MASTER THESIS

Mathematical Models for the Progression of Leishmaniasis

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by

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"Mathematical model building is now taking the 'center stage' in biology, and its use and importance is likely to grow."

Covert et al., 2001.

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ABSTRACT

The WHO considers leishmaniasis as one of the six most important tropical diseases worldwide. It is caused by parasites of genus *Leishmania* which are passed on to humans or animals by sandflies of genus Phlebotomus. Although research is ongoing, there is still a lack of understanding concerning both the parasite's metabolism as well as progression of the disease. However, detailed biological knowledge would be needed in order to find effective therapy methods.

Based on experimental data of *Leishmania amazonensis* in BALB/c mice, in which clinical symptoms, immunological response (innate and specific response) and parasite load were studied, a mathematical model for the progression of the disease is developed. The biologically most significant variables are chosen in order to elaborate a deterministic differential equation model based on the GMA power-law formalism. Parameters that minimize the error between model and experimental data are determined.

The model enables to detect qualitative relations between the selected variables, specified by kinetic orders and rate constants. We consider two models with the same model structure, the first one without assuming a-priori-knowledge and using all 22 parameters, and a second model which is a simplification of the first, containing 18 parameters.

Sensitivity analysis yields parasite growth rate and influx of parasites as most sensitive parameters with respect to parasite load.
An optimization with the aim of minimal parasite load yields augmenting parasite growth rate, augmenting the influence of lymphocytes on their own growth or increasing parasite degradation as best therapeutic targets.
Optimization changing two parameters at a time confirms the feasibility of augmenting parasite growth rate in order to minimize parasite load. Also, simultaneous increase in the influence of IgG2a on parasite death and decrease of lymphocyte degradation is yielded feasible.
Furthermore, drugs currently used against the disease and new therapeutic targets are discussed.
Finally, two other mathematical models of the immune response to Leishmania are presented and the results are compared to the results of our analysis.

ZUSAMMENFASSUNG

Leishmaniose ist eine der sechs bedeutendsten Tropenkrankheiten (Quelle: WHO). Sie wird von Parasiten der Gattung *Leishmania* ausgelöst, die durch Sandfliegen der Gattung Phlebotomus auf Menschen oder Tiere übertragen werden. Trotz des hohen Forschungsinteresses sind sowohl der Stoffwechsel des Parasiten als auch der Krankheitsverlauf noch nicht vollständig erforscht. Biologisches Detailwissen wäre allerdings nötig, um effektive Therapiemöglichkeiten zu finden.

Basierend auf experimentellen Daten eines Versuchs mit *Leishmania amazonensis* in BALB/c Mäusen, bei dem klinische Symptome, die Immunantwort (zelluläre und spezifische) und Parasitenbelastung untersucht wurden, wird ein mathematisches Modell für den Krankheitsverlauf entwickelt. Die biologisch am meisten relevanten Variablen werden ausgewählt, um mithilfe des GMA Power-law Formalismus ein deterministisches Differentialgleichungssystem aufzustellen. Daraufhin werden die Modellparameter so bestimmt, dass sich die Modelldaten bestmöglich an die experimentellen Daten anpassen.

Durch das Modell können qualitative Relationen zwischen den ausgewählten Variablen bestimmt werden, die durch kinetische Ordnungen und Übertragungsraten charakterisiert sind. Wir betrachten zwei gleichstrukturierte Modelle, wobei das erste kein a-priori-Wissen voraussetzt und alle 22 Parameter verwendet und das zweite eine Vereinfachung des ersten mit 18 Parametern ist.

Die Sensitivitätsanalyse liefert die Parasiten-Wachstumsrate und die Zunahme an Parasiten als Parameter höchster Sensibilität.
Optimierung mit dem Ziel minimaler Parasitenbelastung liefert eine Erhöhung der Parasiten-Wachstumsrate, eine Erhöhung des Einflusses der Lymphozyten auf ihr eigenes Wachstum und eine Erhöhung des Parasitenabbaus als beste Therapieansätze.
Die Optimierung unter gleichzeitiger Änderung zweier Parameter bestätigt, dass eine erhöhte Parasiten-Wachstumsrate die Parasitenbelastung minimiert. Außerdem zeigt sich, dass eine gleichzeitige Erhöhung des Einflusses von IgG2a auf die Zerstörung von Parasiten und eine Dämpfung des Lymphozyten-Abbaus möglicherweise einen sinnvollen Therapieansatz darstellt.
Weiters werden Pharmaka, die derzeit zur Leishmaniose-Bekämpfung eingesetzt werden, vorgestellt und neue Therapiemethoden diskutiert.
Schließlich werden zwei andere Modelle für die Leishmania-Immunantwort vorgestellt und die Ergebnisse mit denen dieser Arbeit verglichen.

RESUMEN

Leishmaniasis es una enfermedad producida por parásitos del género *Leishmania* que se transmiten a humanos o animales a través de flebótomos. En este trabajo se ha desarrollado un modelo matemático del curso de la enfermedad, en el que se integran datos experimentales obtenidos en ratones BALB/c infectados con *Leishmania amazonenzis*. Se empleó para ello el formalismo power-law en su versión GMA. El modelo permite detectar cualitativa y cuantitativamente las relaciones existentes entre las variables e identificar aquellas que son críticas en el desarrollo de la misma y se sientan las bases para el diseño racional de estrategias terapéuticas.

Contents

1 Introduction

The WHO ranks leishmaniasis (besides malaria, esquistosomiasis, filariasis, trypanosomiasis and tuberculosis) among the six tropical diseases of major importance for research in the field of prevention, diagnosis and treatment [20]. About 12 million people worldwide suffer from the disease, whereby this official number does not include not-declared or asymptomatic cases. Out of 88 affected countries, 72 are developing countries, and declaration is only obligatory in 32 countries. The annual incidence is 2 million people, among those 1.5 million cases of cutaneous leishmaniasis, 0.5 million cases of visceral leishmaniasis and an evanescent part of mucocutaneous leishmaniasis. 350 million people worldwide are at risk [59]. The disease claims 50 000 deaths annually [60].
Leishmaniasis is mainly spreading in poor regions; 80% of the people suffering from visceral leishmaniasis, the most severe form of the disease, have less than USD 2 a day to live on [17]. Besides clinical symptoms which may lead to death, exclusion from society because of leprosy-like skin lesions and consequent inability to work causing economical ruin are additional problems coming along with the disease. Moreover, many Leishmania regions show an insufficient healthcare infrastructure.

There are three main forms of leishmaniasis:

- Cutaneous leishmaniasis,
- Mucocutaneous leishmaniasis and
- Visceral leishmaniasis.

Cutaneous leishmaniasis has an incubation time of two to six weeks [10]. It begins with a growing papule that typically transforms into a non-hurting ulcer. Spontaneous healing can occur within weeks or months, but lesions can also persist for years [3]. More lesions in form of ulcers, papules or nodules can occur, above all on the extremities and in the face. If skin lesions are widespread and resemble leprosy, this form of the disease is also called *diffuse cutaneous leishmaniasis*. 90% of all cases of cutaneous leishmaniasis occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria [59].
Mucocutaneous leishmaniasis is characterized by partial or total destruction of the mucous membranes of nose, mouth, throat cavities and the surrounding tissues. In contrast to cutaneous leishmaniasis it is characterized by multiple non-ulcerative nodules [45]. Disease starts usually with an infection of the nasal septum. If untreated, the disease progresses and affects the pharynx, palate, larynx and lips, in this order [30]. Mucocutaneous leishmaniasis can lead to death caused by bacterial infection, starvation, aspiration or occlusion of the respiratory system [3]. 90% of all infections with mucocutaneous leishmaniasis occur in Bolivia, Brazil and Peru [59].
Visceral leishmaniasis, also known as "kala azar", "black fever" or "Dumdum fever", is the most severe form of the disease. It especially affects hosts with a weak immune system, like children or people suffering from malnutrition or HIV. After an incubation time of three weeks to eighteen months [3, 20] typical symptoms are fever, diarrhea, body weight loss, lymphadenopathy, hepatomegaly and splenomegaly. Mucosal ulcers, fatigue and anemia are further symptoms. 90% of all visceral leishmaniasis cases occur

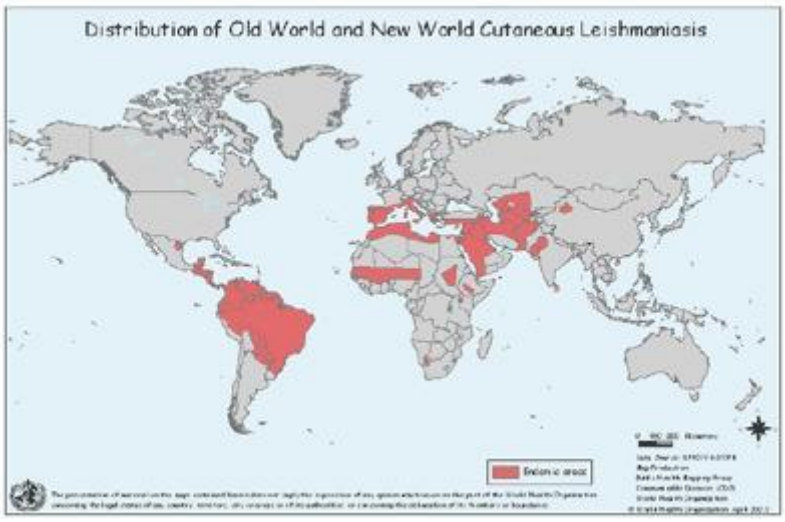

Figure 1: Countries affected by Cutaneous leishmaniasis [59]

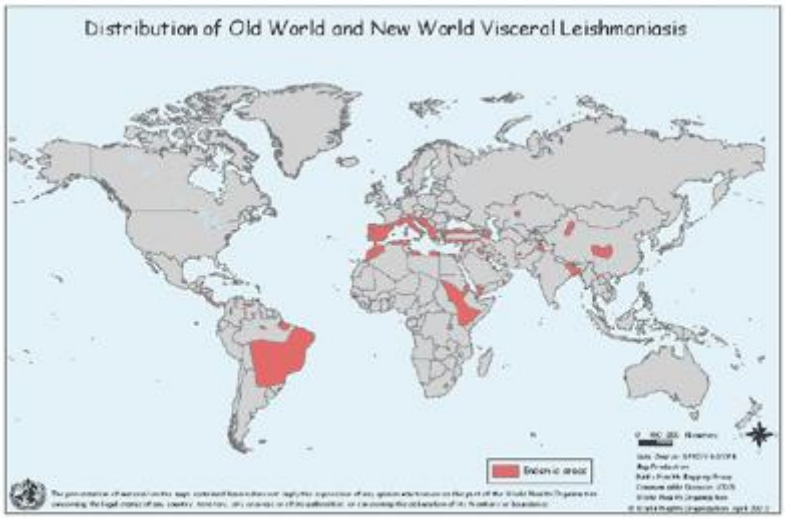

Figure 2: Countries affected by Visceral leishmaniasis [59]

in Bangladesh, Brazil, India, Nepal and Sudan [59].

Fig. 1 and 2 show the spread of cutaneous and visceral leishmaniasis, respectively. A complete table of countries affected by the different kinds of Leishmania can be found in [3].

The type of leishmaniasis that follows infection also depends on the immunological condition of the host. In an immunologically weak person (e.g. a person suffering from AIDS) or in children, a particular stem of leishmania can cause a different form of the disease than in a healthy adult individuum. In Spain for example, *Leishmania infantum* causes visceral leishmaniasis in children and cutaneous leishmaniasis in adults.

1.1 Parasites and Vectors

Transmission of the disease in most cases happens through the vector, sandflies of genus *Phlebotomus* in the "old world" and genus *Lutzomyia* in America, respectively. These sandflies have a size of about 2-3mm. Only 20 out of 500 sandfly species can transfer the disease, and among them only the female subpopulation. The parasite lives within the sandfly for 4 to 25 days until it goes to a mammal host stung by the sandfly.

Leishmania have two forms during their life cycle, a promastigote (flagellated) form in sandfly and an amastigote (non-flagellated) form in mammals.
The life cycle proceeds as follows (Fig. 3):

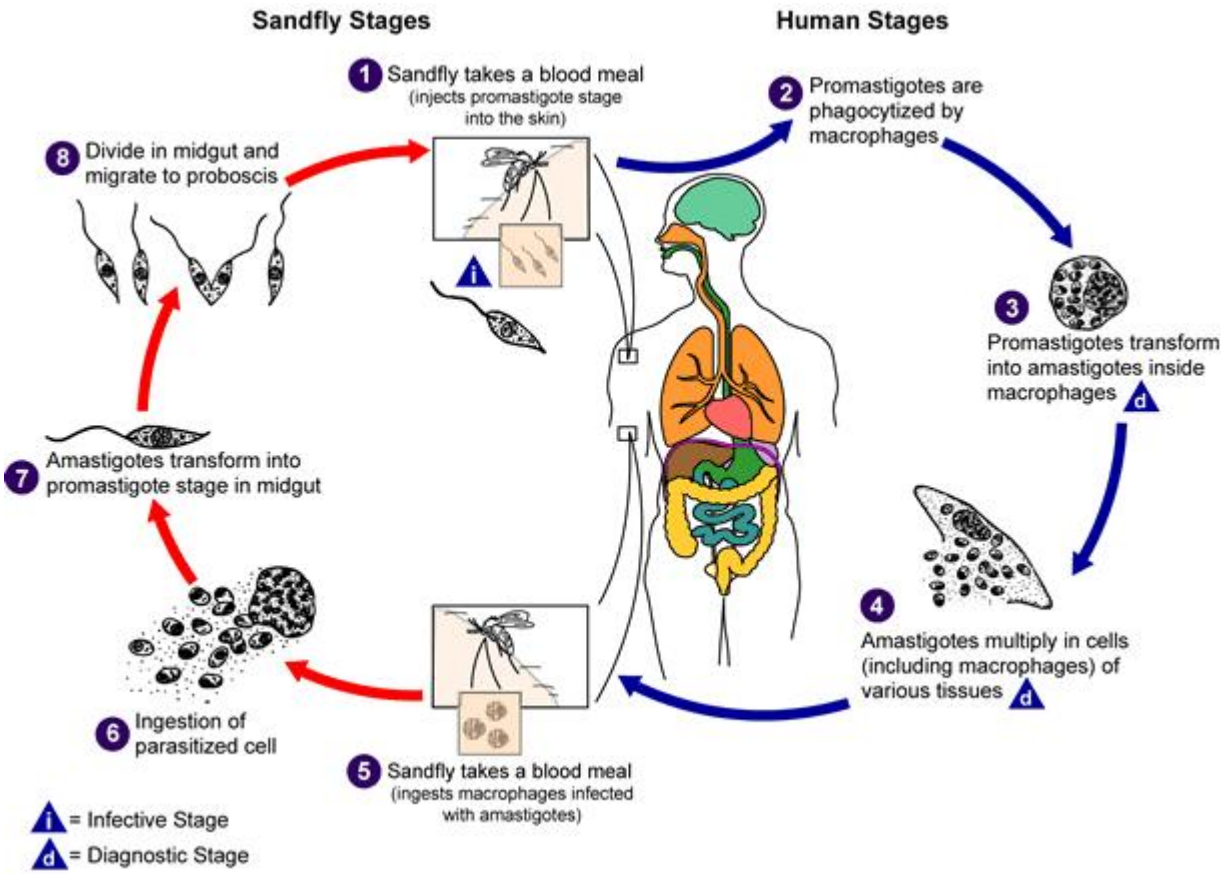

Figure 3: Life cycle of Leishmania [58]

1. An infected sandfly takes a blood meal and thereby injects promastigotes into the human skin.
2. Subsequently, promastigotes are phagocytized by human macrophages.
3. Inside the macrophages, promastigotes transform into amastigotes.
4. Amastigotes proliferate in cells, inhibiting immune defense mechanisms.
5. Another sandfly is taking a blood meal.

6. Infected amastigotes are taken up by the sandfly.

7. In the sandfly's body, amastigotes go to the midgut where they transform back into promastigotes.

8. These divide in the midgut and move to the proboscis in order to be inserted into the human skin (1) and thus we have come full circle.

1.2 Transmission, Diagnosis and Therapy

Transmission of the disease happens mainly through the vector. In some cases people are infected by blood transfusions or contaminated needles. Less often the disease is spread by infection of mother to fetus or from human to human.

Even more dangerous is a co-infection with Leishmania and HIV. If a HIV-positive person is infected with Leishmania, AIDS can develop faster because leishmaniasis suppresses the human immune system and stimulates replication of the virus. 34 countries worldwide are affected by HIV-VL co-infection. [59]

Because sandfly eggs need an environment of organic matter, heat and humidity, risk factors include dry wood stored inside the house, holes in windows or insufficient fencing material in walls, stone houses (resting holes) or houses close to waterways (humidity). Prevention from Leishmania is possible by use of long-lasting insecticide-treated nets. Normal mosquito nets are not sufficient since this type of sandflies is so small that they are able to penetrate the nets [22].

The standard method for diagnosis is a biopsy of the affected tissue, lymph nodes, splenic or bone marrow aspirate (positive rates around 80%) [55]. Other methods include the direct *agglutination* test (sensitivity 95%), the rK39 urinary *antigen* test (sensitivity 87%) [42], the indirect fluorescent *antibody* test or *PCR* techniques [50].

Cutaneous leishmaniasis is usually self-healing and leads to lifelong immunity against re-infection.
Currently, the not self-healing forms of the disease are treated by pharmaceuticals that are usually injected over a period of 15-30 days under medical supervision. Unfortunately this kind of treatment is expensive, time-consuming and inaccessible in poor regions. In 2002 the first oral drug against visceral leishmaniasis was developed. It is based on maintaining the levels of miltefosine, an antiprotozoic agent in the body. A problem of all pharmaceuticals are side-effects e.g. pancreatitis, cardiovascular toxicity and diabetes. Second-line drugs (i.e. agents used when standard "first line" therapy fails [56]) trigger reactions that can even be lethal. Moreover, vectors can develop resistance to first-line drugs (e.g. India: 60% resistance). However, in Syria providing insecticide-impregnated bed nets achieved a 50% reduction in incidence of cutaneous leishmaniasis [59].
Dogs and monkeys can be vaccinated against leishmaniasis. For humans, until now two vaccines (one inducing live, one killed parasites) were licensed [39].
There is an autonomous-living tribe in Peru, the so-called Machiñengas, who are resistant to leishmaniasis. However, investigation on them is difficult since they live isolated from civilization. Moreover the main leishmaniasis research centers are outside Peru, and it is

difficult to transport blood or cellular samples by plane since they need to be maintained frozen.
There are also leishmaniasis-resistant mice stems and investigation has been performed on their immune response to Leishmania. However, until now it has not been possible to establish conditions yielding immunity in humans.
Hopefully, animal models of leishmaniasis will lead to a better understanding of the immune response to Leishmania in the future in order to perform well-directed experiments on human blood samples.

1.3 Control Strategies

There are several programs trying to control the leishmaniasis problem.
One of those is the Brazilian Leishmaniasis Control Program (BLCP) which tries to fight the disease by

1. Diagnosis and early treatment,
2. Immunological screening and eliminating of seropositive dogs (alternate hosts) and
3. Insecticide spraying against sandflies.

However, the BLCP has not improved the situation since 1950 and better control strategies are required.

The WHO has designated leishmaniasis as category 1 (emerging and uncontrolled) disease. Its prevention focuses on objectives (1) and (3) of the BLCP; furthermore on the following points [59]:

- Providing impregnated bed nets
- Health education and training materials
- Early diagnosis of HIV/leishmaniasis co-infections.

The WHO has built up a worldwide network of 28 institutions which use standardized guidelines for diagnosis, as well as computerized case reports.
Doctors without Borders started initiatives for faster and cheaper drug treatment. However, according to a survey of drug resistance in India, only 26% of people are treated accordant to WHO guidelines. Moreover, there are patients that stop treatment on their own initiative [21].
Another approach is to improve the standard of living in areas of risk, since the disease is mainly due to deficient water supply, malnutrition and poor living conditions [17].

2 Hypotheses and Objectives

This section gives a motivation of the work, stating hypotheses and objectives.

2.1 Hypotheses

It is supposed that the essential part of leishmaniasis disease progression in BALB/c mice can be represented by a model consisting of four variables, representing parasite load as well as cellular (lymphocytes) and humoral (IgG1, IgG2a) immune response. Quantitative interactions between variables (i.e. positive or negative influences) are derived from biological knowledge. Based on these interrelationships between variables, a power-law ansatz yields a system of differential equations. Since this is a deterministic modeling approach, the model is expected to represent real behavior *on average*. It has to be taken into account that this is a specific model for *Leishmania amazonensis* in BALB/c mice. Since the far-end aim is to find new therapy methods for leishmaniasis in *humans*, the hypothesis is that disease progression in humans is similar to that in BALB/c mice.
C. Pou states in [1]: *"El objetivo principal al establecer un modelo animal de leishmaniosis, es poder reproducir hasta donde sea posible, las manifestaciones clínicas que se presentan en el humano y de esta manera estudiar los componentes inmunes implicados en el desarrollo de esta enfermedad, y así a partir de este conocimiento se pueda evaluar nuevas terapias farmacológicas, vacunas, etc."* which means:
"The principal objective of establishing an animal model of leishmaniasis is to enable reproduction of the clinical manifestations present in human as far as possible, in order to study immune components involved in disease progression and to evaluate new pharmaceutical therapies, vaccines, etc. based on this knowledge."

