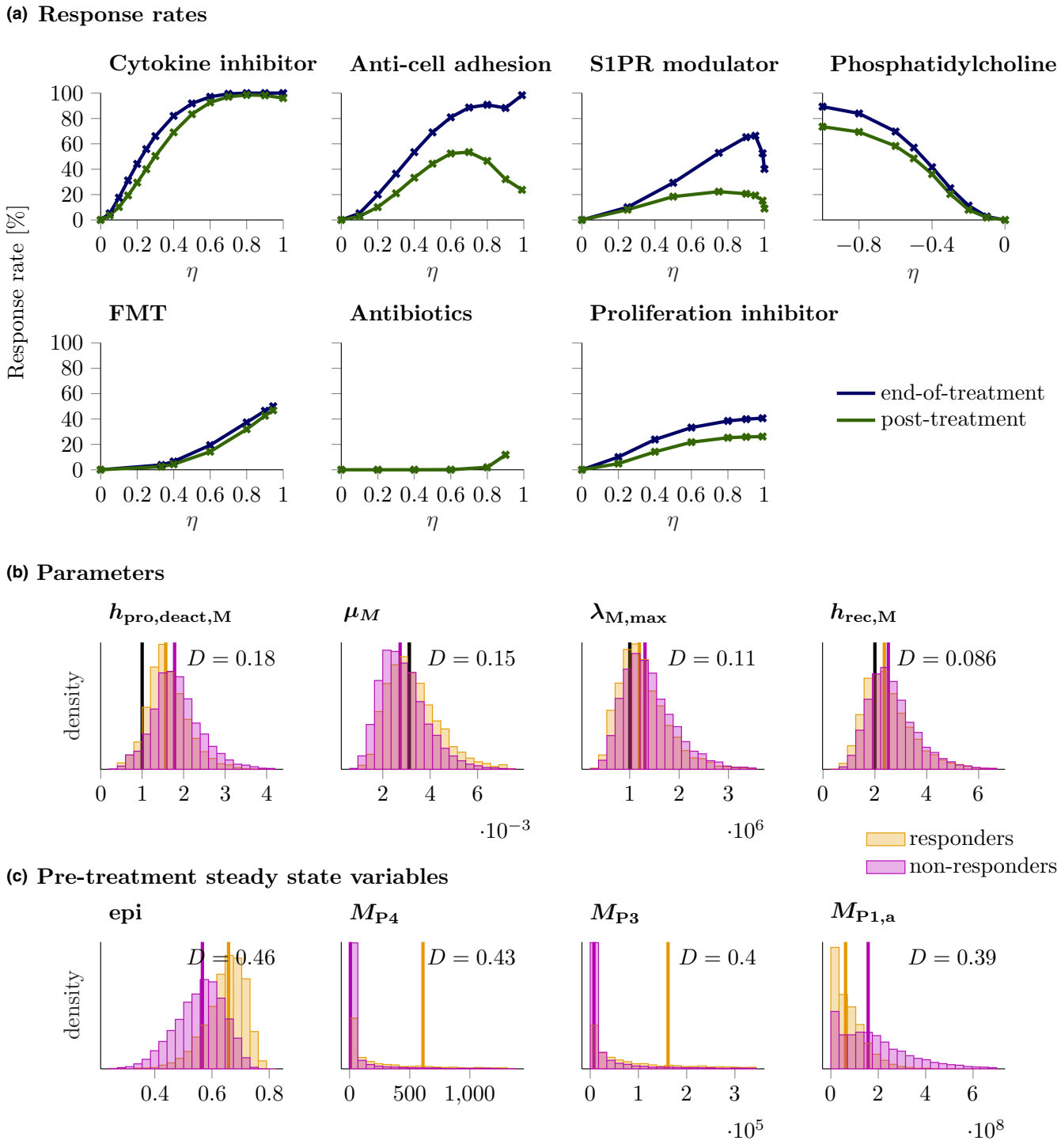

FIGURE 6 Responsiveness of virtual patients with IBD to treatments. (a) Response rates as a function of the treatment effect parameter η. See text and Figure 1 for implementation of the different treatment effects. Note that, naturally, not the full range of displayed treatment effect parameters η is physiologically relevant. Response rate was calculated as the percentage of patients with IBD with CDEIS < 4 at end of treatment (blue) or post-treatment steady-state (green). The difference between end-of-treatment response rate and post-treatment response rate is the relapse rate. For cytokine inhibitors, histograms show (b) parameter value distributions and (c) pretreatment steady-state distributions for the four parameters/pretreatment steady-state variables with highest Kolmogorov–Smirnov test statistic D (testing for difference between responders and nonresponders), for responders (orange) and nonresponders (violet). Orange and violet vertical lines indicate the corresponding population medians; the black line indicates the reference value. Outliers (values above 1.5 times the 95th quantile [/75th quantile for C, second and third columns]) are not shown to improve readability of the plot. CDEIS, Crohn's disease endoscopic index of severity; FMT, fecal microbial transplant; IBD, inflammatory bowel disease; S1PR, sphingosine-1-phosphate receptor type 1.