Hamsters and dogs are considered the most appropriate models for human visceral leishmaniasis. BALB/c mice are considered the best model for visceral leishmaniasis caused by infection with *L. major*. Accordingly, BALB/c mice are feasible for preliminary experiments on any Leishmania stem in order to formulate hypotheses which afterwards can be verified in animals more similar to humans.

2.2 Objectives

The aims of this work can be summarized as follows:

1. To represent the immune response following injection of a certain amount of parasites by means of a system of differential equations. The model is derived from the power-law; for parameter estimation a genetic algorithm is used which enables to choose the set of parameters minimizing the error between experimental data and model approximation.

2. To quantify biological interrelations between variables and to perform predictive simulations.

3. To make a sensitivity analysis reflecting the importance of certain parameters and reactions.

4. To solve an optimization problem with the objective of minimizing parasite load. This yields the conditions that are the most favorable for healing of the disease.

5. According to (3) and (4) crucial targets for the interaction of pharmaceuticals can be identified and it is tried to find drug agents that yield these conditions which suggest possible therapy methods for leishmaniasis.

3 Antecedents and State-of-the-Art

In this Section statistics of research on leishmaniasis around the world are given and similar studies are resumed.

3.1 Leishmaniasis Research Statistics

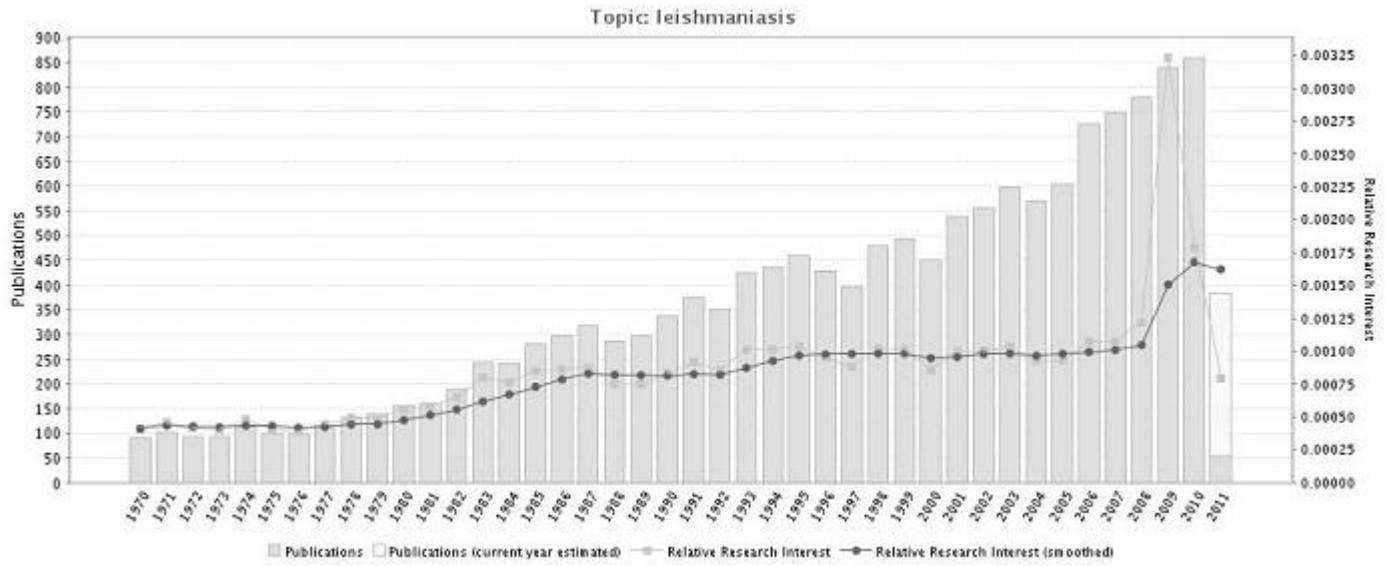

Figure 4: Publications (left axis) and relative research interest (right axis) concerning leishmaniasis over time (1970-02.02.2011) [57].

Leishmaniasis research is done in 132 countries around the world, including all continents. The country with the highest number of publications between 1970 and 2010 is Brazil (1788), followed by the USA (1415), India (991), the United Kingdom (677), Spain (618) and France (576). The rest of the countries show less than 500 publications. In Austria, 28 articles about Leishmania have been published yet. All these data refer to the state-of-the-art at February 2^{nd}, 2011.

Fig. 4 shows a diagram of the number of publications over time; in Fig. 5 the regions in which research on leishmaniasis is performed are marked on a world map.
Interest in visceral leishmaniasis is higher than interest in cutaneous leishmaniasis which in turn is higher than that in mucocutaneous leishmaniasis. Mucocutaneous leishmaniasis is the form of the disease affecting the least number of people. In contrast, it is surprising that there is more investigation about visceral than about cutaneous leishmaniasis, since the annual incidence of the cutaneous form is three times as high as that of the visceral form of the disease (see Section 1). However, visceral leishmaniasis is the most severe form of the disease.

3.2 Antecedents

The majority of existing mathematical models are either of epidemiological kind or investigate the parasite Leishmania e.g. its genome or metabolism but not the disease

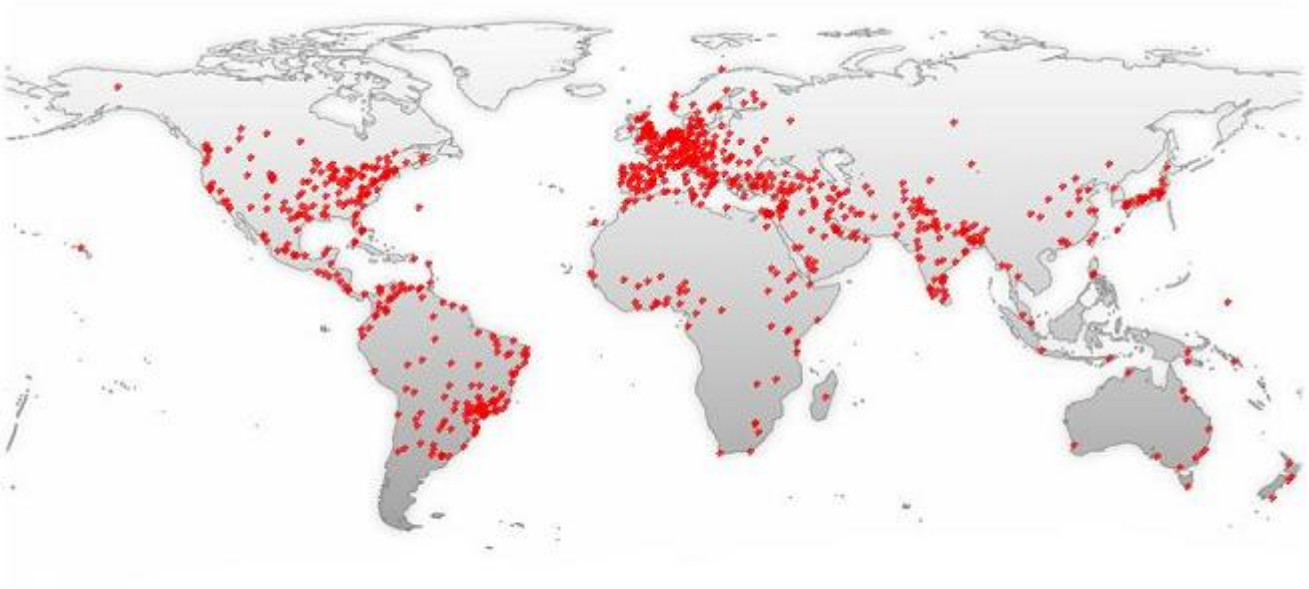

Figure 5: Locations where research on leishmaniasis is done [57].

Leishmaniasis. Concerning experimental models, there are works similar to that of Pou [1].

The only mathematical models of leishmaniasis found which is not an epdemiological study are the agent-based model of *Leishmania major* by Dancik et al. (2006, 2010) [15, 16] and a theoretical model of the immune-response to *Leishmania* by Nelson and Velasco-Hernández [36]. In the papers by Dancik et al. an agent-based stochastic model of immune response to *Leishmania major* infection in the ear of mice is described. The biggest difference to our model is that macrophages are taken into account and the model is a compartment model. Activities (like parasite growth and maturation of T cells) and movement of components (macrophages, chemokines, antigen-presenting cells, T cells) are described in terms of probabilities. Sensitivity analysis using FANOVA is performed yielding the six most important weights of the pathogen load which explain 99% of its variance. Calibration of the model to field data is performed. The main conclusions concern the influence of parameters on pathogen load and macrophage levels. The model is discussed in further detail in Section 6.2.1.
The model of Nelson and Velasco-Hernández [36] considers the variables: resident macrophages, activated macrophages and parasites; later the model is extended by the additional variables IL-12 and T-helper cells. A differential equation system is set up and a steady-state analysis yields a parasite-free unstable steady-state and a stable steady-state where macrophages and parasites coexist. The model is discussed in more detail in Section 6.2.2.

Concerning experimental models, a brief summary of similar models is given here which does not claim completeness. Further references can be found in [1].
Probably the most similar experimental work is that of Courret et al. [13] who describe lesion development, cellular response, expression of cytokines as well as parasite load

in spleen and liver in BALB/c mice infected with *Leishmania amazonensis*. Another model that investigates BALB/c mice is that of Arrais-Silva et al. [4] which concerns the hypoxia-inducible factor-1α resulting from an infection with *Leishmania amazonensis*. This factor is exclusively produced by macrophages infected with Leishmania and therefore can serve as a marker of infection.
Lira et al. studied symptoms, parasite load and immune response in C57BL/6 mice infected with *Leishmania major* [28].
Symptoms of leishmaniasis were further investigated in [19] which is a study on canine visceral leishmaniasis. Rodríguez-Cortés et al. [44] present a long-term study on six dogs infected with visceral leishmaniasis which identifies key parameters of leishmaniasis and correlations among those.
[41] and [18] are two models on *Leishmania infantum* in golden hamsters. Both papers show the feasibility of hamsters for in-vivo experiments on leishmaniasis whereby Requena et al. [41] investigate clinical symptoms, parasite loads as well as antibody levels in susceptible, oligosymptomatic and resistant hamsters. Dea-Ayuela et al. [18] compare those parameters as well as lymphocyte population in two groups infected with a different amount of parasites and a non-infected control group.
Porrozzi et al. conducted a study on rhesus monkeys infected with *Leishmania infantum* in order to investigate symptoms that also occur in human infection [40].

Concerning epidemiological models, Chaves and Hernandez present one model of american cutaneous leishmaniasis [12]. They developed a general model for the dynamics of a vector-borne disease, considering a population of incidental hosts (humans) and a population of reservoir hosts (mammals). Population dynamics are described by a system of differential equations. The threshold for persistence of the disease is calculated. Stability analysis yields one equilibrium solution apart from the trivial. If the disease is endemic, this solution is globally stable otherwise it is unstable and the system converges to disease extinction.
N. Bacaër and S. Guernaoui present a model of cutaneous leishmaniasis with respect to seasonality [5]. The basic reproduction number i.e. the threshold to stop the disease is calculated using data of the province of Chichaoua, Marocco.
In [9] Carneiro et al. classify regions in the province of Bahia, Brazil, according to their risk of American visceral leishmaniasis. The aim is to use data from spatial-temporal scanning to develop control strategies in endemic areas of the disease.
In [38] Palatnik-de-Sousa et al. show the effectiveness of a dog vaccination with Leishmune in Brazil which reduces both canine and human incidence of leishmaniasis in endemic regions.

Other mathematical models concerning Leishmania include a model of *Leishmania major*, developed by Chavali et al. [11]. They present an 8-compartment model of Leishmania's stage-specific metabolism. Equations are derived from flux-balance analysis. By means of gene-knock-outs, genes that are lethal for *Leishmania major* but not present in human are identified. Moreover, they identify a "minimal medium" i.e. the minimal set of substrates that is necessary for Leishmania to express all genes. Furthermore, previously uncharacterized genes are functionally annotated.
Leifso et al. developed a model which represents 94% of Leishmania genes in its two different life stages, promastigote and amastigote stage [27]. They discovered that the

majority of mRNAs is constitutively expressed i.e. their activity is constant and active. 98.5% of genes are similar in promastigotes and amastigotes.
Despite this similarity which was also yielded by Rochette et al. [43], this group showed that 25% of the genes involved in metabolism are differentially expressed in promastigotes and amastigotes. Whereas promastigotes yield their energy from glucose metabolism and overexpress microtubules for locomotion, amastigotes use fatty acids as energy source and overexpress membrane transporters.
McNicoll et al. investigated stage-specifity of genes in *Leishmania infantum* promastigotes and amastigotes [31]. 2D-gel analysis with an amastigote-specific, a promastigote-specific and a master gel showed that the majority of proteins is stage-specific. The level of a protein corresponds to the expression of a determined gene. The authors divide proteins in three classes: Proteins involved in stress response, metabolic enzymes and proteins involved in proteolysis.

4 Material and Methods

In this section the experiments which yielded data for analysis, and the mathematical methods used are described.

4.1 Experimental Data

This work is based on experimental data from the doctoral thesis of C. Pou Barreto [1]. BALB/c mice were infected with *Leishmania amazonensis*, a species of Leishmania affecting Brazil, Costa Rica, Panama, Columbia, French Guyana, Ecuador, Peru, Bolivia and Venezuela. This species of parasites can cause all known forms of leishmaniasis (see Section 1 and [3]). *Leishmania amazonensis* was chosen in order to investigate both cutaneous as well as visceral leishmaniasis, as this parasite stem causes at first cutaneous and later visceral leishmaniasis in BALB/c mice. In human *Leishmania amazonensis* usually causes cutaneous and mucocutaneous leishmaniasis.
Animals were supervised over a period of 28 weeks. There were three groups of 20 mice each; in one group 10^3 promastigotes were injected, in another group 10^6 promastigotes. The third group was not infected and used as a control. The parasites in stationary promastigote stage were inoculated in the tarsal bone of the mice's hind paw.
Measurements of the following 15 variables were taken (see Appendix A for abbreviations):

1. Clinical variables:
    - lesion type (classification from small changes up to metastatic lesions)
    - lesion size (at inoculation site)
    - body weight gain
    - organ weight
        - popliteus ganglion
        - inguinal ganglion
        - spleen
        - liver
2. Parasite load:
    - popliteus ganglion
    - inguinal ganglion
    - spleen
    - liver
3. Immune response:
    - humoral
        - IgG (set of immunoglobulins)
        - IgG1

  - IgG2a
- cellular: IE (population of lymphocytes)

Starting four weeks after infection, lesion type, size, body weight and immune response were measured in a sample of eight mice every two weeks. The immune response was additionally measured at week 2. The rest of the variables (organ weight and parasite load) were measured every eight weeks by sacrificing four mice, respectively.
In natural infection, sandflies inject about 10-1000 promastigotes into the human skin, thus the group injected with 1000 promastigotes is the closest to natural circumstances. There were no signs of recovery in any of the mice - lesions were growing with time. In the group infected with 10^3 promastigotes the disease was developing more slowly than in the highly-infected group.
Since the 10^3 parasites group is the most representative for natural infection

the following analysis focuses on the group infected with 1000 parasites.

4.2 Variable Selection

In order to build a schematic model for disease progression, the biologically most important variables are chosen. The essential mechanism in disease progression is the body immune response. To model the latter with respect to parasite load, we choose the following variables:

1. Parasite load (mean of parasite load in popliteus ganglion, inguinal ganglion, spleen and liver)
2. Number of lymphocytes (variable IE)
3. IgG1 and
4. IgG2a.

These variables were measured as follows (see [1] for details):

- Parasite load was measured via microtiter. Animals were dissected, and tissue samples of popliteus ganglion, inguinal ganglion, liver and spleen were extracted. The samples were weighed; subsequently dilutions from 1 to $\frac{1}{3}\cdot 10^{-6}$ were performed. After incubation the best dilution containing at least one living promastigote was determined. Using
$$\frac{N°parasites}{g} = \frac{\frac{1}{D+}}{w},$$
whereby $D+$ denotes the "best" dilution and w the organ weight in grams, the number of parasites per gram could be determined.
- The response of the specific antibodies IgG1 and IgG2a to recombinant proteins (LbHSP83, LbL6R and LbL6R-HSP83, as well as LbAgS) was measured via indirect ELISA. In this technique, antigen is added to wells of a microtiter plate. Subsequently, the unknown amount of primary antibody is added. A second antibody

which is marked by a signaling enzyme is added. After addition of the respective substrate, the enzyme emits a signal (e.g. fluorescence) which is measured in order to determine the amount of primary antibody for which measurement is required.

- Population of lymphocytes was measured via the optical density which in turn was measured by use of a colorimetric method using sulfonamide B. To set a cutoff-value, at first infected or immunized mice cells were compared to normal mice cells. The cutoff-value was determined as the arithmetic mean of treated minus untreated cells divided by three times the standard deviation. Finally the IE was calculated as the difference of the mean optical density of treated and untreated cells divided by the cutoff value i.e.

$$IE = \frac{mean(OD_{treated} - OD_{control})}{\text{cutoff}}.$$

Apart from the fact that the chosen variables are the most relevant ones from a biological point of view, they are also the ones that are most relevant for immunotherapy i.e. killing of parasites by the immune system whereas clinical variables e.g. lesion type, lesion size or body weight gain (see Section 4.1) represent disease symptoms. Parasite load is the variable representing the disease burden that shall be minimized in the end.
IgG (including various immunoglobulins) is the only measured immunological variable that is not included in the model but instead IgG1 and IgG2a which correlate with IgG. IgG1 and IgG2a are chosen because IgG2a is directly inhibiting parasite load and IgG1 is an important marker of infection in contrast to healing. The latter does not decrease parasite load because, although trying to identify and destroy the parasite, it is not capable to detect parasites inside macrophages. A cellular response (proliferation of lymphocytes) is necessary to activate macrophages which leads to destruction of the parasite.

The mean value of all mice in which measurements are taken is considered for calculations. This is crucial for parasite load and the number of lymphocytes since these variables require sacrifice of mice and therefore cannot be measured in the same mouse at different time points. Another strategy would be to consider the median. This would be the more robust method, excluding outliers.

4.3 Power-law Model

With the variables chosen in the foregoing section, a mathematical model consisting of an ordinary differential equation system is derived using the GMA power-law ansatz.

Power-law modeling is based on the formula

$$\frac{dX_i}{dt} = \sum_j \sigma_{ij}\gamma_j \prod_k X_k^{g_{jk}}, \tag{1}$$

[53], whereby X_i are the system variables, σ_{ij} are the coefficients of the stoichiometric matrix, γ_j are rate constants and g_{jk} kinetic orders.
The stoichiometric matrix contains $\sigma_{ij} = 1$ if a particle goes from site j to site i and $\sigma_{ij} = -1$ if a particle goes from site i to site j. If there is no interaction between site i and site j, $\sigma_{ij} = 0$.
Rate constants are directly proportional to the variables' influx and outflow.

Kinetic orders γ_i refer to the influences among parameters: A kinetic order g_{jk} of zero means that there is no influence of variable k on variable j. A negative kinetic order means that variable k has a negative influence on variable j, i.e. if x_k decreases, x_j augments and vice versa. A positive kinetic order refers to positive influence of variable k on variable j, i.e. if x_k increases (decreases), x_j increases (decreases).

4.4 Mathematical Model

Due to biological considerations based on the experiments of C. Pou [1], a mathematical model for the progression of Leishmaniaisis is derived using the power-law approach.

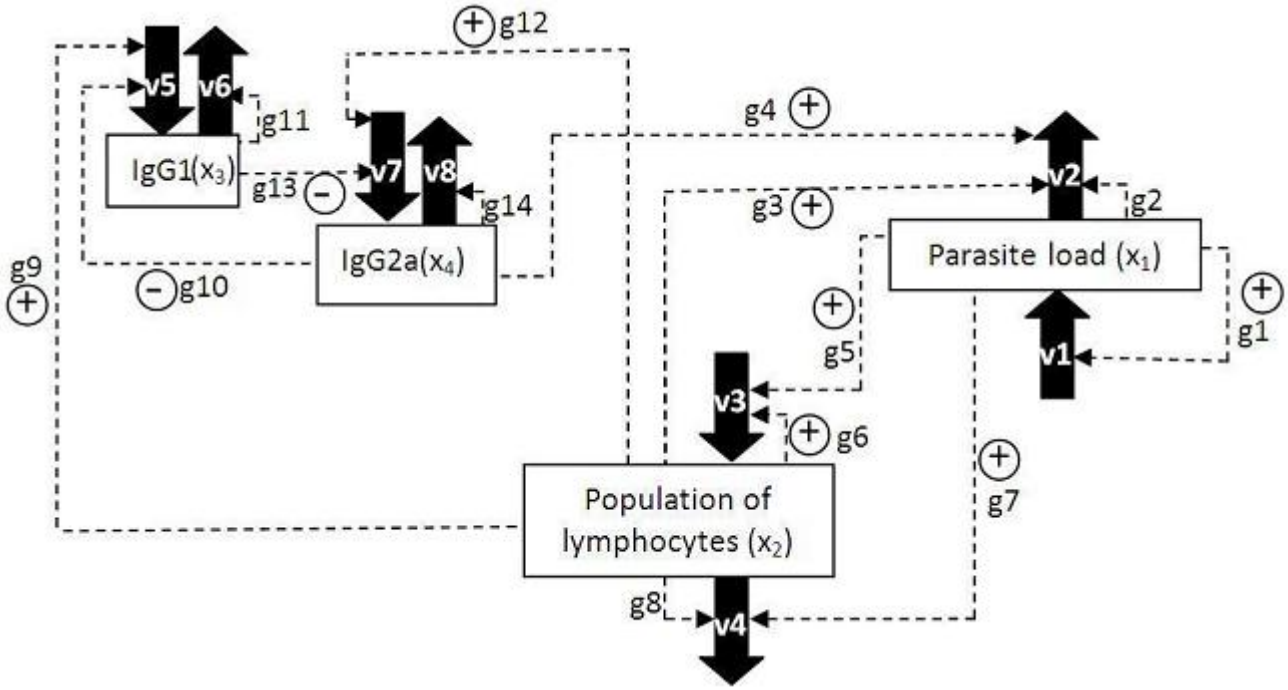

Figure 6: Simplified model of leishmaniasis progression. Thick arrows: production / degradation (flux of matter), dashed arrows: influence/signal. v_i: generation/degradation velocities with γ_i proportional to v_i; g_i: kinetic orders.

Fig. 6 shows the model scheme, consisting of the chosen variables $x_1, ..., x_4$ and the influences among them which are denoted by arrows and parameters. $g_1, ..., g_{14}$ stand for kinetic orders representing influences on the creation or degradation of the four variables. Consider that we use "signaling modeling" i.e. information transfer rather than mass transfer [23]. I.e. a dashed arrow from variable A to variable B does not mean that one molecule A transforms into a molecule B or that a particle is going from A to B, but that A influences B.

Immune reaction starts when parasites are injected at the site of inoculation, which is the hind paw in C. Pou's experiments [1]. Parasites proliferate by division, which means exponential growth i.e. parasites have a positive influence on their proper generation (g_1). Parasite injection causes an immune response which means proliferation of lymphocytes (g_5). On the other hand, increasing parasite load may also lead to degradation of lymphocytes, because parasites are acting against the immune system (g_7). However,

it is not known if they increase degradation of lymphocytes or inhibit creation of new lymphocytes. In our model, g_5 is the parasite load's influence on generation of lymphocytes and g_7 is the parasite's load influence on their degradation. Thus, from a mathematical point of view, increasing degradation of lymphocytes by the parasite load would mean $g_7 > 0$, whereas inhibition of the creation of new lymphocytes would mean that g_5 is possibly negative. In Section 4.8 we will see that $g_5 > g_7$ thus g_5 is nonnegative in our case.
Since lymphocytes also proliferate by cell division, they grow exponentially which means that the number of lymphocytes has a positive influence on its proper generation (g_6). Moreover, lymphocytes kill parasites which reduces parasite load (g_3). The host immune system produces antibodies IgG1 and IgG2a to identify Leishmania antigens. For the model this means a positive influence of the number of lymphocytes on the generation of IgG1 (g_9) and IgG2a (g_{12}).
The two immunoglobulins are antagonists, so each of them has a negative influence on the other's generation (g_{10}, g_{13}). This is confirmed by a negative partial correlation coefficient.
Finally, IgG2a helps lymphocytes to identify parasites and therefore contributes to a decrease in parasite load (g_4).
All the variables are assumed to be proportional to their own degradation - a higher amount of the variable also increases its degradation rate (g_2, g_8, g_{11}, g_{14}).
IgG1 is also an antibody, but it does not inhibit parasite load. It does not act as a parasite marker because it is not able to locate parasites hidden inside macrophages. IgG2a and the cytokines produced by IgG2a are markers of cellular response, which means activation of macrophages consequently causing parasite death. It is assumed that there is a moment when the population of lymphocytes does not increase further.

In our case, every variable has an influx and an outflow, so the stoichiometric matrix is

$$S = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix}$$

In this matrix, rows refer to the four model variables $x_1, ..., x_4$ and columns refer to the influx and outflow of each variable: $\gamma_1, ..., \gamma_8$.
The kinetic orders g_m denote influences among variables. Without loss of generality, g_m are assumed to be non-negative. Negative kinetic orders are annotated by a minus.
According to the model structure (Fig. 6) we obtain the following flux matrix:

	x_1	x_2	x_3	x_4
	parasite load	lymphocytes	IgG1	IgG2a
v_1	g_1	0	0	0
v_2	g_2	g_3	0	g_4
v_3	g_5	g_6	0	0
v_4	g_7	g_8	0	0
v_5	0	g_9	0	$-g_{10}$
v_6	0	0	g_{11}	0
v_7	0	g_{12}	$-g_{13}$	0
v_8	0	0	0	g_{14}

variable	x_i
parasite load	x_1
lymphocytes (IE)	x_2
IgG1	x_3
IgG2a	x_4

Table 1: Variable identifiers

With the annotation given in Tab. 1 the equation system for the fluxes is:

$$v1 = \gamma_1 \cdot x_1^{g_1}$$
$$v2 = \gamma_2 \cdot x_1^{g_2} \cdot x_2^{g_3} \cdot x_4^{g_4}$$
$$v3 = \gamma_3 \cdot x_1^{g_5} \cdot x_2^{g_6}$$
$$v4 = \gamma_4 \cdot x_1^{g_7} \cdot x_2^{g_8}$$
$$v5 = \gamma_5 \cdot x_2^{g_9} \cdot x_4^{-g_{10}}$$
$$v6 = \gamma_6 \cdot x_3^{g_{11}}$$
$$v7 = \gamma_7 \cdot x_2^{g_{12}} \cdot x_3^{-g_{13}}$$
$$v8 = \gamma_8 \cdot x_4^{g_{14}}$$

The maximum ranges for γ_i and g_m are:

$$0 \leq \gamma_i \leq \infty \text{ and } 0 \leq g_k \leq 3 \tag{2}$$

because kinetic constants g_k rarely reach a value greater than two. However, since our model considers influences rather than fluxes of matter, higher g_k may be appropriate, too. For our simulations we choose the ranges:

$$0 \leq \gamma_i \leq 10 \text{ and } 0 \leq g_k \leq 3. \tag{3}$$

We could choose a higher value e.g. 100, as an upper bound for γ_i but expenditure of computation time would increase exponentially, since the parameter space using a tenfold range for γ_i is 10^n times greater for a system in n parameters.

Because the derivative of each variable can be calculated as the difference of influx and outflow ($x_i = v_{2i-1} - v_{2i} \forall i \in \{1, ..., 4\}$), we obtain the set of equations

$$\begin{aligned}
\frac{dx_1}{dt} &= \gamma_1 \cdot x_1^{g_1} - \gamma_2 \cdot x_1^{g_2} \cdot x_2^{g_3} \cdot x_4^{g_4} \\
\frac{dx_2}{dt} &= \gamma_3 \cdot x_1^{g_5} \cdot x_2^{g_6} - \gamma_4 \cdot x_1^{g_7} \cdot x_2^{g_8} \\
\frac{dx_3}{dt} &= \gamma_5 \cdot x_2^{g_9} \cdot x_4^{-g_{10}} - \gamma_6 \cdot x_3^{g_{11}} \\
\frac{dx_4}{dt} &= \gamma_7 \cdot x_2^{g_{12}} \cdot x_3^{-g_{13}} - \gamma_8 \cdot x_4^{g_{14}}
\end{aligned} \tag{4}$$

This is a system of 4 ordinary differential equations in 4 variables, containing 22 parameters ($\gamma_1, ..., \gamma_8, g_1, ..., g_{14}$).

All variables except for parasite load are normalized with respect to a control group of mice (group without inoculation of parasites). Normalized values are calculated according to

$$x_{norm} = \frac{x(group)}{x(control)}. \tag{5}$$

Furthermore, to have all variables in the same range (mean 1), their values are divided by their proper mean, i.e.

$$x_i = \frac{x_i}{mean(x_1, ..., x_n)} \tag{6}$$

whereby 1,...,n denote the considered time points.

As a next step, estimation of parameters is performed using a genetic algorithm.

4.5 Parameter Estimation

Parameter estimation is performed using a genetic algorithm derived by J. Hormiga (see [23] for details). In each step, a random set of parameters ("population") is generated. Parameters are optimized by minimizing the objective function

$$\sum_{i=1}^{n} \sum_{j=1}^{s} (X_i(t_j) - sol_i(t_j))^2, \tag{7}$$

whereby n is the number of variables, s the number of measurement points, X_i denote variables, t_j time points and sol_i the approximation of X_i by the model. In other words, the least-squares solution is calculated i.e. the solution that minimizes the sum of squared errors between data and approximation.
It is important to note that the objective function only takes into account the actual measurement points, neglecting interpolated points in order not to falsify the result. The fit of all four variables is weighted equally, i.e. for parasite load and number of lymphocytes

each of the 4 measurement points has weight $\frac{1}{4}$ and for IgG1 and IgG2a, each of the 13 measurement points has weight $\frac{1}{13}$. Thus, the weight matrix is given by:

$$\begin{pmatrix} \frac{1}{4} & \frac{1}{4} & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ 0 & 0 & \frac{1}{13} & \frac{1}{13} \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{13} & \frac{1}{13} \end{pmatrix}, \tag{8}$$

whereby columns mean the 4 variables in the order: parasite load - number of lymphocytes - IgG1 - IgG2a and rows denote different time points 0,2,...,24 weeks. Measurements were taken over a period of 28 weeks, however, since of lymphocytes and parasite load the last measurement was taken after 24 weeks, we only consider a period of 24 weeks to avoid extrapolation because the latter would falsify the result.

The genetic algorithm used for parameter estimation has the data as well as the system of equations (4) as inputs. The variables have to be vectors of the same length i.e. all variables have to be measured at the same points in time. Furthermore, parameter ranges have to be specified in advance (see (2), (10), (11)) and (12). The program's output includes the parameter values, the value of the objective function (7) as well as plots of the data versus solution, sorted values of the objective function and the error of estimated versus real data. The algorithm is iterated several times whereby the best solution is chosen in each step. The number of iterations can be specified by the user. Calculations are performed in Matlab 7.1 and Matlab R2007a.

The genetic algorithm requires vectors of equal size as inputs. Therefore missing values in our data have to be compensated.

IgG1 and IgG2a were measured every two weeks, whereas the number of lymphocytes and the parasite load were only measured every eight weeks. Moreover, we do not have an initial value for parasite load - this would be zero, since parasites were injected into the paw and not into one of the four organs considered in the model. However, if we used the real value zero as an initial value, parasite load and number of lymphocytes would remain constant the whole time due to the system of equations given in (4). This is the case because without initial parasite load disease does not evolve. A more accurate representation would be yielded considering the site of inoculation as additional variable and building a compartment-model. However, parasite load was not measured at the inoculation site so there are no data for this variable except the initial values: 10^3 and 10^6 parasites, respectively.

An initial value of parasite load in the four organs considered in the model cannot be yielded by normalization with respect to the control group either, because the latter does

not show any parasite load, which would yield division by zero in (5). In contrast, for the normalized value of x_2 (number of lymphocytes) we can assume an initial value of one, since before inoculation of parasites the number of lymphocytes is assumed to be the same in all groups.

A way to handle initial parasite load zero is to use a small initial value for parasite load. Although this does not correspond to the real situation because initially there are no parasites in any of the four organs, it enables data fitting in the interval between week 0 and week 8, and therefore serves as a model for a surveillance period of 24 weeks.
A different strategy would be to represent only the period between week 8 and week 24. This is a better representation of reality and would probably also yield better fit, however, the model would lack information about disease progression in the first eight weeks after inoculation. Since a model for disease progression from the disease outset is required, the first strategy is applied i.e. a small initial parasite load is used.
Parasite load is originally given logarithmically which does not matter though because the variable is normalized with respect to its proper mean. Because of logarithmic scaling its true absolute initial value is zero. Initial values between 10^{-10} and 0.01 are implemented, comparing them in terms of the objective function (7). The smaller the minimum of the objective function, the better the model with the respective initial value fits experimental data.

4.6 Model Selection Strategies

The topology of the model is assumed to be like in Fig. 6. However, there are different models concerning the number of parameters used. The most general model uses all 22 parameters (kinetic orders and rate constants). Other models are simplifications of this general model, using between 15 and 21 parameters, according to the respective use of more or less a-priori-knowledge which fixes the value of certain parameters to zero or one. In Section 4.8 the different models will be presented.
Four strategies of model selection are proposed in order to find the one that yields the "best" fit:

1. To specify a determined number of iterations and choose the model with the smallest objective function value.

2. To iterate until convergence of the error i.e. until the error gradient falls below a determined value.

3. To specify a fixed value of the objective function and designate models that fall below this value as "appropriate". Out of these, choose the most significant (in our case: smallest initial value) or most simple (e.g. the model with the least number of parameters) model, respectively.

4. To perform (1) and subsequently iterate simpler models using the objective function value of the best model according to (1) until they either reach a smaller value or a determined number of iterations.

Strategy 1 would probably yield the most complex model as best solution, because among all models it has the most degrees of freedom and therefore yields the highest accuracy. Moreover, the lower the number of iterations, the more likely random effects affect and falsify the result.
Strategy 2 would not necessarily consider the most complex model as the best, however, it may get stuck in a local minimum of the error.
Strategy 3 is a reliable alternative although the cut-off value for the objective function is chosen arbitrarily.
Strategy 4 does not yield this problem, however, it has the disadvantage of random effects in (1) and can be time-consuming using a high number of iterations.

After evaluation of the different alternatives it is concluded that strategy 3 is the most appropriate one. The objective function is based on the sum of errors (differences between real and modeled data); therefore its cut-off value represents a certain error level.

4.7 Initial Values

In this step of model selection, models for which the minimum of the objective function is smaller than 0.2 are considered appropriate. In terms of the error between experimental and model data, $f_{obj} = 0.2$ occurs for example if the mean squared error in each of the four variables is 0.05 due to

$$f_{obj} = \sum_{i=1}^{4} error_i,$$

whereby $error_i$ is the mean squared error of variable i, given by

$$error_i = \sum_{j=1}^{n} \lambda_{ji} \cdot (exact(t_j) - model(t_j))^2$$

with i being the variable number, n the number of data points of the respective variable and λ_{ji} the weight of variable i at time point j according to the weight matrix in (8).
Of the initial values for which simulations were performed, 10^{-4}, 10^{-6} and 10^{-8} meet the criterion $f_{obj} < 0.2$ within 100 iterations. Objective function values together with their computation times are given in Tab. 2. Out of them 10^{-8} is nearest to the real value zero. However, using this initial value none of the remaining five models considered in Section 4.8 (models with reduced number of parameters) achieves $f_{obj} < 0.2$ whereas for the initial value 10^{-6} two of the six models yield $f_{obj} < 0.2$.

Therefore 10^{-6} *is used as initial value for parasite load in the group infected with* 10^3 *parasites.*

In this way a comparison between those two models that fulfill $f_{obj} < 0.2$ is possible.
Using 100 as the maximal number of iterations, computation times are between 11 and 20 hours (Computer: Intel(R) Core(TM)2 CPU, T6400 @ 2.00GHz, 1.99GHz, 1.99GB RAM with Matlab R2007a).

The following section describes simplifications of the model in order to reduce the number of parameters.

initial pload	10^{-4}	10^{-6}	10^{-8}
f_{obj}	0.1860	0.1408	0.1374
time (h)	11.19	15.71	16.29

Table 2: Best models with respect to initial values of parasite load using 100 iterations of the genetic algorithm in the 10^3 parasites group. Computer: Intel(R) Core(TM)2 CPU, T6400 @ 2.00GHz, 1.99GHz, 1.99GB RAM with Matlab R2007a. Parameter ranges are [0,3] for g and [0,10] for gamma. *initial pload:* initial parasite load, f_{obj}*:* minimal value of the objective function, *time:* overall execution time of parameter estimation algorithm.

4.8 Parameter Reduction

Annotation of fluxes refers to the model scheme Fig. 6, Section 4.4.

A first simplification is achieved if we consider the outflow of each variable to be directly proportional to the variable value. This means that the exponent of the variable's influence on its proper degradation is 1, yielding

$$g_2 = g_8 = g_{11} = g_{14} = 1$$

for our set of parameters. In this way we have reduced the system from 22 to 18 parameters.

Furthermore, two of the variables, namely parasite load and number of lymphocytes, have an influence on their proper influx. Like above, we can consider these variables to be directly proportional to their proper generation, which yields

$$g_1 = g_6 = 1$$

and a reduction to 16 parameters.

In our model parasite load has a positive influence both on an increase as well as a decrease of the number of lymphocytes. This is biologically supported since on the one hand, parasites act contra lymphocytes, trying to avoid any defense mechanism of the body against the illness but on the other hand, the higher parasite load, the more lymphocytes are produced (immune-response). This is why both parameters are implemented. If parasites only inhibit generation of lymphocytes or only promote lymphocyte death, we expect the other parameter to be zero, respectively. Thus, knowing which of the two compensative influences on the influx and outflow has a greater effect, we could leave out the other one.

Fig. 7 shows that the more parasites there are, the greater the population of lymphocytes, i.e. the influence of parasite load on the number of lymphocytes is positive. Because of this, we can summarize the two arrows in the diagram to the one increasing the influx of lymphocyte population (g_5). In terms of parameters this means

$$g_7 = 0.$$

So the model has been reduced to 15 parameters.
Combination of the just mentioned parameter reduction strategies results in the six models given in Tab. 3.

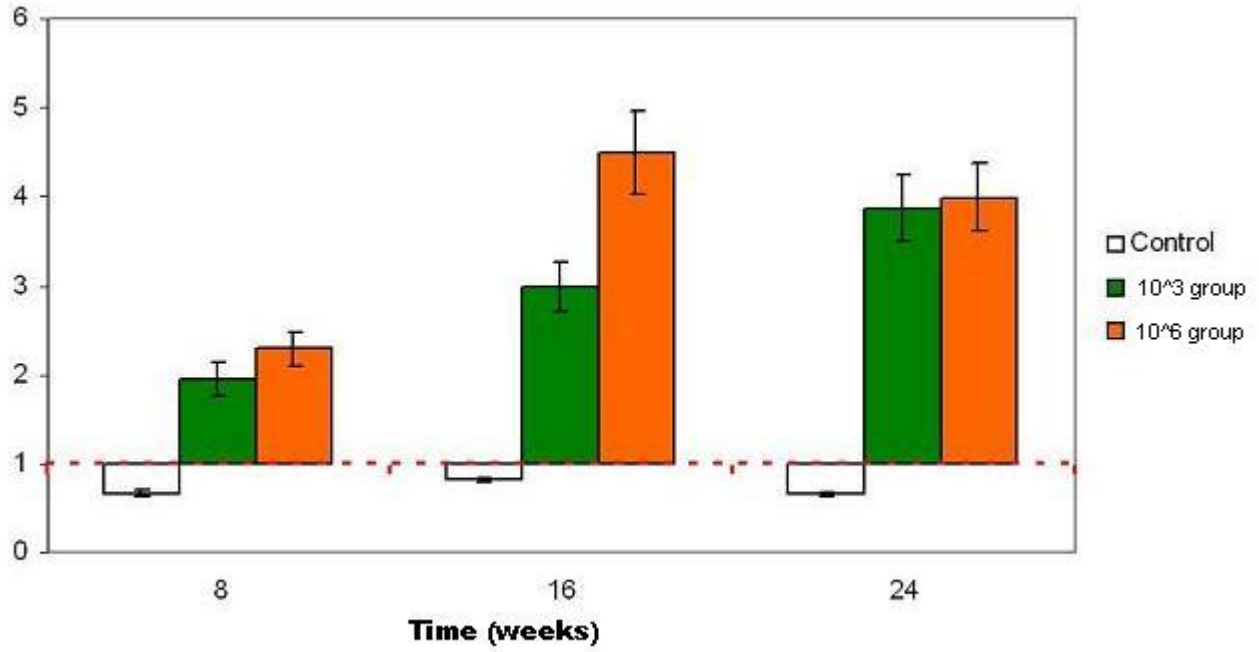

Figure 7: Number of lymphocytes in control group (white, left bar), 10^3 parasites group (green, middle bar) and 10^6 parasites group (orange, right bar). Data from C. Pou [1].

n° parameters	all parameters	outflow prop.	outflow + influx prop.
all parameters	22	18	16
$g_7 = 0$	21	17	15

Table 3: Number of parameters for different models. *all parameters:* original model without simplifications, *outflow prop.*: variables directly proportional to their proper outflow ($g_2 = g_8 = g_{11} = g_{14} = 1$), *outflow + influx prop.*: variables directly proportional to their proper influx and outflow ($g_1 = g_2 = g_6 = g_8 = g_{11} = g_{14} = 1$), $g_7 = 0$: leaving out parasites' influence on degradation of lymphocytes.

4.9 Dynamic Sensitivity Definition

Sensitivity of a parameter is the extent to which variable values change if the respective parameter is perturbed on a small scale. It can be seen as a measure for the influence of a parameter on model behavior.
The far-end aim of this work is to find pharmaceutical agents decreasing parasite load. In order to identify crucial points where these could interact, we have to study the influences of our model parameters on parasite load. It is supposed that drugs are able to change both kinetic orders g_i and rate constants γ_i.