To identify potential reasons for (non-)responsiveness, we compared the parameter distributions between responders and nonresponders, shown in Figure 6b exemplified for cytokine inhibitors. On the single parameter level, the distributions are highly overlapping, indicating that the causes determining the responsiveness are multifactorial. The parameters most different between responders and nonresponders to cytokine inhibitors are related to macrophages: lower macrophage death rate and higher macrophage inflow rate are correlated with lower response, as are higher Hill factors for the deactivation and recruitment of macrophages. As it would be highly desirable to identify nonresponders before the start of treatment, we compared the pretreatment levels between responders and nonresponders; see Figure 6c exemplified for cytokine inhibitors. We could clearly observe the correlation of lower epithelium functionality (i.e., more destruction), lower concentrations of anti-inflammatory, and higher concentrations of pro-inflammatory macrophage subtypes with a lower response to treatment. Again, however, the distributions overlapped considerably. To find the best predictors for responsiveness, we performed a regularized LDA, which allows to find the best linear models with a limited number of predictors: a linear model of only two predictors (pro-inflammatory macrophages and epithelium) had a prediction accuracy of 74% (in a population of the same number of responders and nonresponders to cytokine inhibitors). To find potential biomarkers for the responsiveness to a specific drug, the treatment effect parameter η can be adapted according to the known response rate, and the described approach (comparison of parameter distributions and regularized LDA) can then be used to identify the best predictors.

Model-guided choice of second-line treatments and combination therapies

Finally, we analyzed how to increase overall response rates in the virtual IBD population by either starting with the first-line treatment and—if the first treatment failed—the optimal second-line treatment, or by directly using simultaneous combination therapies. To this end, we again assumed all treatment effects to be equally effective (i.e., resulting in 30% response rate in the virtual IBD population). (We also performed the analysis with 50% response rate, and the main results continue to hold.)

To identify optimal second-line treatments, we simulated the response rates to alternative treatment effects in the subpopulation of nonresponders to a first-line treatment (termed first-line nonresponders; i.e., the response rate to a treatment conditioned on nonresponsiveness of another treatment). Note that patients that initially responded and subsequently relapsed are considered responders. If the response rates to different treatments were independent within the virtual IBD population, we would expect the response rate to any alternative treatment to be 30% in the population of first-line nonresponders; as a consequence, the particular choice of second-line treatment would not matter. If the response rates were completely dependent within the virtual population (i.e., if an individual does not respond to one treatment), it also does not respond to any other treatment, we would expect the response rate to be 0% for any alternative treatment. Figure 7a shows that the response rates to alternative treatments differ substantially between the treatment effects. The knowledge about the nonresponsiveness of a patient to the first-line treatment is therefore informative for choosing the best second-line treatment. If first-line treatment is given by cytokine inhibitors, anti-cell adhesion treatment or orally administered phosphatidylcholine, the best second-line treatment is given by an S1PR modulator, resulting in response rates of 25.1%–28.3% in the population of first-line nonresponders, which is close to the response rate of 30% in the virtual IBD population, indicating that the response rates are almost independent. If first-line treatment is given by FMT or proliferation inhibitors, the best second-line treatments are given by an S1PR modulator or phosphatidylcholine. However, the resulting response rates in the population of first-line nonresponders are lower (18.4%–21.9%).

To identify the best combination treatments, we simulated the response rates to combinations in the virtual IBD population. For sake of illustration, we considered only combinations of a cytokine inhibitor with other treatment effects, see Figure 7b (red bars). According to our model predictions, combination therapies of cytokine inhibitors with other cytokine inhibitors, anti-cell adhesion treatment, orally administered phosphatidylcholine, or FMT are very promising. In these cases, a large proportion of the nonresponders to the sequential treatment (i.e., to either first- or second-line treatment) respond to the combination treatment (see yellow bar). The specific choice should of course include safety and toxicity considerations. For the combination of cytokine inhibitor plus S1PR modulator, the model predicted the combination therapy to be less successful than using an S1PR modulator as second-line treatment in the population of nonresponders to the cytokine inhibitor. The main reason is that a large proportion of patients that respond to the cytokine inhibitor (25.9%) do not respond to the combination of cytokine inhibitor plus S1PR modulator (see green bar), whereas S1PR modulators are quite effective as second-line treatment. This effect was also observed to a lower extent for the combination of cytokine inhibitor plus T cell proliferation inhibitor.