The size of parameter perturbation is specified by a factor between 0.9 and 1.1 which is multiplied with the parameter (thus representing changes of +/- 10% of the parameter). The concept of dynamic sensitivity is explained by means of the rate constant for parasite load generation, γ_1. The components of the vector

$$[0.9, 0.92, 0.94, 0.96, 0.98, 1.02, 1.04, 1.06, 1.08, 1.1] \tag{9}$$

are used as parameter coefficients. Because the considered parameter γ_1 is directly proportional to parasite load (see (4) in Section 4.4), factors smaller than one refer to less parasite load with respect to the original value whereas factors higher than one refer to an augmentation in parasite load.

Fig. 8 shows a graphical interpretation of dynamic parameter sensitivity with respect to a variable: For an infinitesimal change in the system parameter, the absolute value of sensitivity is the area between the original variable curve and the new variable curve ("new" denotes the curve calculated using the infinitesimally changed parameter). The sign of the sensitivity is positive if the area below the new curve is greater than that below the original curve and negative if it is smaller.

A plot of change factor versus size of the area below the *parasite load* curve represents the dynamic sensitivity of a parameter with respect to the variable. Interpolation e.g. by splines is performed to yield a continuous line. The sensitivity of this parameter is the value of this curve at abscissa 1 because we are interested in the question if variable behavior changes significantly with *infinitesimal perturbations* of the original parameter which correspond to coefficients near one.

Since the aim is to reduce parasite load in an effective way, our interest focuses on sensitivities with respect to x_1. Sensitive parameters are possible therapeutic targets.

For sensitivity analysis of parameters, we use the following algorithm:

1. Parameter values from simulation of the "best" model are loaded and the integration step size is specified.
2. The solution (i.e. approximation of variables by the model) is calculated using the specified integration step size.
3. For each of the model variables (a)-(g) is executed:
   (a) The area A_1 between the original curve and the abscissa (absolute value) is calculated using the trapezoidal method.

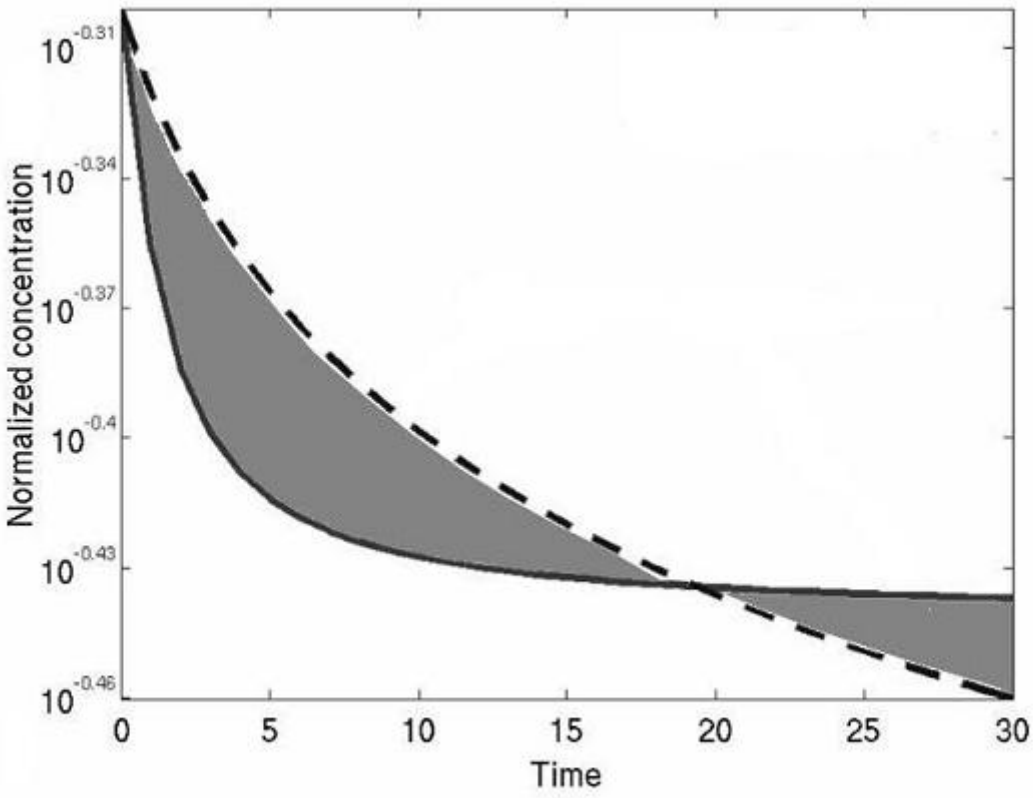

Figure 8: Graphical representation of dynamic sensitivity. The continuous line shows the original system dynamics, while the dotted one corresponds to the time course after an infinitesimal change of parameter p_k. The gray area represents the absolute value of sensitivity.

(b) One of the parameters is multiplied with a factor between 0.9 and 1.1, the rest of the parameters do not change their values.

(c) The area A_2 between the new curve and the abscissa (absolute value) is calculated using the trapezoidal method.

(d) The area D between the original and the new curve is calculated using the trapezoidal method.

(e) The sign of sensitivity is determined according to

$$sign = signum \frac{A_2 - A_1}{p_2 - p_1},$$

whereby p_1 means the original parameter value and p_2 the disturbed parameter.

(f) Parameter sensitivity is calculated according to

$$sensitivity = sign \cdot \frac{D}{\frac{|p_2 - p_1|}{p_1}}.$$

(g) Steps (a) to (f) are repeated for all coefficients in (9).

4. Data of factors versus sensitivities are interpolated using cubic spline interpolation.

5. The dynamic sensitivity of the parameter is the interpolated value at factor=1.

6. Maximal minus minimal sensitivity in the interval $[0.9 \cdot parameter, 1.1 \cdot parameter]$ yields a measure for the error.

7. Sensitivities and errors for all parameters and variables are represented in an image scan.

As an alternative to (9) any vector containing values in $[1 - \epsilon, 1 + \epsilon] \backslash \{1\}$, can be used whereby x=1 is excluded because this would yield division by zero in (3e) and (3f).

The area between the two curves calculated in (3d) is used as a measure of change in model behavior after infinitesimal change of a parameter value, thus indicating the influence of the respective parameter on the considered variable.
According to (3e) positive sensitivity means direct proportionality of parameter change and change of the area below the curve. This means that if the parameter increases, the area below the curve augments which means an increase in the sum of all values of the respective variable. If the parameter decreases, the area below the curve is reduced which means diminishment of the sum of all values of the respective variable. In the same way negative sensitivity stands for indirect proportionality. A sensitivity of zero means a change of the parameter does not have any influence on the sum of values of the respective variable.
The denominator in (3f) expresses the relative change of the parameter value. The whole fraction expresses the change in variable behavior with respect to a small change in the parameter value. This is the definition of sensitivity.
An interpolation method has to be used since we are interested in sensitivity at the original parameter value (factor=1) but formula (3f) would yield $\frac{0}{0}$ at factor=1 (no change in parameter, original curve).

A high absolute value of sensitivity means that a small change in the respective parameter is enough to change the variable curve significantly. In contrast, a sensitivity value near to zero signifies that variable values almost stay the same despite small parameter changes. Thus, the higher the sensitivity's absolute value, the more influential a parameter is considered.

As alternative to the area between curves, the difference in terms of mean, median, maximum or final value could be used as a measure for the "change" in model behavior.

4.10 Optimization Algorithm

Minimization of parasite load is tried changing the value of kinetic constants g_i and rate constants γ_i. Only one of the parameters is changed at a time whereas the others maintain their values. The parameter is successively multiplied with factors $0.1 \cdot n$, whereby $n \in \mathbb{N}$ is increased as long as the parameter is in its range [0,3] (g_i) or [0,10] (γ_i), respectively. For each factor, solutions are calculated according to equations (4). Mean, maximal and final parasite load serve as measures of effectiveness of the parameter change. All three values are used because the mean parasite load is reflecting the average severity of the disease, the maximal value is supposed to have a certain limit according to the maximal number of parasites the organism is able to bear and the final parasite load represents the final outcome of the disease or treatment. The latter is considered the most important reference value since it represents healing or non-healing. A possible optimization strategy would be to set a limit for maximal parasite load, representing the highest parasite load the organism is able to handle, and minimize final parasite load within a certain time span.
Moreover the derivative of parasite load has to be born in mind. Treatment should lead to a decrease in parasite load i.e. the gradient of parasite load should be negative in the end.

Furthermore, optimization changing two parameters simultaneously is performed. For this analysis, the parameters yielding the lowest final parasite load (i.e. a parasite load lower than 2) in one-dimensional optimization are chosen and up- or downregulated by division by 10 and multiplication with 10, respectively, depending on if they were up- or downregulated in one-dimensional-optimization. In every case, kinetic constants are maintained in the range $[0, 3]$. Final, maximum and mean parasite load are determined whereby final parasite load and parasite load gradient are considered the crucial measures for effectiveness of the respective parameter change. Finally, these results are compared to results of a systematic 2D-optimization (i.e. optimization changing all pairs of parameters) by B. Wimmer [54].

5 Results

In this section the best models are presented together with the best sets of parameters. Special focus is put on the identification of relevant parameters and their biological significance and interpretation. Moreover, sensitive parameters are chosen and using them, strategies for the minimization of parasite load are investigated. The latter yield new ideas for the design of drugs against Leishmania.

5.1 Model Selection

parameters	22	18	16	21	17	15
iterations	38	95	100	100	100	100
objective function	0.1412	0.1962	0.4701	0.3963	0.4652	0.7557
execution time (h)	3.06	8.18	9.85	1.38	22.68	19.52
computer	1	1	1	2	2	2

Table 4: Comparison of the six models given in Tab. 3 (Section 4.8) in terms of the number of iterations, objective function value, maximal error between interpolated and model data as well as execution time. The stopping criterion is $f_{obj} < 0.2$. Computer 1: Intel(R) Core(TM)2 Duo CPU, E7400 @ 280GHz, 2.80GHz, 1.96GB de RAM with Matlab 7.1, computer 2: Intel(R) Core(TM)2 CPU, T6400 @ 2.00GHz, 1.99GHz, 1.99GB RAM with Matlab R2007a. Parameter ranges are [0,3] for g_i (kinetic orders) and [0,10] for γ_i (rate constants).

Out of the models proposed in Tab. 3 (Section 4.8), we choose the one that reflects best experimental data. In order to do this, we make use of model selection strategy 3 introduced in Section 4.6 (i.e. performing 100 iterations and selecting the simplest model with $f_{obj} < 0.2$).
Tab. 4 shows results obtained for the six models.

Models that meet $f_{obj} < 0.2$ are the model with outflow proportional to variable value (18 parameters) and the model with 22 parameters. Since the aim is to choose the simplest model that reflects data sufficiently well, the 18-parameter-model is considered as the best one in the first place. However, in order to study the influences of all parameters, the 22-parameter-model is further investigated, too, and a comparison between the two models is done.

As a next step, we use the model's parameters and their standard deviation to construct individual parameter ranges. The latter consist in the parameters of the best model +/- their standard deviation. Simulations are done on the 18- and the 22-parameter-model. In order to provide generality of the model, the unit subset of the parameter ranges of both models, restricted to their maximal parameter ranges, [0,2] and [0,3] for kinetic orders and [0,10] for rate constants, respectively, is used:

$$([g_{18} - \sigma_{g_{18}}, g_{18} + \sigma_{g_{18}}] \cup [g_{22} - \sigma_{g_{22}}, g_{22} + \sigma_{g_{22}}]) \cap [0, 2] \tag{10}$$

or

$$([g_{18} - \sigma_{g_{18}}, g_{18} + \sigma_{g_{18}}] \cup [g_{22} - \sigma_{g_{22}}, g_{22} + \sigma_{g_{22}}]) \cap [0, 3], \tag{11}$$

and

$$([\gamma_{18} - \sigma_{\gamma_{18}}, \gamma_{18} + \sigma_{\gamma_{18}}] \cup [\gamma_{22} - \sigma_{\gamma_{22}}, \gamma_{22} + \sigma_{\gamma_{22}}]) \cap [0, 10] \tag{12}$$

whereby g_{18} and g_{22}, respectively γ_{18} and γ_{22} denote the best parameters from the 18- and 22-parameter-model, respectively, and the respective σ their standard deviations within all iterations.
Simulation is iterated until $f_{obj} < 0.2$.
The following two models yield the best results:

- 18-parameter-model using the unit subset of parameter ranges restricted to $g \in [0, 2]$ (10), (12) and
- 22-parameter-model using the unit subset of parameter ranges restricted to $g \in [0, 2]$ (11), (12).

In conclusion, the 18-parameter-model using the subset of individual parameter ranges of the 18- and 22-parameter-model restricted to $g \in [0, 2]$ i.e. (10), is considered the best model ($f_{obj} = 0.0704$), whereas the most general model i.e. the model using the least a-priori-knowledge, is the 22-parameter-model with $g \in [0, 3]$. These two models are investigated in the following. They are simulated with 1000 iterations, whereby parameter ranges are (10) and (12) for the 18-parameter-model and (11) and (12) for the 22-parameter-model.

5.2 18-Parameter Model: Degradation Directly Proportional to Variable

This section provides an analysis of the 18-parameter-model including identification of key parameters, sensitivity analysis and optimization with the aim of minimizing parasite load.

After 1000 iterations and an execution time of 96.42 hours the genetic algorithm yields an objective function value of 0.0517 for this model. The maximal absolute error between interpolated data and model data is 0.5828.

Fig. 9 and Fig. 10 represent the size of rate constants and kinetic coefficients, respectively, by means of bar plots.

Plots of data and model prediction of the four model variables are given in Fig. 11.

To check feasibility of the model we apply the model with the same parameters to data of the 10^6 parasites group. This yields an objective function value of 0.1973 (in contrast to 0.0517 for the 10^3 parasites group). Plots are given in Fig. 12.

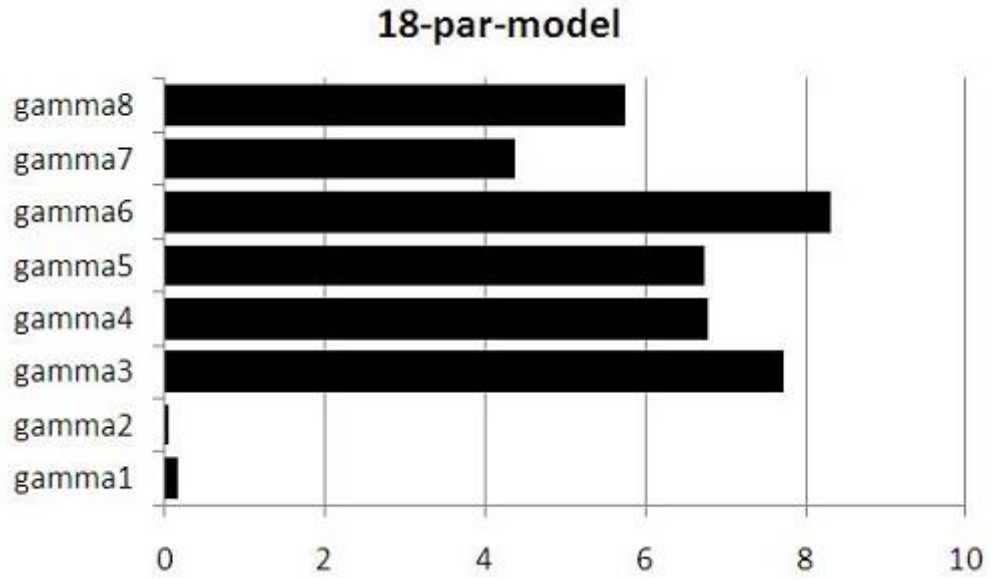

Figure 9: Size of rate constants resulting from parameter estimation with parameter ranges (10) and (12) in the 18-parameter-model (outflow proportional to variable), 1000 iterations.

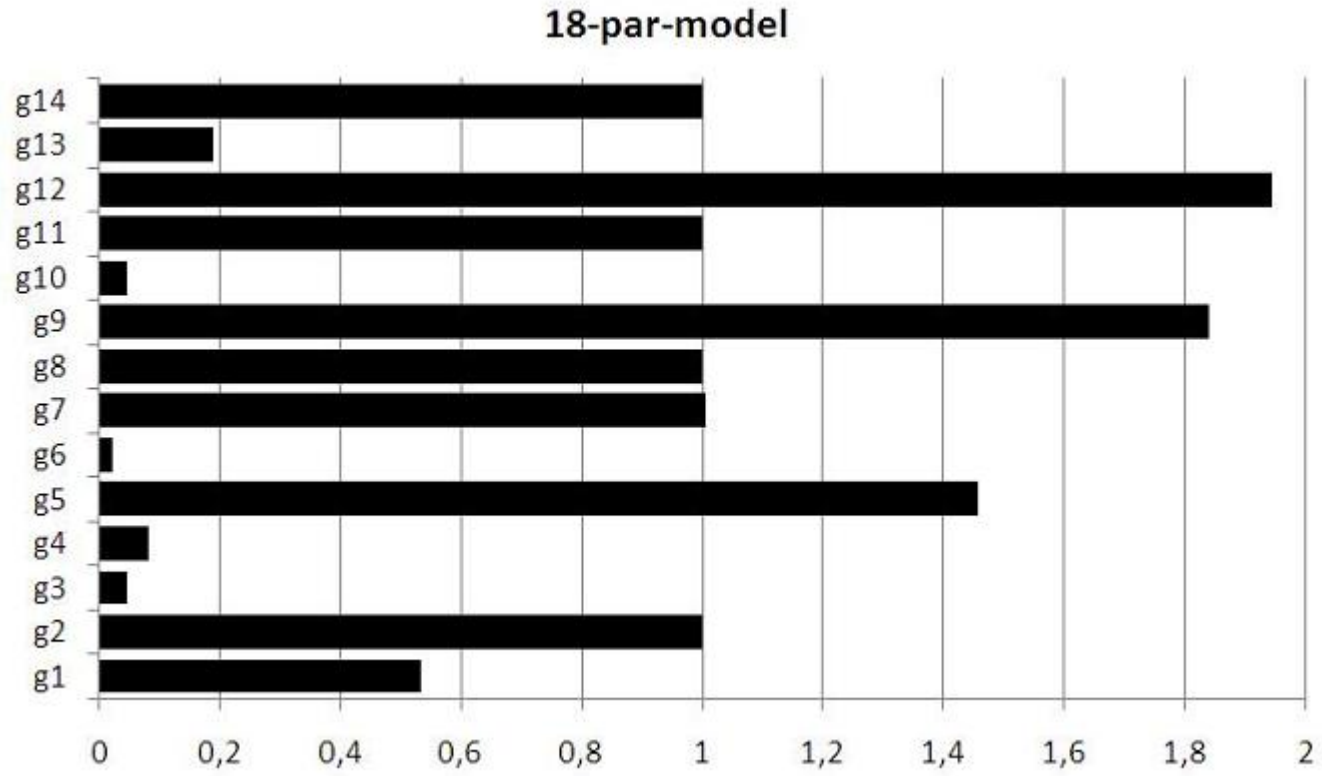

Figure 10: Size of kinetic constants resulting from parameter estimation with parameter ranges (10) and (12) in the 18-parameter-model (outflow proportional to variable), 1000 iterations.

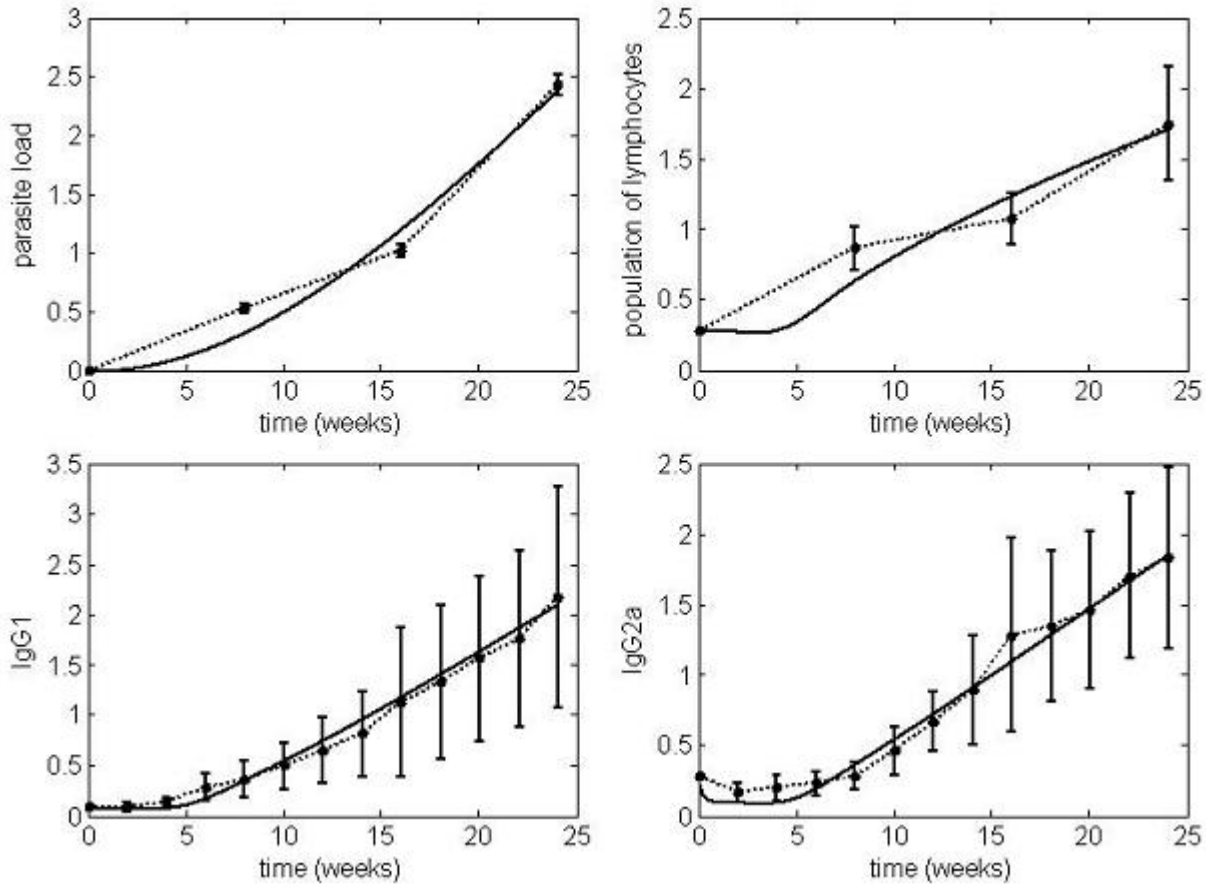

Figure 11: Experimental data of the 10^3 parasites group (points) and model approximation (line) in 18-parameter-model (outflow proportional to variable), parameter ranges (10) and (12), 1000 iterations. **I** represents the standard deviation of experimental data.

parameter	value	parameter	value
γ_1	0.16884464	g_4	0.08101011
γ_2	0.04320366	g_5	1.45712004
γ_3	7.73529493	g_6	0.02273563
γ_4	6.77372398	g_7	1.00487798
γ_5	6.74165524	g_8	1
γ_6	8.31217464	g_9	1.84130268
γ_7	4.36878533	g_{10}	0.04564140
γ_8	5.75473286	g_{11}	1
g_1	0.53341135	g_{12}	1.94380751
g_2	1	g_{13}	0.18999831
g_3	0.04628653	g_{14}	1

Table 5: Parameter values for outflow-proportional-to-variable-model (18-parameter-model), considered as most appropriate model, using the genetic parameter estimation algorithm with 1000 iterations and individual parameter ranges for each g_i and γ_i i.e. (10) and (12). Data correspond to an objective function value of $f_{obj} = 0.0517$.

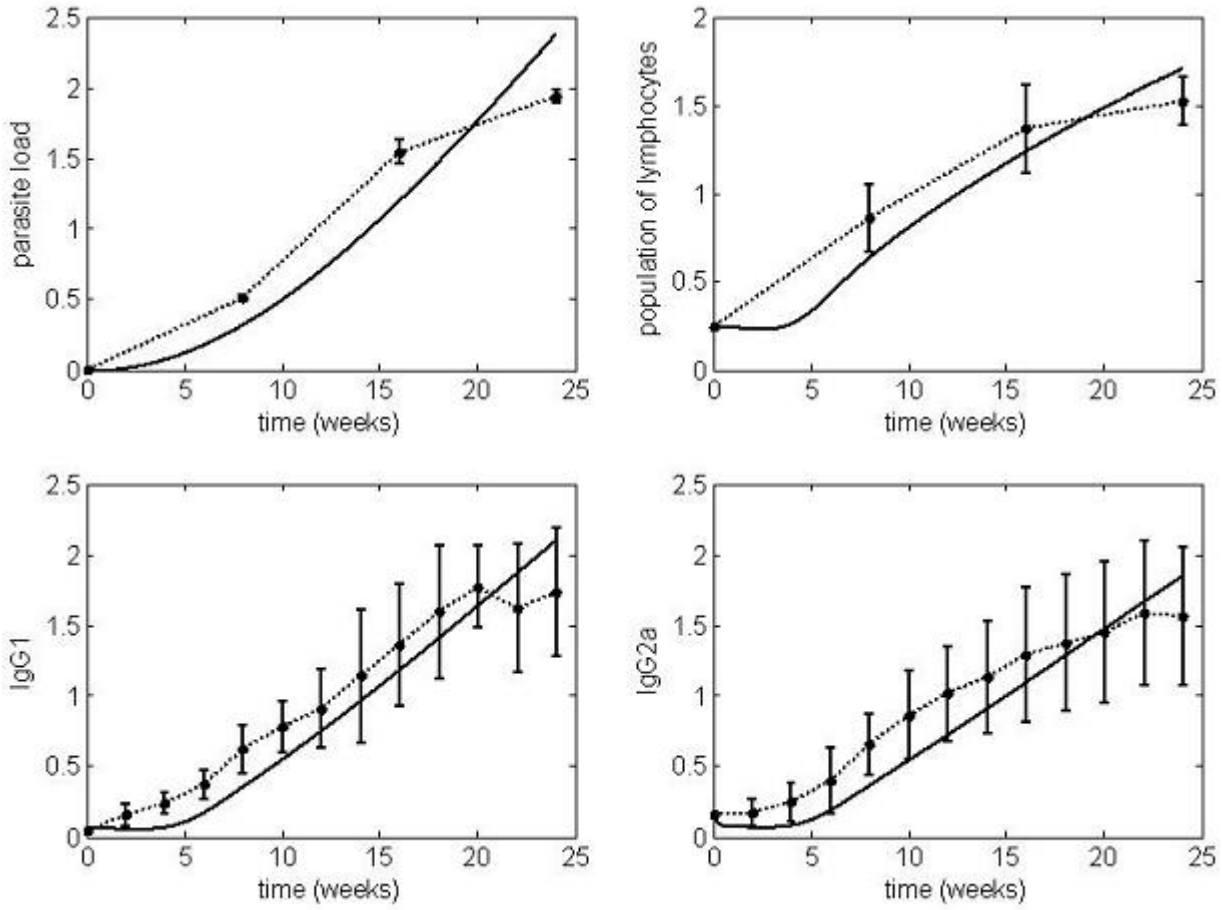

Figure 12: Experimental data of the 10^6 parasites group (points) and model approximation (line) in 18-parameter-model (outflow proportional to variable), parameters estimated using data of the 10^3 parasites group, parameter ranges (10) and (12), 1000 iterations. **I** represents the standard deviation of experimental data.

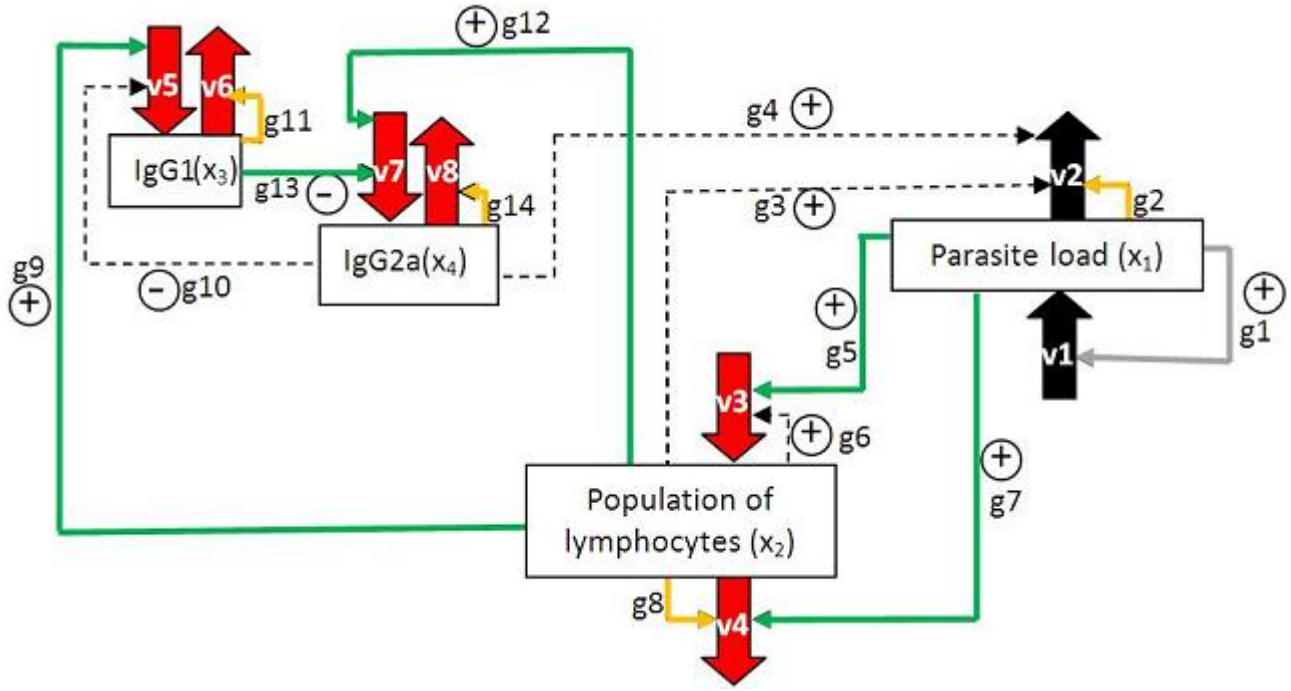

Figure 13: Parameter sizes in the 18-parameter-model. Yellow: $g_i = 1$ (g_2, g_8, g_{11}, g_{14}); dark green: $g_i > 1$ ($g_5, g_7, g_9, g_{12}, g_{13}$); gray: $0.1 < g_i < 1$ (g_1); dashed: $g_i < 0.1$; red: $\gamma_i > 4$ ($\gamma_3, ..., \gamma_8$); black: $\gamma_i < 1$ (γ_1, γ_2).

5.2.1 Significant Parameters

Fig. 13 shows the model, indicating parameter influence with different arrow line types and colors.

Interestingly, γ_1 and γ_2 which correspond to parasite proliferation and degradation, respectively, are the smallest rate constants, equating 0.1688 and 0.0432 respectively, whereas for all the other rate constants $4.36 < \gamma_i < 8.32$ holds, which means that they have a much higher influence.

g_3 and g_4 which are directly related to degradation of parasites, are not of significant size (i.e. < 0.1). Moreover, the number of lymphocytes is rather dependent on parasite load (g_5, g_7) than on its proper value (g_6): $g_5 > g_6$, $g_7 > g_6$.

IgG2a does not seem to have an influence on the rest of the variables: Its influences on the other variables (g_4, g_{10}) have an absolute value smaller than 0.1. Interestingly, g_5 and g_7 i.e. the parasite load's influence on production and degradation of lymphocytes are both significant (i.e. > 1), whereby g_7 i.e. the influence of parasite load on degradation of lymphocytes nearly equals one (1.0049) indicating that degradation of lymphocytes is directly proportional to parasite load. Setting $g_7 = 1$ would yield a model with 17 parameters.

The highest kinetic orders correspond to the lymphocyte population's influence on production of IgG1 and IgG2a. This result stresses the high importance of immunoglobulins in disease progression.

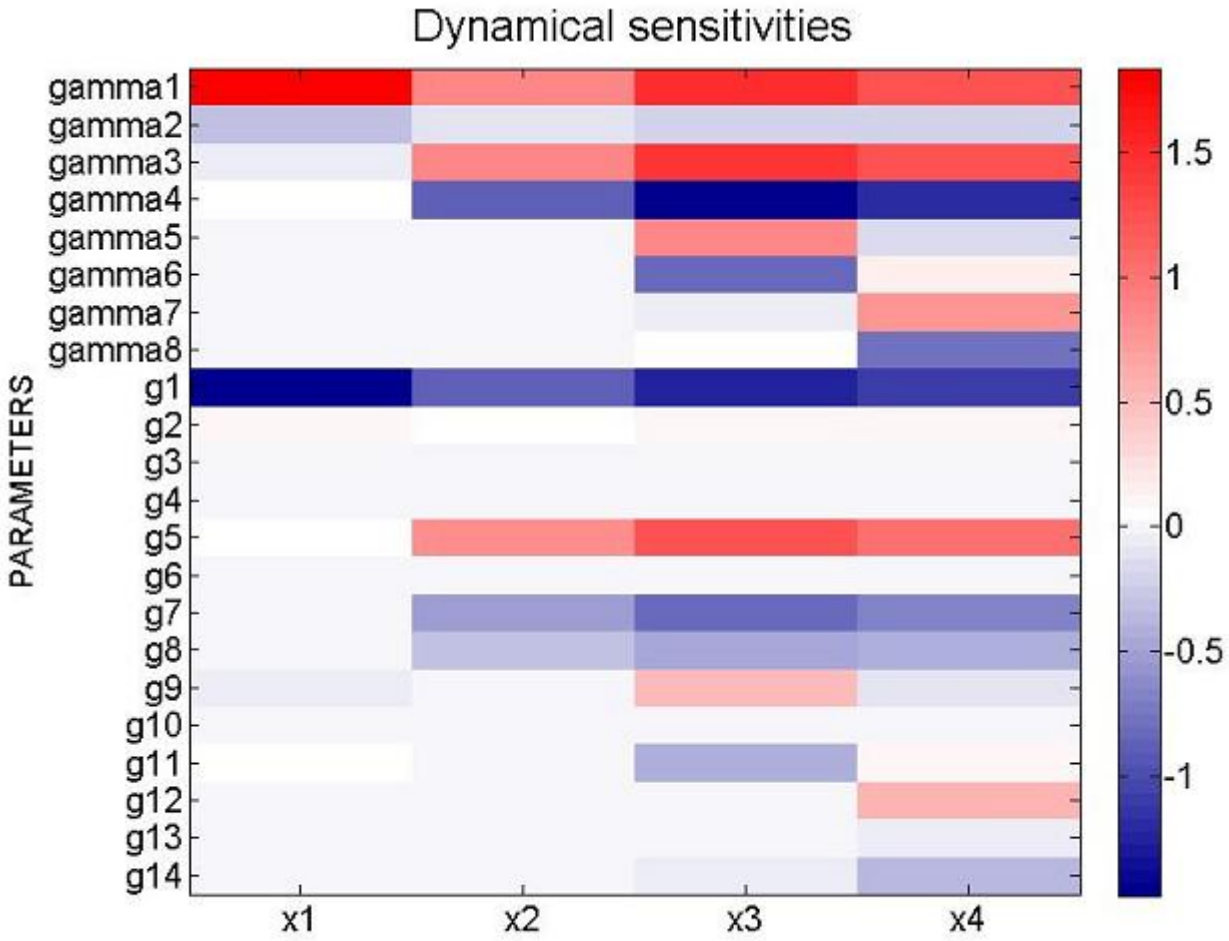

Figure 14: Parameter sensitivities in the 10^3 parasites group, 18-parameter-model.

5.2.2 Sensitivity Analysis

The algorithm introduced in Section 4.9 is now applied to the parameters of the 18-parameter-model with 1000 iterations (see Tab. 5 for parameter values) in order to identify the parameters having the highest influence on parasite load.
In the model scheme (Fig. 6, 13) we can see that the parameters γ_1, γ_2, g_1, g_2, g_3 and g_4 have a direct influence on parasite load.
Sensitivities are denoted as follows: $S(g_i, x_j)$ is the sensitivity of parameter g_i with respect to the variable x_j.

A graphical representation of the sensitivities of all parameters with respect to all variables is provided in Fig. 14 (see Fig. 6, 13 for the meaning of parameters). The deviation of parameter sensitivities in the interval [0.9 1.1] is used as a measure for the error of parameter sensitivity values. Errors of sensitivities with respect to parasite load are between 0.0002 ($S(g_4, x_1)$) and 0.2457 ($S(g_1, x_1)$) with a median of 0.01225. For the other three variables sensitivity error medians are 0.00895 (x_2), 0.02865 (x_3) and 0.0199 (x_4), respectively.
All obtained sensitivities have absolute values between 0.00004 ($S(g_{10}, x_2)$ = sensitivity

of g_{10} with respect to the number of lymphocytes) and 1.8358 ($S(g_1, x_1)$ = sensitivity of g_1 with respect to parasite load). The median of absolute values of sensitivities is 0.0671 which signifies high robustness of the model. The mean of absolute sensitivities is 0.3541. The difference between median and mean favors the existence of outliers i.e. few parameters have high sensitivities (sensitivity$>$ 1 for $\gamma_1, \gamma_3, \gamma_4, g_1, g_5$) whereas most parameters yield small sensitivities.
For each variable, the sensitivity with greatest absolute value, indicating the parameter with highest influence on this variable corresponds to the variable at which the corresponding arrow is pointing in the model scheme except for the parameters directly influencing the number of lymphocytes ($\gamma_3, \gamma_4, g_5, g_6, g_7$ and g_8). The latter show higher sensibilities for IgG1 than for the number of lymphocytes. This indicates that a change in the mentioned parameters is more likely to produce a change in immunoglobulin levels than in the number of lymphocytes i.e. that the amount of lymphocytes is more stable than immunoglobulin levels. This is reasonable because the IE includes all types of lymphocytes, which is supposed to be more general and thus more stable than expression of specific immunoglobulins.
Whereas rate constants γ_i are proportional to variable values i.e. a change in γ_i is directly related to a change in the variable it is influencing, kinetic parameters g_i represent *influences* among variables but do not implicate changes in *variable values*. An increase in g_9 for example does not necessarily cause an increase in IgG1 because it could be the case that IgG1 outflow is greater than IgG1 influx due to other parameters, or that IgG1 is decreased by greater g_9 because its value is less than one. An increase in g_9 just means that the influence of lymphocytes on IgG1 production increases. A positive/negative sensitivity is therefore not directly related to direct/indirect proportionality of two variables, but to proportionality of one parameter (representing an influence) and one variable.

Our interest focuses on sensitivities with respect to parasite load, since this is the variable desired to be reduced by drugs. Fig. 15 shows the model, using different colors for parameters according to their sensitivities.

Sensitivities with respect to parasite load: $S(g_i, x_1)$, $S(\gamma_i, x_1)$

Using a cutoff-value of one for significance, significant parameters for parasite load are γ_1 (positive sensitivity) and g_1 (negative sensitivity). This result signifies that generation of parasites is directly proportional to parasite load and the higher the influence of parasites on their own degradation, the lower parasite load.
The sensitivity of γ_2 (corresponding to parasite degradation) on parasite load is much lower than that of γ_1, namely -0.2958 in contrast to $S(g_1, x_1) = 1.8358$. The influence of parasite load on its proper degradation (g_2) yields a sensitivity of $S(g_2, x_1) = 0.1186$. All the other parameters yield sensitivities with absolute values of less than 0.1 for parasite load.

Other sensitivities

On average, sensitivities of parameters with respect to the other three model variables

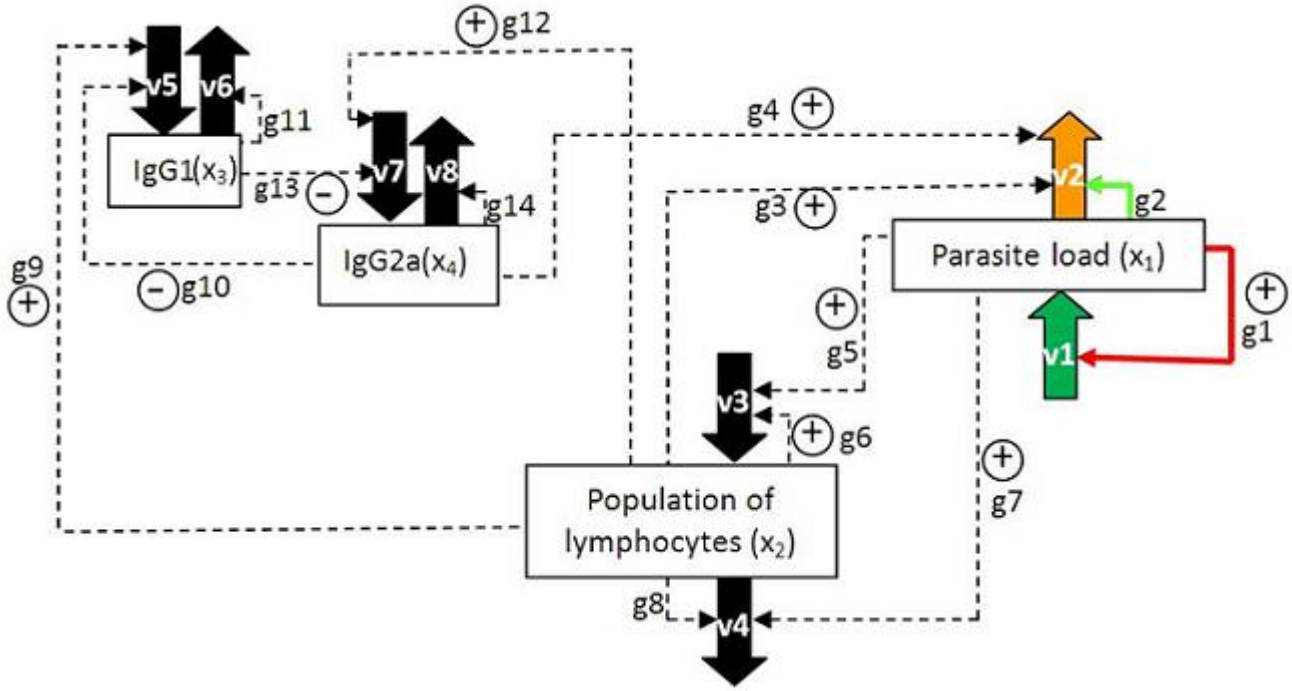

Figure 15: Parameter sensitivities with respect to parasite load in the 18-parameter-model: Dark green: $S(\gamma_i, x_1) > 1$ (γ_1); light green: $0.1 < S(g_i, x_1) < 1$ (g_2); orange: $-1 < S(\gamma_i, x_1) < -0.1$ (γ_2); red: $S(g_i, x_1) < -1$ (g_1); dashed, black: $|S(., x_1)| < 0.1$.

are higher than sensitivities on parasite load: The mean absolute value of sensitivity with respect to parasite load is 0.1840, whereas the mean absolute value of sensitivity with respect to lymphocytes is 0.2503, to IgG1 0.5153 and to IgG2a 0.4668. This means that facing an infinitesimal change in one of the model parameters, on average parasite load is more robust than the population of lymphocytes which in turn is more robust than the amount of immunoglobulins.