We observed that response rates to combination therapies were larger when cytokine inhibitors were combined

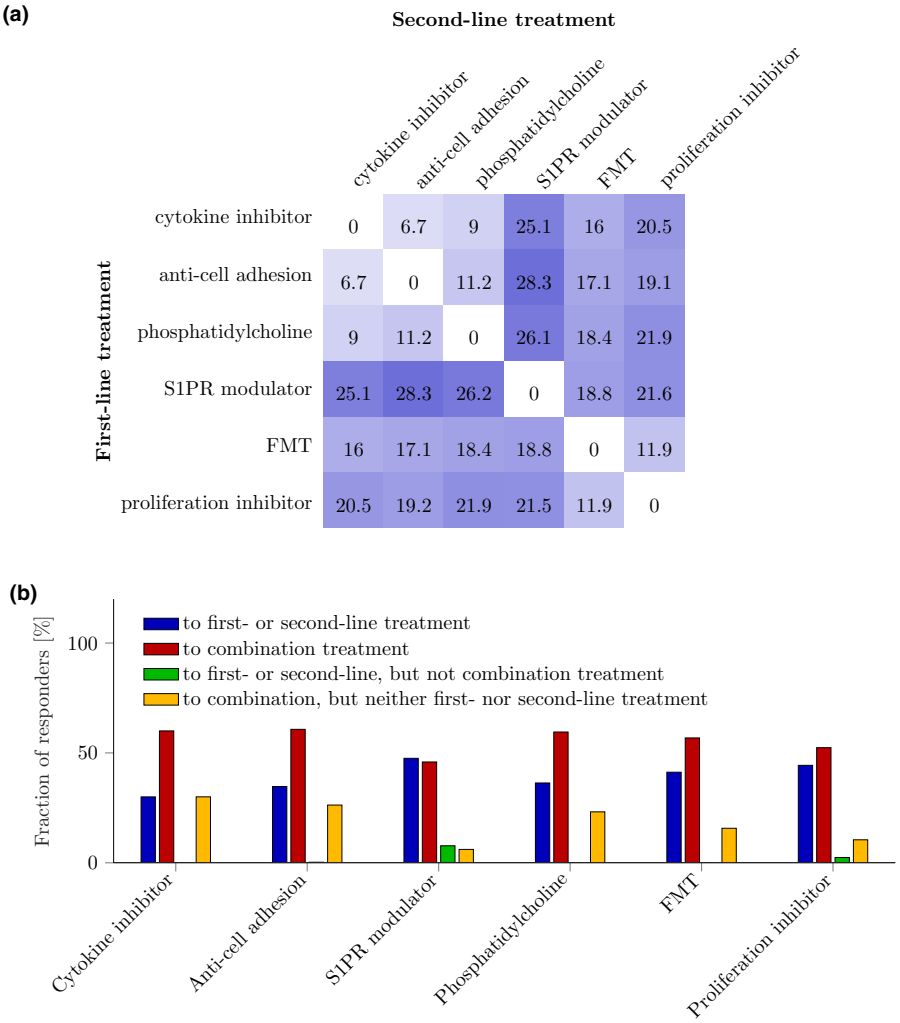

FIGURE 7 Evaluation of alternative and combination treatments. (a) Fraction of patients (%) responding to second-line treatment in the population of first-line non-responders. (b) Fraction of patients (%) responding to (blue) first-line treatment with a cytokine inhibitor or second-line treatment indicated on the x-axis (or both) and (red) simultaneous combination treatment of a cytokine inhibitor and the treatment indicated on the x-axis. Green and yellow bars show the fractions of patients responding to the first- or second-line treatment, but not the combination treatment or those that respond to the combination, but not the first- or second-line treatment, respectively, in the percentage of the virtual IBD population. a, b All treatment effects were assumed to be similarly effective in the virtual IBD population (i.e., the treatment effect parameters η were chosen so that the response rate was 30% for each of the single treatments). FMT, fecal microbial transplant; IBD, inflammatory bowel disease; S1PR, sphingosine-1-phosphate receptor type 1.