With respect to the other three variables, significant parameters are above all those that have a direct influence on the respective variable. However, sensitivity analysis (Fig. 14) yields some interesting results:
The parameter with the highest sensitivity is γ_1 which yields sensitivities between 0.9030 (with respect to lymphocytes) and 1.8358 (with respect to parasite load) for all variables. Since the fundamental model variable (representing severity of the disease) is parasite load, and its input is directly related to γ_1, this result is obvious. An augmentation of γ_1 corresponds to an augmented parasite load which yields a higher immune response, thus augmenting the value of all variables. A lower value of γ_1 i.e. a decrease in parasite load provokes a lower immune response thus decreasing the values of all variables.
g_1, the influence of parasite load on its own generation, has a negative sensitivity with respect to all parameters. This means that the more parasite load is stimulating its proper influx, the lower the immune response, but also the lower parasite load.

Sensitivities with respect to the number of lymphocytes: $S(g_i, x_2)$ and $S(\gamma_i, x_2)$

There are no sensitivities with absolute value greater than one with respect to the number

of lymphocytes (x_2). This suggests that the variable is stable facing small changes of parameters.

Sensitivities with respect to IgG1: $S(g_i, x_3)$ and $S(\gamma_i, x_3)$

For all of the sensitivities with respect to IgG1 $|S(g_i, x_3)| > |S(g_i, x_2)|$ and $|S(\gamma_i, x_3)| > |S(\gamma_i, x_2)|$ hold and 17 of 22 parameters yield the same sign in sensitivity corresponding to lymphocytes and IgG1. Only $\gamma_8, g_9, g_{10}, g_{11}$ and g_{12} yield different signs. These are parameters directly influencing either IgG1 or IgG2a. This kind of similarity between sensitivities of lymphocytes and immunoglobulins is reasonable because immunoglobulins are produced by lymphocytes.
$\gamma_1, \gamma_3, \gamma_4, g_1$ and g_5 yield sensitivities with absolute values greater than 1. Positive sensitivity $S(\gamma_1, x_3)$ means that the higher γ_1, thus the more parasites are produced, the higher the IgG1 level. γ_3 and γ_4 are proportional to lymphocyte proliferation and degradation, respectively. It is interesting that these are more sensitive to IgG1 than γ_5 and γ_6, corresponding to influx and outflow of IgG1. Also none of the kinetic parameters g_i directly influencing IgG1 yields a significant (> 1) sensitivity with respect to x_3 which indicates that influence of parasite load- and lymphocyte-related parameters is higher than influences directly related to IgG1 like its inhibition by IgG2a.

Sensitivities with respect to IgG2a: $S(g_i, x_4)$ and $S(\gamma_i, x_4)$

IgG2a yields the same significant (> 1) sensitivities as IgG1. Like IgG1 it is produced by lymphocytes but in contrast to IgG1, IgG2a is able to detect the parasite and act as a marker (see Section 4.4). The fact that significant sensitivities with respect to IgG1 and IgG2a have the same sign suggests that their expression is up- and downregulated accordingly.

5.2.3 Optimization

For optimization, the algorithm presented in Section 4.10 is used. Whereas sensitivity analysis investigates reaction of the model to infinitesimal changes, optimization refers to changes on a larger scale.
The aim is to find the most effective parameters for minimization of parasite load. These parameters shall be changed by pharmaceuticals in order to heal the disease. Pharmaceuticals are supposed to be able to change kinetic parameters g_i and rate constants γ_i.
Section 5.2.2 yields γ_1 and g_1 as the most influential parameters on parasite load. These parameters are supposed to be the most feasible for optimization. However, since sensitivity analysis refers to factors around one whereas optimization refers to factors of up to 100, the latter could consider different parameters to be feasible. In the following, parameters will be changed one by one and the most effective ones to minimize parasite load will be chosen. Subsequently, it is tried to find pharmaceutical agents that change these parameters.

Kinetic Orders

g_1 is negatively sensitive to parasite load which means that augmentation of g_1 causes a decrease in the number of parasites. In order to maintain it in the range [0,3] its value is augmented until it reaches the upper limit 3.

parasite load	$0.1 \cdot n_{final}$	final	$0.1 \cdot n_{max}$	maximal	$0.1 \cdot n_{mean}$	mean
g_1	5.6	4.48256E-07	2.1	0.000001000	5.6	6.87165E-07
g_2	3	1.667032487	3	1.667032487	0.5	0.692322337
g_3	64.8	1.578499851	64.8	1.578499851	64.8	0.785303725
g_4	37	1.663950171	37	1.663950171	37	0.845514882
g_5	2	2.312166691	2	2.312166691	0.1	0.825555051
g_6	45.1	0.004074094	43	1.486163502	44.6	0.611352966
g_7	0.1	2.351514887	0.1	2.351514887	2.9	0.85072119
g_8	0.2	2.226298661	0.2	2.226298661	2.1	0.866837037
g_9	0.2	2.373430506	0.2	2.373430506	1.6	0.867753344
g_{10}	9.5	2.374781935	9.5	2.374781935	6.5	0.868539562
g_{11}	1.9	2.37421382	1.9	2.37421382	0.2	0.860540859
g_{12}	1.5	2.360407849	1.5	2.360407849	0.1	0.864744016
g_{13}	15.7	2.363380862	15.7	2.363380862	15.7	0.827862499
g_{14}	0.4	2.373807119	0.4	2.373807119	2.4	0.862851656

Table 6: Lowest values for final, maximal and mean parasite load, using parameters $g_i \cdot 0.1 \cdot n$ in the range [0,3], 18-parameter-model.

Tab. 6 and Fig. 16 show the best (i.e. lowest) values for mean, maximal and final value of parasite load, multiplying kinetic constants g_i with a factor ≥ 0.1, while maintaining them in the range [0,3]. After the change all parameters yield a lower final parasite load than the original one.
Among all the coefficients $0.1 \cdot n$ for g_i, the one yielding the lowest value for mean, maximal and final parasite load, respectively, is chosen. n does not have to be the same for mean, maximum and final value with respect to one parameter. Some variables show e.g. a decrease in final parasite load and maximum with increasing coefficients, whereas the mean parasite load increases with increasing coefficients. In this case the highest coefficient is chosen for final and maximal parasite load, whereas the smallest one is chosen for mean parasite load. For g_2, g_6, g_8, g_{11} and g_{14} smaller ranges ([0.5,3], [0,1.03], [0.2,3], [0.2,3], [0.2,3]) are used because parameter values outside these ranges yield complex solutions of the system of equations.
The smallest values of parasite load are reached controlling g_1. Out of the rest of the parameters g_6 yields the least parasite load.

Changing g_1

Among all kinetic constants g_i, g_1 is the one with the highest absolute value of sensitivity

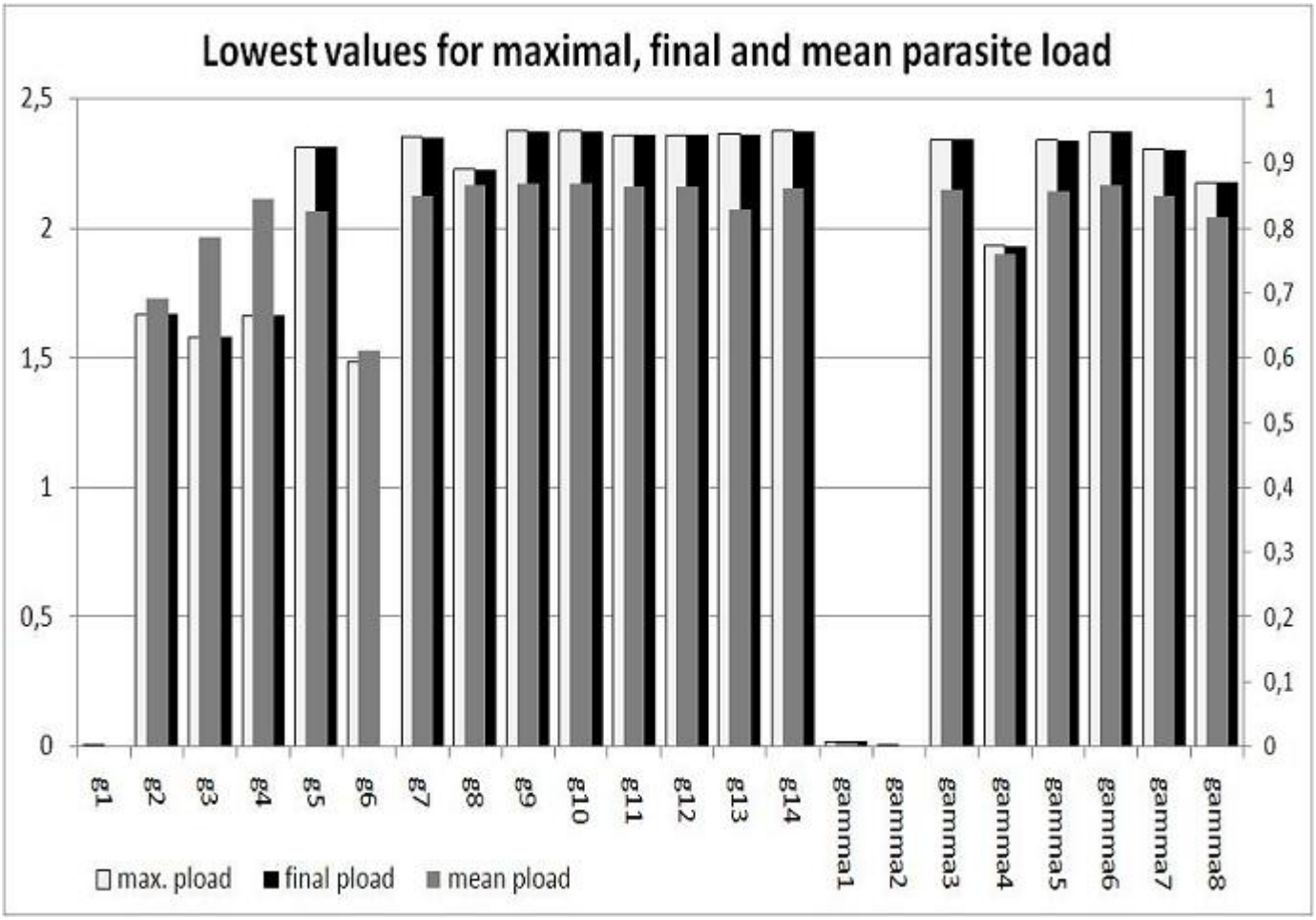

Figure 16: Lowest maximal (white, left axis), final (black, left axis) and mean (gray, right axis) parasite load for kinetic parameters g_i and rate constants γ_i after multiplication with a factor $\in [0.1, \frac{3}{g_{best}}]$ (for g_i) or a factor $\in [0.1, \frac{10}{\gamma_{best}}]$ (for γ_i), respectively, whereby g_{best} and γ_{best} denote parameter values estimated in the 18-parameter-model with 1000 iterations.

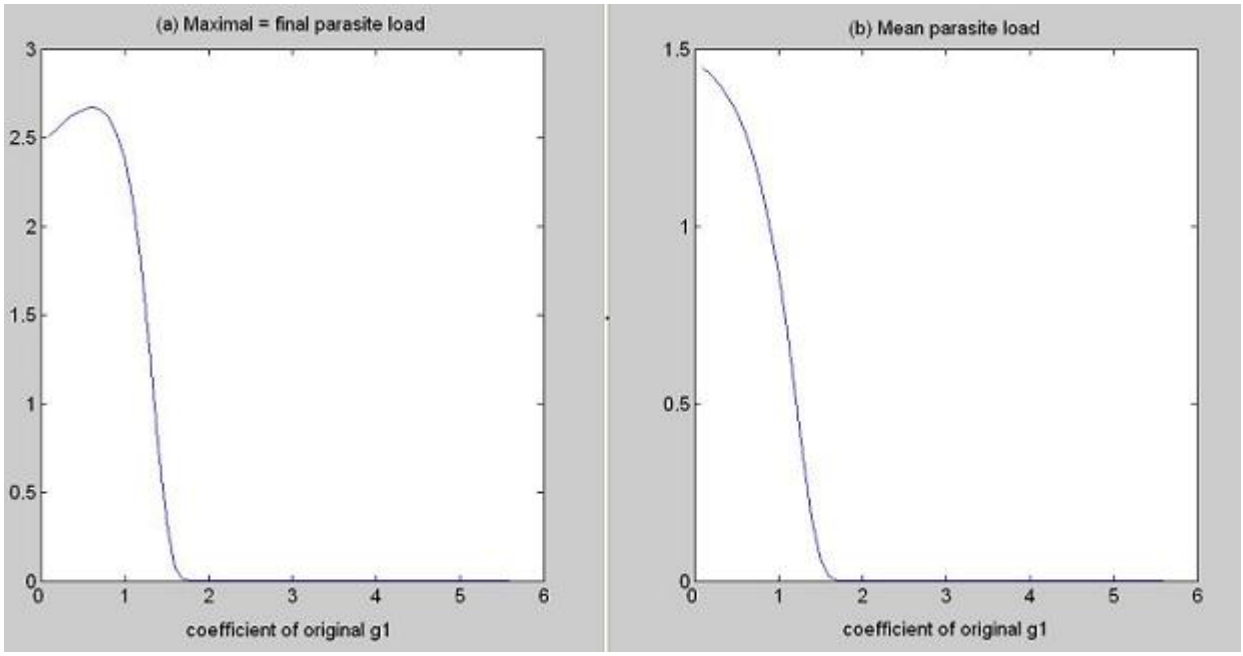

Figure 17: 18-parameter-model: left: Augmentation factor of g_1 versus maximal = final value of parasite load, right: augmentation factor of g_1 versus mean parasite load.

(see Fig. 14). Due to the model scheme (Fig. 6, 13), g_1 is the influence of parasites on their proper proliferation. Tab. 6 shows that g_1 is capable to reduce parasite load to a final value of $4.48 \cdot 10^{-7}$, a maximal value of 10^{-6} and a mean value of $6.87 \cdot 10^{-7}$ in a period of 24 weeks after infection. This result is obtained by multiplication of g_1 with 5.6, thus if its value is elevated to a value of 2.9871. (Its original value from parameter estimation is 0.5334.)

Fig. 17 shows a plot of the coefficient of g_1 (0.1 until 5.6) versus mean, maximal and final parasite load. Sensitivity $S(g_1, x_1)$ of g_1 with respect to parasite load is negative (Fig. 14), so an increase in g_1 should lead to a decrease in parasite load. For the mean parasite load (considering a time period of 24 weeks) this is true. In sensitivity analysis we basically investigate the sum of all variable values, which is equivalent to considering the mean. Therefore negative sensitivity corresponds to a decrease in mean with increasing coefficients. In contrast, maximum and final value of parasite load are monotonic only in a certain range for g_1, namely [0.7, 3]. For $g_1 \in [0, 0.6]$ the maximum and final value of parasite load are increasing for increasing g_1, whereas mean parasite load is decreasing. This means given that $g_1 \in [0, 0.6]$ parasite load reaches a higher maximum, but on average values are lower.

As stated above, minimal values for final parasite load are reached at a g_1 of 2.9871. Fig. 18 shows the progression of parasite load during a time period of 24 weeks for this parameter value. Decrease in parasite load corresponds to healing which means feasibility of g_1 as therapeutic target. Parasite load decreases nearly linearly with respect to g_1.

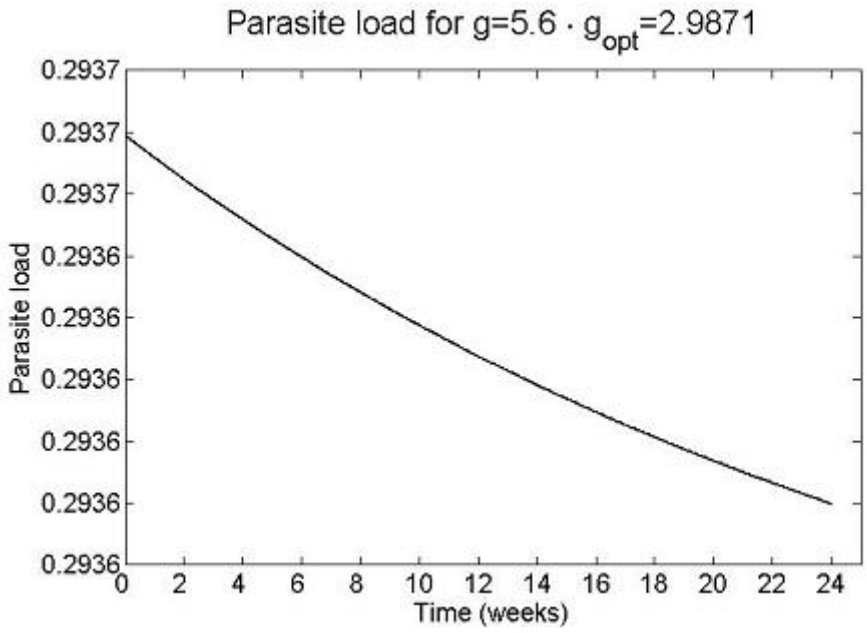

Figure 18: Parasite load over time for $g_1 = 2.9871$ (minimal final parasite load) and remaining parameters like in Tab. 5.

Changing g_6

g_6 stands for the influence of lymphocytes on their proper production. It seems surprising that g_6 yields good results in optimization despite its sensitivity with respect to parasite load of 0.0004. However, g_6 has a small original value (resulting from the 18-parameter-model with 1000 iterations) of 0.0227. Therefore it can be multiplied by factors of up to 131.9, remaining in the range of [0,3]. This high variability of the parameter is probably the reason for its high potential to reduce parasite load. Using a multiplication factor of 45.1, thus a g_6 of 1.0254, yields a final parasite load of 0.0041. The lowest maximal parasite load (1.4862) is reached using $g_6 = 0.9776$ (43 times the original g_6); the lowest mean parasite load is yielded by $g_6 = 1.0140$ which corresponds to multiplication of the original g_6 with 44.6.

Fig. 19 shows a plot of the augmentation coefficient of g_6 versus parasite load. Whereas the final parasite load is monotonically decreasing with increasing g_6, maximal parasite load is decreasing only until a g_6 multiplication factor of 43, whereas greater factors yield a higher final parasite load. The mean parasite load is not monotonic either with respect to g_6.

Fig. 20 shows progression of parasite load over time using values for g_6 which minimize final, maximal and mean parasite load, respectively. In all three cases, parasite load augments at the beginning, reaches its maximum after 18 to 20 weeks, and decreases afterwards. Parasite load that is decreasing towards the end corresponds to healing. The g_6 optimizing mean parasite load yields a final parasite load of 1 (which because of normalization corresponds to the mean) and therefore is not considered feasible for a treatment period of 24 weeks. However, it may yield lower parasite load over a larger time period.
The small sensitivity of g_6 results from the fact that the gradient of coefficient versus mean parasite load is nearly zero at x=1. This shows the different significance of sensi-

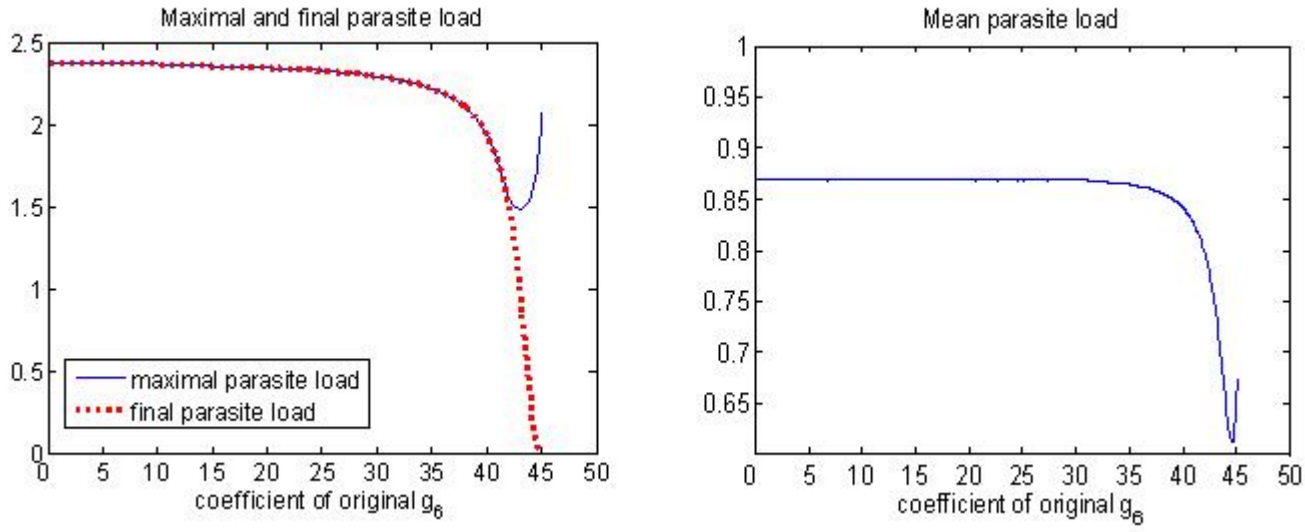

Figure 19: 18-parameter-model: left: Augmentation factor of g_6 versus maximal respectively final value of parasite load, right: augmentation factor of g_6 versus mean value of parasite load.

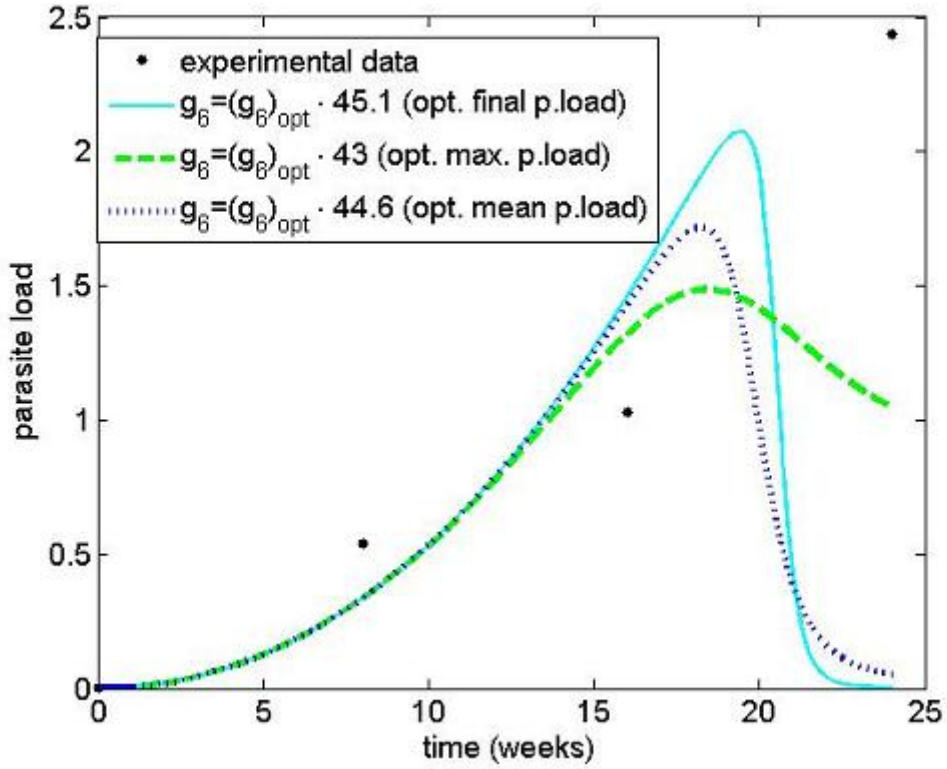

Figure 20: Parasite load over time for $g_6 = 0.0254$ (minimizing final parasite load, factor=45.1, blue), $g_6 = 0.9776$ (minimizing maximal parasite load, factor=43, green) and $g_6 = 1.0140$ (minimizing mean parasite load, factor=44.6, magenta), respectively, as well as real data (points). Remaining parameters like in Tab. 5.

parasite load	$0.1 \cdot n$	final	maximal	mean	end gradient
γ_1	0.1	0.019493325	0.019493325	0.006844242	1.73E-05
γ_2	100	0.00170651	0.00170651	0.001579836	-1.36E-07
γ_3	1.2	2.34206245	2.34206245	0.859497942	1.84E-03
γ_4	0.1	1.93189199	1.93189199	0.760880972	0.001276251
γ_5	0.1	2.338070643	2.338070643	0.858133141	0.001835432
γ_6	1.2	2.372377656	2.372377656	0.867377879	0.001885131
γ_7	2.2	2.303201305	2.303201305	0.849222174	0.001792951
γ_8	0.1	2.175910702	2.175910702	0.815925481	0.001619941

Table 7: Lowest values for final, maximal and mean parasite load and difference between the last two estimations for parasite load ("end gradient"), using parameters $\gamma_i \cdot 0.1 \cdot n$ in the range [0,10], 18-parameter-model.

tivity analysis compared to optimization: Since the range is much wider in optimization, a parameter can have a high influence if augmented sufficiently, despite a sensitivity of nearly zero.
$S(g_6, x_1)$ has an error of 0.0004 which is 100% of the parameter value and due to the small derivative at *factor=1.*

Optimization yields two extreme cases:

- Augmentation of g_1 decreases parasite load constantly right from the beginning (see Fig. 18). This behavior is similar to the body's innate immune response which is non-specific and fast: Lymphocytes check macrophages randomly in order to identify parasites inside them.
- On the contrary, augmentation of g_6 could refer to the specific immune response which is slower but more effective. In this process, lymphocytes identify specific pathogens with the help of antibodies. There is a time lag between the start of immune response and the identification of parasites, but once identified, the parasite is eliminated rapidly. Thus parasite load is augmenting in the beginning until it reaches a certain point where it is suddenly decreased (see Fig. 20).

Rate Constants

Concerning rate constants γ_i, γ_1 is considered sensitive to parasite load. This section investigates changes in all rate constants, one at a time, varying them within the range [0,10] by multiplying them with factors $0.1 \cdot n$, whereby $n \in \mathbb{N}$ and $n \leq \frac{10}{\gamma_{best}}$.
Tab. 7 and Fig. 16 show final, maximal and mean parasite load for the respective n where their minimum is reached. After change, all parameters yield a lower final parasite load than the original one.
The variable yielding the minimum for final, maximal and mean parasite load is γ_2 i.e. the rate constant for parasite degradation.
Due to the model it would be expected that γ_1 and γ_2 are the most feasible variables for

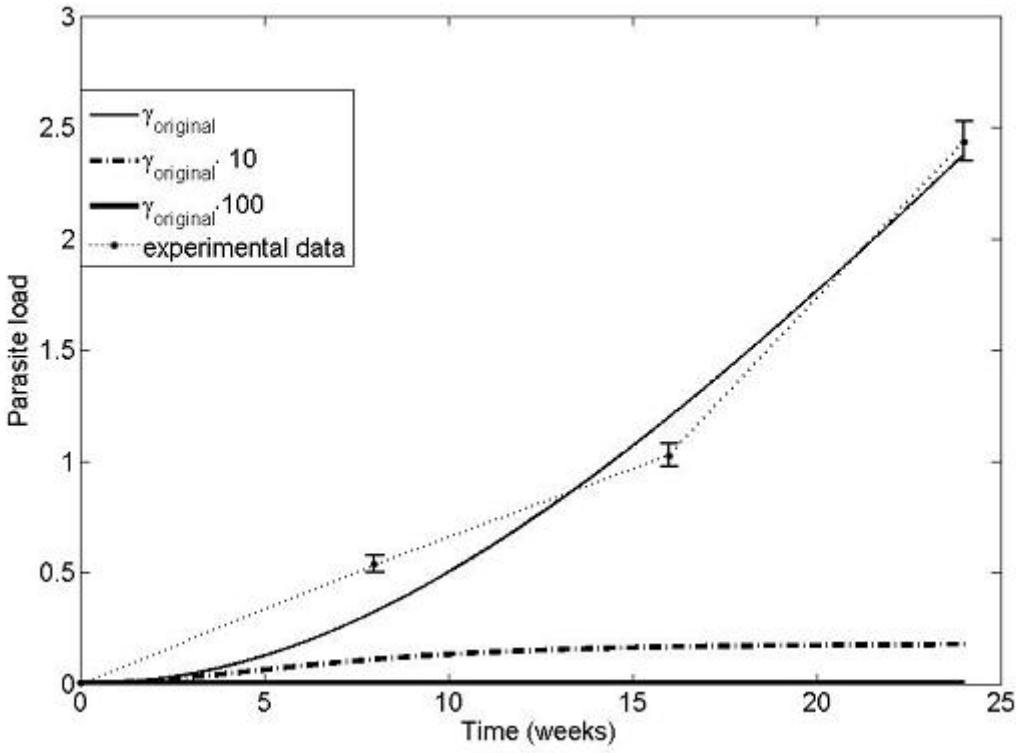

Figure 21: 18-parameter-model: Parasite load over time for original γ_2 (continuous line), 10 times augmented γ_2 (alternating dots and dashes) and 100 times augmented γ_2 (thick line, almost zero) and experimental data (dots) with interpolation (dotted line). **I** represents the standard deviation of experimental data within the group of mice.

optimizing parasite load, since they represent generation and degradation of parasites, respectively.
Our analysis shows that γ_2 is the most feasible parameter to decrease and clear parasite load, which implies that influencing γ_2 is more feasible than influencing γ_1. Mathematically, this is probably due to the fact that γ_2 can be multiplied by a wide range of factors while still remaining in the range [0,10]. Sensitivity analysis yielded γ_1 as the most influential parameter, but sensitivity analysis considers changes around a coefficient of one whereas in optimization coefficients of up to 100 were used for γ_2.

Fig. 21 shows a plot of γ_2, augmented by a coefficient of 1, 10 and 100, respectively, with respect to the original value. Fig. 22 shows that diminished γ_1 yields a far lower parasite load than originally obtained, which is logical since γ_1 represents parasite influx. However, also with diminished γ_1 parasite load is increasing over the whole time interval, but its final value is 0.0195 instead of 2.4359 which was originally obtained.

Summing up, g_1, g_6, γ_1 and γ_2 are considered the most feasible anti-Leishmania therapeutic targets. Other parameters that diminish final parasite load significanltly (to a value < 2) are g_2, g_3, g_4 and γ_4.

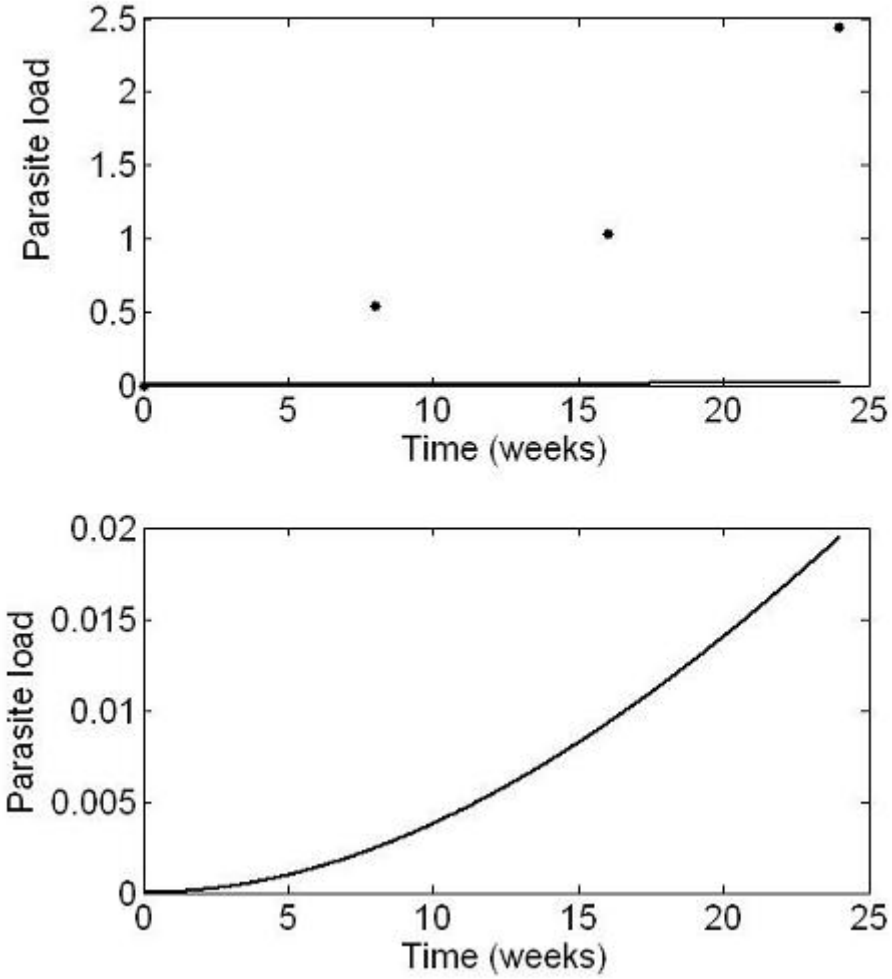

Figure 22: 18-parameter-model. Upper: Parasite load over time for γ_1 multiplied by a factor of 0.1 with respect to the original value (line) and experimental data (points). Lower: Parasite load over time for γ_1 multiplied by a factor of 0.1 with respect to the original value.

5.3 22-Parameter Model

In addition to the 18-parameter-model selected in Section 5.1 and analyzed in Sections 5.2.1 to 5.2.3, the 22-parameter-model with parameter ranges (11) and (12) is analyzed. This model represents the most general case, using all parameters without assuming a-priori-knowledge.

parameter	value	parameter	value
γ_1	0.127106222	g_4	0.715699797
γ_2	0.003132907	g_5	0.891210814
γ_3	8.422517763	g_6	0.032316599
γ_4	7.08422542	g_7	0.060918958
γ_5	4.954271405	g_8	1.717637746
γ_6	6.657315678	g_9	2.759989786
γ_7	7.323158228	g_{10}	0.133600746
γ_8	9.677579918	g_{11}	1.480741078
g_1	0.456662477	g_{12}	2.876439055
g_2	0.307580207	g_{13}	0.150938502
g_3	0.354307548	g_{14}	1.836502565

Table 8: Parameters of the 22-parameter-model, 1500 iterations, parameter ranges (11) and (12).

Parameter estimation is executed using the mentioned parameter ranges and 1500 iterations. After a computation time of 105.74 hours, the minimal value of the objective function is 0.0444. As expected, this value is lower than that obtained from the 18-parameter-model with 1000 iterations (0.0517).

Tab. 8 shows the parameters obtained from 1500 iterations of the genetic algorithm. Fig. 23 shows the model scheme whereby influences are marked with different colors corresponding to the size of respective parameters. Plots of data and model prediction of the four model variables are given in Fig. 24. Fig. 25 represents adjust for data from the 10^6 parasites group to the model with parameters obtained from data of the 10^3 parasites group.

Model quality is checked applying the model using the parameters obtained from fitting to experimental data of the 10^3 parasites group, on the initial conditions of the 10^6 parasites group. The resulting value of the objective function is 0.2167 (in contrast to 0.0444 for the 10^3 parasites group, 22-parameter-model). Thus the 18-parameter-model yields an even better fit for experimental data of the 10^6 parasites group ($f_{obj} = 0.1973$).

5.3.1 Significant Parameters

As in the 18-parameter-model, we now study parameter values in detail in order to find out which influences are important in our model.

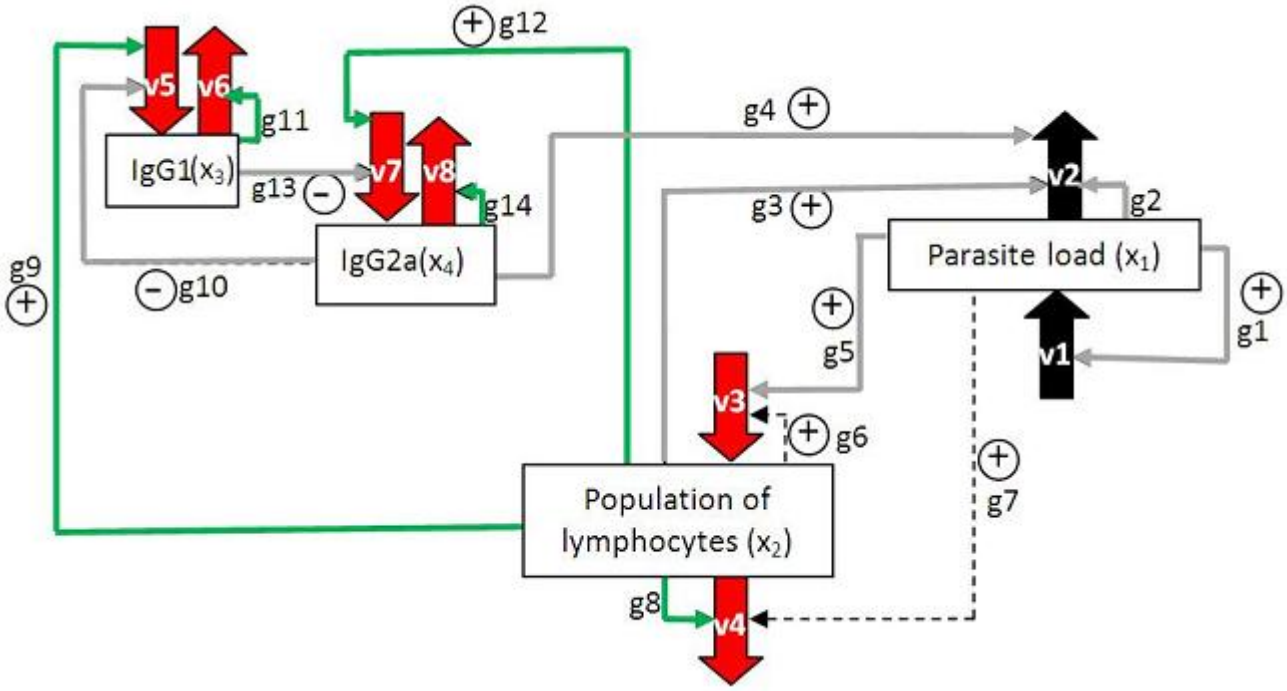

Figure 23: Size of parameters in the 22-parameter-model. Dark green: $g > 1$ ($g_8, g_9, g_{11}, g_{12}, g_{14}$), gray: $0.1 < g < 1$ ($g_1, g_2, g_3, g_4, g_5, g_{10}, g_{13}$); dashed: $g < 0.1$, red: $\gamma > 4$ ($\gamma_3, ..., \gamma_8$), black: $\gamma < 1$ (γ_1, γ_2).

Concerning kinetic parameters the 22-parameter-model yields the following:
All parameters g_i directly related to parasite load are smaller than 1 which means that they have a minor influence on disease progression. This is an interesting result because we assumed parasite load to be the crucial variable for disease progression. The model yields that the latter depends to a greater extent on the immune response than on the number of parasites injected. On the contrary, the 18-parameter-model (see Fig. 13) considered influence of parasite load on both proliferation (g_5) and degradation (g_7) of lymphocytes as significant. Since g_5 and g_7 are opponents, this could be a defect of the model yielding two different solutions with similar fit. In this case it would be interesting to investigate the model with $g_7 = 0$ in order to see if both models yield a similar value for g_5. However, both the 18- and the 22-parameter-model assume that g_5 has a higher influence than g_7 which corresponds to the fact that a higher parasite load leads to an elevated number of lymphocytes (see Fig. 7). This fact is stressed by the small value of $g_7 = 0.0609$ in the model with 22 parameters.
In both models, influence of lymphocytes on their own production (g_6) yields a value smaller than 0.1 (0.0227 and 0.0323 in the 18- and 22-parameter-model, respectively). Lymphocyte number is therefore rather controlled by parasite load than by itself.
Parameters g_9 and g_{12} which represent the influence of lymphocyte number on generation of the antibodies IgG1 and IgG2a have a value > 1 in both models, signifying that these influences are important for disease progression.
All parameters except for parasite load have a significant influence on their proper degradation. Influence of parasites on their own degradation is minor - degradation of parasites is also influenced by the population of lymphocytes and IgG2a.
Inhibition of IgG1 by IgG2a (g_{10}) and vice versa (g_{13}) is minor than stimulation of an-

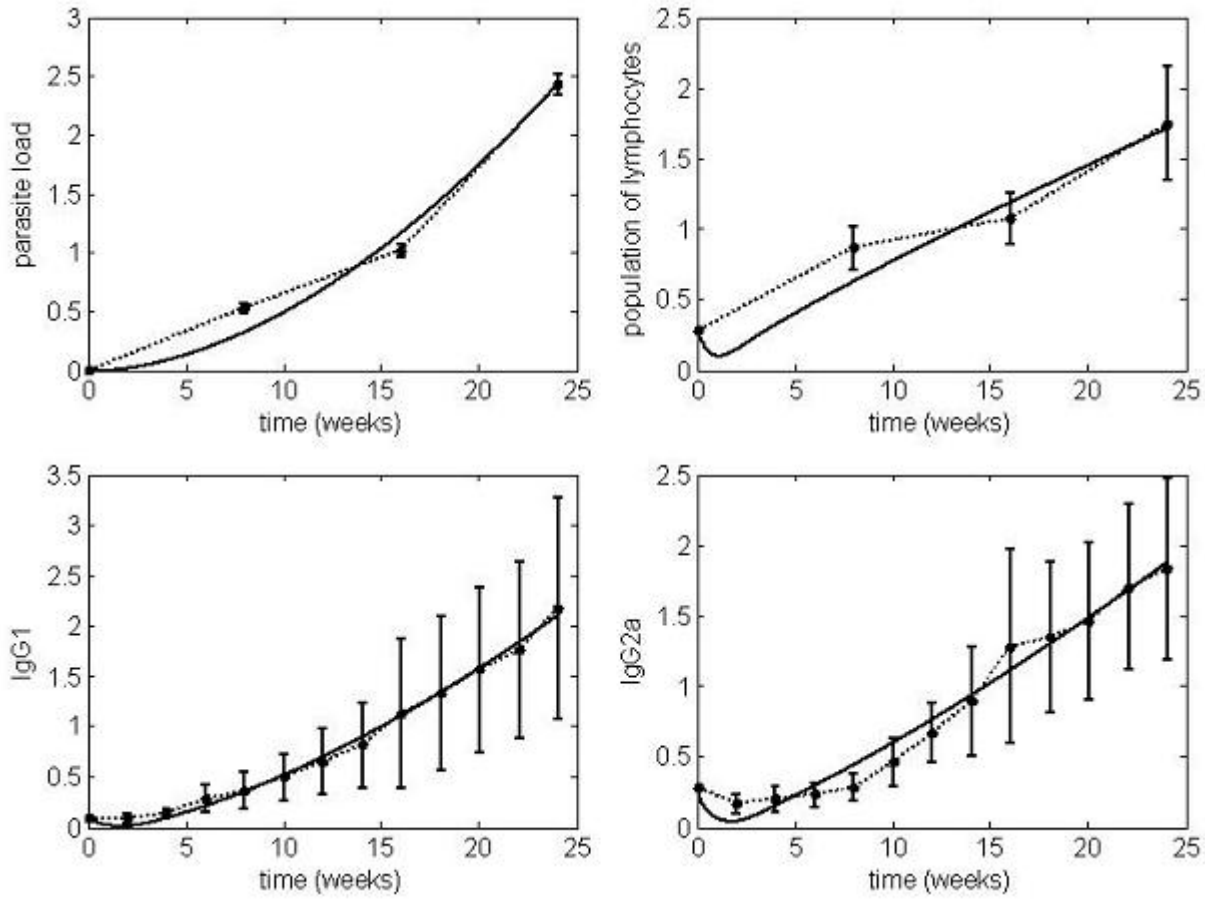

Figure 24: Experimental data of the 10^3 parasites group (points) and model approximation (line) in 22-parameter-model, parameter ranges (11) and (12), 1500 iterations. **I** represents the standard deviation of experimental data.