with treatment effects that resulted in lower response rates when used as second-line treatments for nonresponders to cytokine inhibitors. In other words, if two treatments are successful in the same subpopulation of patients, our analysis suggests to use them in a combination therapy, whereas if two treatments are successful in different subpopulations of patients, our analysis suggests to rather start with one of them and switch to the other as second-line treatment. This has important implications for therapy protocols: the combination of two treatments with high single response rates in the overall IBD population does not guarantee the combination therapy to have a response rate close to the sum of the single response rates. The same holds for the use of second-line treatments. What matters is the conditional response of a treatment within the population of first-line nonresponders.

DISCUSSION

We developed a novel systems biology model in the context of IBD that can be viewed a comprehensive summary of available knowledge on the mucosal immune response. We used the model to study a virtual population representing healthy individuals and patients with IBD. Importantly, we defined IBD via its phenotype (i.e., the response of the patient's immune system to a potential trigger), rather than in terms of hypothesized changes of a

number of parameter values. This approach allowed us to study potential IBD predispositions in the virtual patient population.

A few systems biology models for IBD have been previously published (e.g., refs. 1–4, 13). The unique feature of our model is that its structure and parameters were chosen to describe the healthy mucosal immune response, without including any data from patients with IBD. Only to identify patients with IBD in a virtual population generated by including variability in our model, we used information on IBD. Through this, we (i) ensured to have included the most important processes of the mucosal immune response, as the healthy immune response can be adequately simulated; and (ii) obtained an "unbiased" IBD population covering the space of possible parameter combinations that (based on the underlying model) could lead to IBD. A related approach was used by Rogers et al. (2020),[3,4] who also developed a systems biology model to describe with the same structural model healthy subjects and patients with IBD using virtual populations. In contrast to our approach, Rogers et al. (i) focused more on specific cytokines, (ii) implemented drug pharmacokinetic/pharmacodynamic (PK/PD) based on specific PK profiles and binding reactions to the targeted cytokines, and (iii) defined a priori, which parameters can be different between healthy subjects and patients with IBD. Interestingly, and in contrast to our findings, Rogers et al.[4] did not observe different steady-states of the model (i.e., after stop of treatment, all subjects returned to the pretreatment steady-state, according to figures 3–5, and 7 in ref. 4).

As every (mathematical) model describing a complex biological process, our systems biology model is based on simplifications and limitations, which naturally also affect its predictive capabilities. Although the complexity of the presented model is already considerably high (133 different reactions and 47 different cell types or subtypes), it is of course far from representing the full complexity of the human mucosal immune system. Including additional processes, cell types or reactions in the model would aim to increase its predictive capacity, but at the same time would require additional data needed for parameterization (which are often not available in literature). We want to point out especially the simplifications regarding the type of disease and spatial location of the inflammation: We did not differentiate between CD and UC, as a thorough description of those differences would have required even more complexity of the model (regarding e.g., cytokine profiles and spatial compartments describing the intestinal wall). The model focuses on the colon (i.e., if available, structure, parameters, and cell concentrations were specifically based on literature data on sigmoidal colon), but is intended to be viewed as representative of the full gastrointestinal tract that can be affected by IBD. In addition, the parameterization of our model is based on a variety of literature sources, and, in some cases, where literature data were insufficient, parameters were set to values of reasonable magnitude ensuring the desired expected behavior of the model. This variability in data sources impacts the quantitative precision that can be expected from the output. Therefore, results from our model simulations are intended rather qualitative (e.g., finding parameters with high potential for IBD predispositions), identifying cell concentrations that could be used as predictors for responsiveness, or testing hypotheses on novel drug targets. To describe treatment effects, we used simplified PK (i.e., assumed a constant treatment effect over the time of treatment). For better representation of the PK of a specific drug, the presented systems biology model should be linked to a PK/PD model describing drug concentrations and resulting target inhibition over time. Our systems biology model does not account for disease progression. This implies that in our simulations of first- and second-line treatments, the first-line treatment does not influence the time course of the response to the second-line treatment, as the patients fall back to the pretreatment steady-state after a non-successful first-line treatment.

Generation and use of virtual populations played a key role in our analysis. In our context, a model is defined by the structural model (the system of differential equations) and the vector of parameter values (including initial conditions). Differences between individuals can be linked to differences in their vectors of parameter values. A common approach to generate an ensemble of vectors of parameter values is to generate random variability around some reference vector of parameter values. Generating virtual populations this way, of course, faces challenges: variability might be too low in some parameters and too high in others; in addition, correlations between parameters might be missing, when variability is included independently on all parameters. In this paper, we used a novel way of defining a virtual population: in a first step, we generated virtual individuals using a relatively large parameter variability relative to some reference individual and without including correlations between parameters. In a second step, we filtered out virtual individuals showing unphysiological cell concentrations or responses corresponding to a different disease. Then, in a third step, we used disease-specific insight to identify those individuals that show typical behavior of IBD, whereas the remaining virtual individuals are considered healthy. Our approach is different from other approaches (including Rogers et al.[3]), where often variability is only included on a subset of parameters. This requires prior knowledge, but more importantly, potentially introduces bias in the analysis. Including variability on all parameters does not only generate IBD individuals, but mostly only additional healthy individuals. The virtual

population should be seen and interpreted as a population of potential individuals exhibiting predefined characteristics in the context of the model. This way, the virtual population allows to find possible parameter differences eliciting the disease and to perform clinical study simulations. It can be expected that some of those parameter differences relate to real-life parameter differences, and some might not be feasible in real life. As a result, simulation studies allow to support existing hypotheses or to generate new. Of course, newly generated hypotheses would have to be confirmed by new experimental studies.

Our study resulted in a number of important findings: (i) the implemented processes sufficed to qualitatively describe the mucosal immune response to different inflammatory stimuli and the occurrence of IBD as result of interindividual variability. (ii) The population of virtual IBD individuals is very heterogeneous (regarding both the time course of cell concentrations and the predispositions). Throughout the enormous diversity of predispositions, IBD can result from both a small number of large parameter changes or a higher number of small parameter changes. (iii) Hill factors (for recruitment or cell activation) seem to play a dominant role in IBD predispositions. (iv) The model allowed to identify predictors for responsiveness to different treatment effects (e.g., pro-inflammatory macrophages and epithelial destruction for cytokine inhibitors), and to provide possible explanations for the high interindividual differences in treatment responsiveness via identification of parameter differences between responders and nonresponders. This does, however, not imply that they all relate to differences in real patients with IBD, but they can give a good overview of possible reasons, and be a good starting point for further analyses in clinical studies via biomarkers. (v) The model provides interesting insights into sequential versus combination treatments: good choices of treatments in a sequential therapy (i.e., choice of a follow-up treatment among nonresponders to a first treatment) are less promising when used as combination treatments. We believe that the presented model and analyses are an important step toward better understanding IBD and the different treatment options.

AUTHOR CONTRIBUTIONS

S.S., C.K., and W.H. wrote the manuscript. S.S. and W.H. designed the research. S.S. performed the research. S.S., C.K., and W.H. analyzed the data.

ACKNOWLEDGMENTS

S.S. kindly acknowledges financial support from the Graduate Research Training Program PharMetrX: Pharmacometrics & Computational Disease Modeling, Berlin/Potsdam, Germany. We kindly acknowledge very fruitful and stimulating discussions with Professor Oliver Pabst and Vuk Cerovic, PhD, from Uniklinik RWTH Aachen/Germany. Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) — Project number 491466077. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

This work was funded by the Graduate Research Training Program PharMetrX: Pharmacometrics & Computational Disease Modeling, Berlin/Potsdam, Germany.

CONFLICT OF INTEREST STATEMENT

C.K. and W.H. report grants from an industry consortium (AbbVie Deutschland GmbH & Co. KG, AstraZeneca, Boehringer Ingelheim Pharma GmbH & Co. KG, Grünenthal GmbH, F. Hoffmann-La Roche Ltd, Merck KGaA, Novo Nordisk A/S and SANOFI) for the PharMetrX PhD program. C.K. reports an additional grant from the Innovative Medicines Initiative-Joint Undertaking (DDMoRe), grants from the Federal Ministry of Education and Research within the Joint Programming Initiative on Antimicrobial Resistance Initiative (JPIAMR) and from the European Commission within in the Horizon 2020 framework programme (FAIR). All other authors declared no competing interests for this work.

ORCID

Wilhelm Huisinga https://orcid.org/0000-0002-5249-3914

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Stübler S, Kloft C, Huisinga W. Cell-level systems biology model to study inflammatory bowel diseases and their treatment options. *CPT Pharmacometrics Syst Pharmacol*. 2023;12:690-705. doi:10.1002/psp4.12932