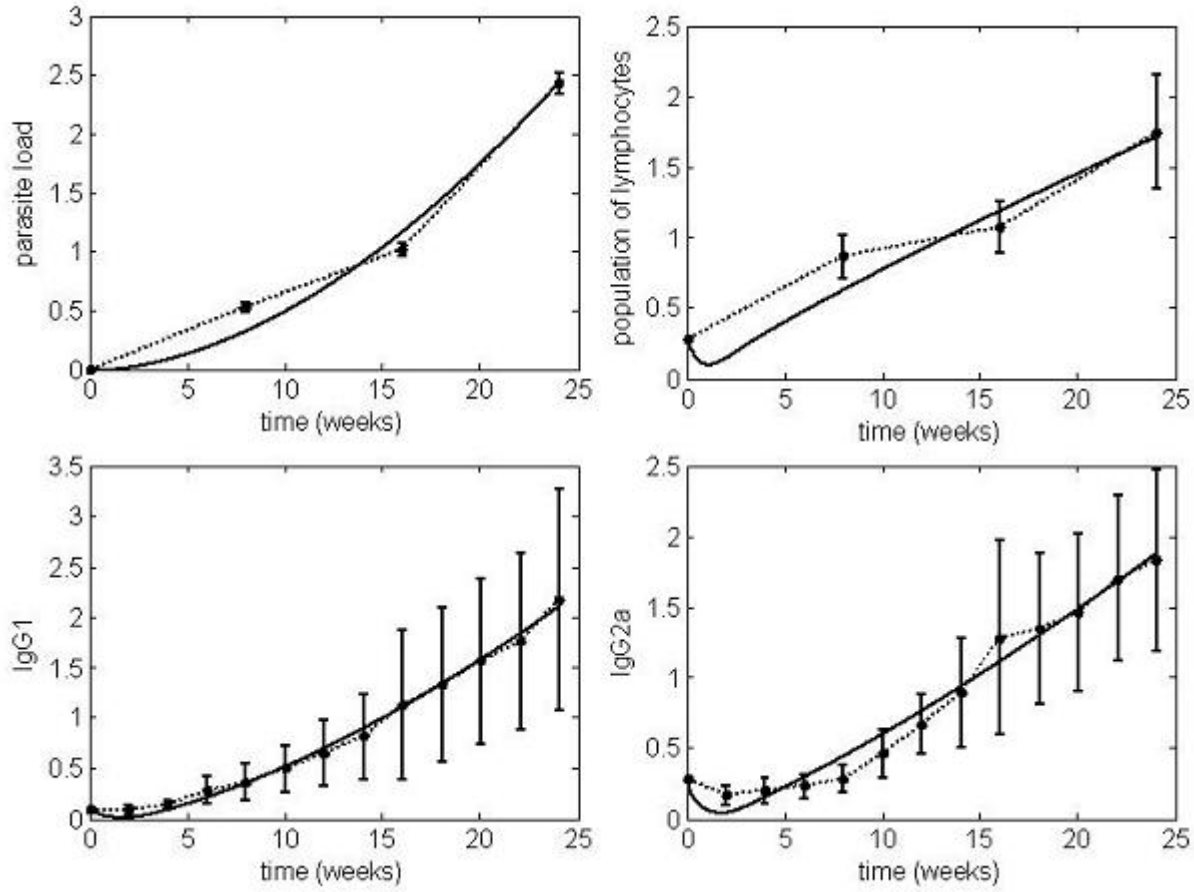

Figure 25: Experimental data of the 10^6 parasites group (points) and model approximation (line) in 22-parameter-model, parameters estimated using data of the 10^3 parasites group, parameter ranges (11) and (12), 1500 iterations. **I** represents the standard deviation of experimental data.

	$x_1(18)$	$x_1(22)$	$x_2(18)$	$x_2(22)$	$x_3(18)$	$x_3(22)$	$x_4(18)$	$x_4(22)$
γ_1	1.8358	1.5952	0.9030	0.8196	1.4794	1.2932	1.2475	1.0634
γ_2	-0.2958	-0.0255	-0.1284	-0.0118	-0.2285	-0.0201	-0.1894	-0.0161
γ_3	-0.0507	-0.0213	0.8822	0.5256	1.4619	0.8288	1.2300	0.682
γ_4	0.0491	0.0195	-0.8674	-0.5224	-1.4454	-0.8233	-1.2149	-0.6767
γ_5	0.0077	0.0009	0.0063	0.0004	0.8579	0.5583	-0.1463	-0.0456
γ_6	-0.0069	-0.0011	-0.0053	-0.0005	-0.8471	-0.5492	0.1452	0.0446
γ_7	-0.0268	-0.011	-0.0138	-0.0048	-0.0612	-0.0492	0.7739	0.4441
γ_8	0.0017	0.0129	-0.0034	0.0054	0.0404	0.0487	-0.7796	-0.441
g_1	-1.4777	-1.1333	-0.8704	-0.699	-1.2613	-0.9636	-1.0911	-0.8327
g_2	0.1186	0.0058	0.0729	0.0037	0.1043	0.005	0.0907	0.0043
g_3	0.0023	0.0019	0.0013	0.0012	0.0019	0.0016	0.0016	0.0014
g_4	0.0087	0.007	0.0051	0.0043	0.0076	0.0061	0.0065	0.0052
g_5	0.0254	0.0146	0.8356	-0.3254	1.2157	0.4402	1.0341	0.3793
g_6	0.0004	0.0001	0.0068	0.0064	0.0110	0.0093	0.0093	0.0078
g_7	-0.0171	-0.0008	-0.5443	0.0231	-0.8212	-0.03	-0.6975	-0.0259
g_8	0.0172	0.0054	-0.3006	-0.3385	-0.4854	-0.4868	-0.4072	-0.408
g_9	-0.0351	0.027	-0.0108	0.0111	0.5232	0.5393	-0.1185	0.0158
g_{10}	0.0001	0.0001	0.0000	0	-0.0170	-0.031	0.0031	0.0028
g_{11}	0.0441	0.0059	0.0224	0.0027	-0.3988	-0.4011	0.1020	0.0714
g_{12}	0.0089	0.0268	0.0062	0.0125	-0.0248	0.0104	0.5440	0.4571
g_{13}	-0.0016	-0.0006	-0.0063	-0.0004	-0.0066	0.0032	-0.0792	-0.036
g_{14}	-0.0173	0.0032	-0.0134	-0.0041	-0.0356	0.0308	-0.3579	-0.3619

Table 9: Sensitivities with respect to indicated model variable in the 18- and 22-parameter-model.

tibody production by lymphocytes (g_9, g_{12}) in both models by a factor of more than 9. This means that expression levels of the two antibodies depend to a much larger extent on the number of lymphocytes than on each other.

5.3.2 Sensitivity Analysis

As for the 18-parameter-model, sensitivity of all 22 parameters will be studied using the algorithm introduced in Section 4.9. Results for sensitivity analysis are compared to those obtained from the 18-parameter-model.

Tab. 9 provides sensitivity values for all parameters with respect to all variables in the 18- and 22-parameter-model. Fig. 26 graphically represents sensitivities in the 22-parameter-model.

Parameter sensitivities are similar to those in the 18-parameter-model. Maximum, minimum, mean and median of the absolute difference in sensitivities between the two models are shown in Tab. 10. For parasite load, the mean deviation between the two models is only 0.0567 and the median even 0.0161. This shows high robustness of the model: If a parameter is changed infinitesimally, model behavior changes slightly (small absolute

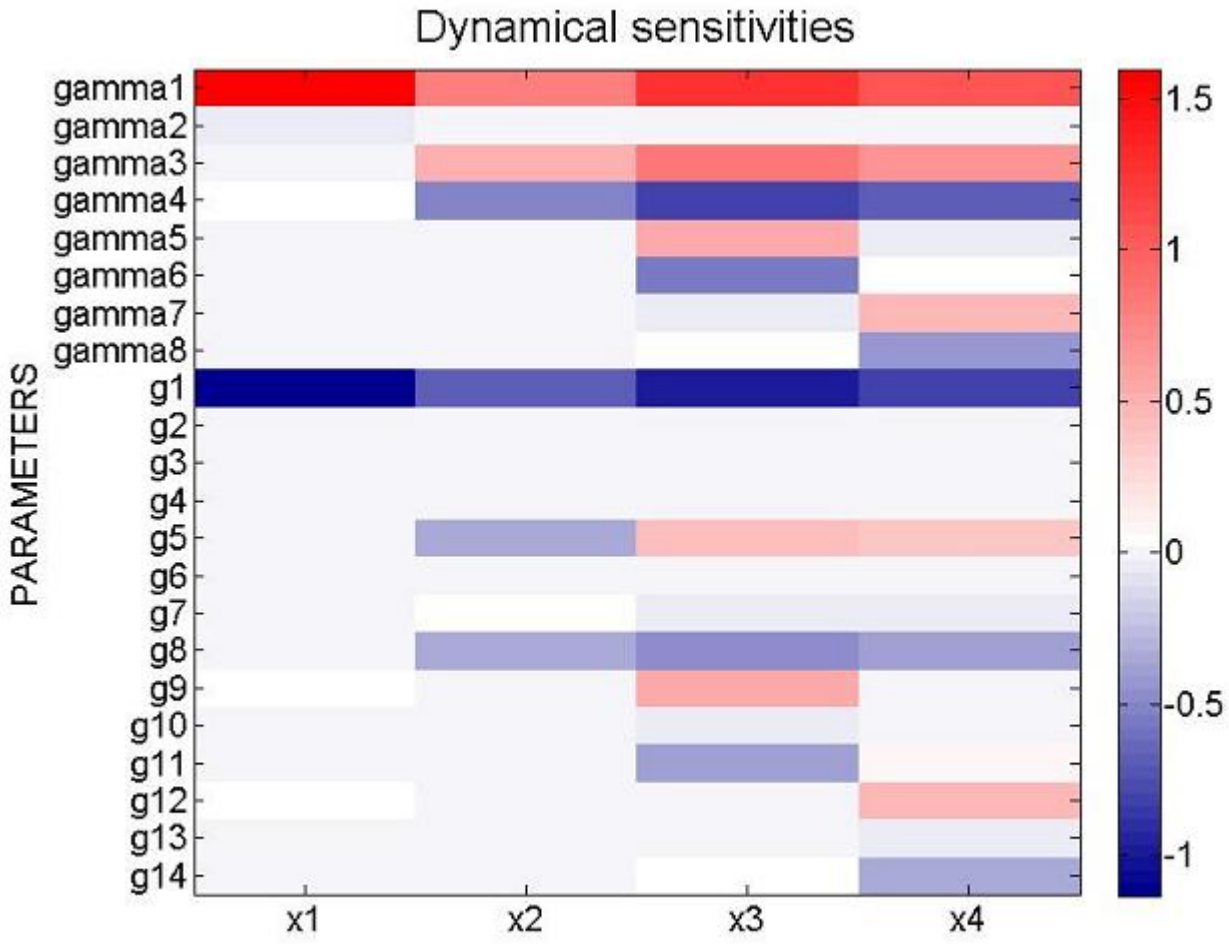

Figure 26: Parameter sensitivities in the 22-parameter-model.

sensitivities	parasite load	lymphocytes	IgG1	IgG2a
min.	0	0	0.0003	0.0002
max.	0.3444	1.161	0.7912	0.6716
mean	0.0567	0.1364	0.1991	0.1949
median	0.0161	0.0145	0.0508	0.1007

Table 10: Minimum, maximum, mean and median of absolute difference in sensitivities between the 18- and 22-parameter-model.

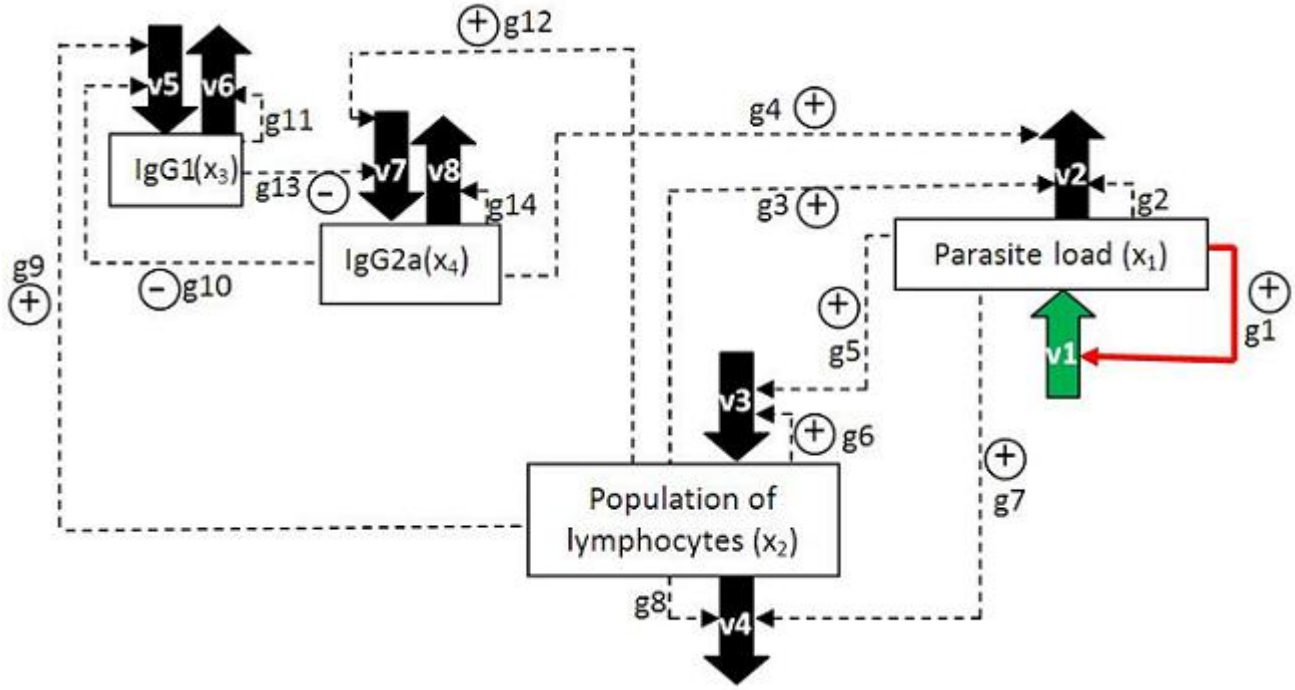

Figure 27: Parameter sensitivities with respect to parasite load in the 22-parameter-model: Dark green: $S(\gamma_i, x_1) > 1$ (γ_1); red: $S(g_i, x_1) < -1$ (g_1); dashed, black: $|S(., x_1)| < 1$.

sensitivity), whereby this change in model behavior is fairly independent of parameter reduction in the model (similar sensitivities in 18- and 22-parameter-model).
On average absolute values of sensitivities are smaller than in the 18-parameter-model. In the latter, the median of absolute values of sensitivities is 0.0671 in contrast to 0.0198 in the 22-parameter-model. Also the mean of 0.2204 is lower in the 22-parameter-model than in the 18-parameter-model (0.3541). This shows a higher robustness of the model with 22 parameters.

Fig. 27 shows the model scheme indicating sensitivity values with respect to parasite load with different colors.

Like in the 18-parameter-model, considering sensitivities with absolute value greater than 1 as significant, γ_1 and g_1 are sensitive with respect to parasite load. In fact, these are even the only parameters yielding an absolute sensitivity value greater than 0.1. None of the parameters is sensitive (sensitivity > 1) with respect to the population of lymphocytes and γ_1 is also sensitive with respect to IgG1 and IgG2a. In contrast to the 18-parameter-model, only this parameter is sensitive to the immunoglobulins, whereas g_1, g_5, γ_3 and γ_4 show sensitivities with absolute value smaller than 1. This underlines the great influence on parasite load on all variables.
Many of the conclusions and interpretations drawn in Section 5.2.2 also hold for the 22-parameter-model: γ_1 is sensitive with respect to parasite load, IgG1 and IgG2a, g_1 is sensitive to parasite load.

parasite load	$0.1 \cdot n_{final}$	final	$0.1 \cdot n_{max}$	maximal	$0.1 \cdot n_{mean}$	mean
g_1	1.5	0.823220094	1.5	0.823220094	1.5	0.196931962
g_2	9.7	2.260472244	9.7	2.260472244	0.3	0.855886647
g_3	8.4	2.35733146	8.4	2.35733146	8.4	0.863228068
g_4	4.1	2.375840171	4.1	2.375840171	0.1	0.854309497
g_5	3.3	2.369765491	3.3	2.369765491	0.1	0.841196092
g_6	52.8	0.050877436	48.5	1.349347111	50.6	0.68461982
g_7	49.2	2.14967675	49.2	2.14967675	49.2	0.723710725
g_8	0.3	2.325644574	0.3	2.325644574	0.3	0.861389146
g_9	0.1	2.435977772	0.1	2.435977772	0.7	0.866898536
g_{10}	3.3	2.436420086	3.3	2.436420086	0.1	0.866687313
g_{11}	0.2	2.435568104	0.2	2.435568104	0.2	0.864381325
g_{12}	0.1	2.429892129	0.1	2.429892129	0.1	0.856256502
g_{13}	19.8	2.207853739	19.8	2.207853739	19.8	0.742245947
g_{14}	0.2	2.360144596	0.2	2.360144596	1.6	0.864152135

Table 11: Lowest values for final, maximal and mean parasite load, using parameters $g_i \cdot 0.1 \cdot n$ in the range [0,3] for the 22-parameter-model.

5.3.3 Optimization

Like in Section 5.2.3, kinetic parameters are now varied in the range [0,3] in order to minimize parasite load. One parameter is changed at a time i.e. multiplied with a coefficient between 0.1 and $\frac{3}{g_i}$, whereby g_i is the model parameter received from the parameter estimation algorithm. Effects of parameter changes on final, maximal and mean parasite load are investigated.
Sensitivity analysis (Section 5.3.2) yielded g_1 and γ_1 as the most sensitive parameters. These are expected to have the highest influence on parasite load; however, since sensitivity analysis investigates infinitesimal changes whereas optimization changes the parameters on larger scales, this does not have to be the case.

Kinetic orders

For minimization of parasite load we use the optimization algorithm introduced in Section 4.10. It yields the following results:

Tab. 11 and Fig. 28 show the best (=minimal) values of maximal, final and mean parasite load. Among all the coefficients $0.1 \cdot n$ for g_i, the one yielding the lowest value for mean, maximal and final parasite load, respectively, is chosen. n does not have to be the same for mean, maximum and final value with respect to one parameter. For g_1, g_6, g_8, g_{11} and g_{14} smaller ranges ([0,0.6850], [0,1.7095], [0.5153,3], [0.2961,3], [0.3673,3]) are used because parameters outside these ranges yield complex solutions.
All parameters except g_9 (positive influence of the population of lymphocytes on IgG1) and g_{10} (negative influence of IgG2a on IgG1) yield a lower final parasite load than originally obtained (2.435938257).

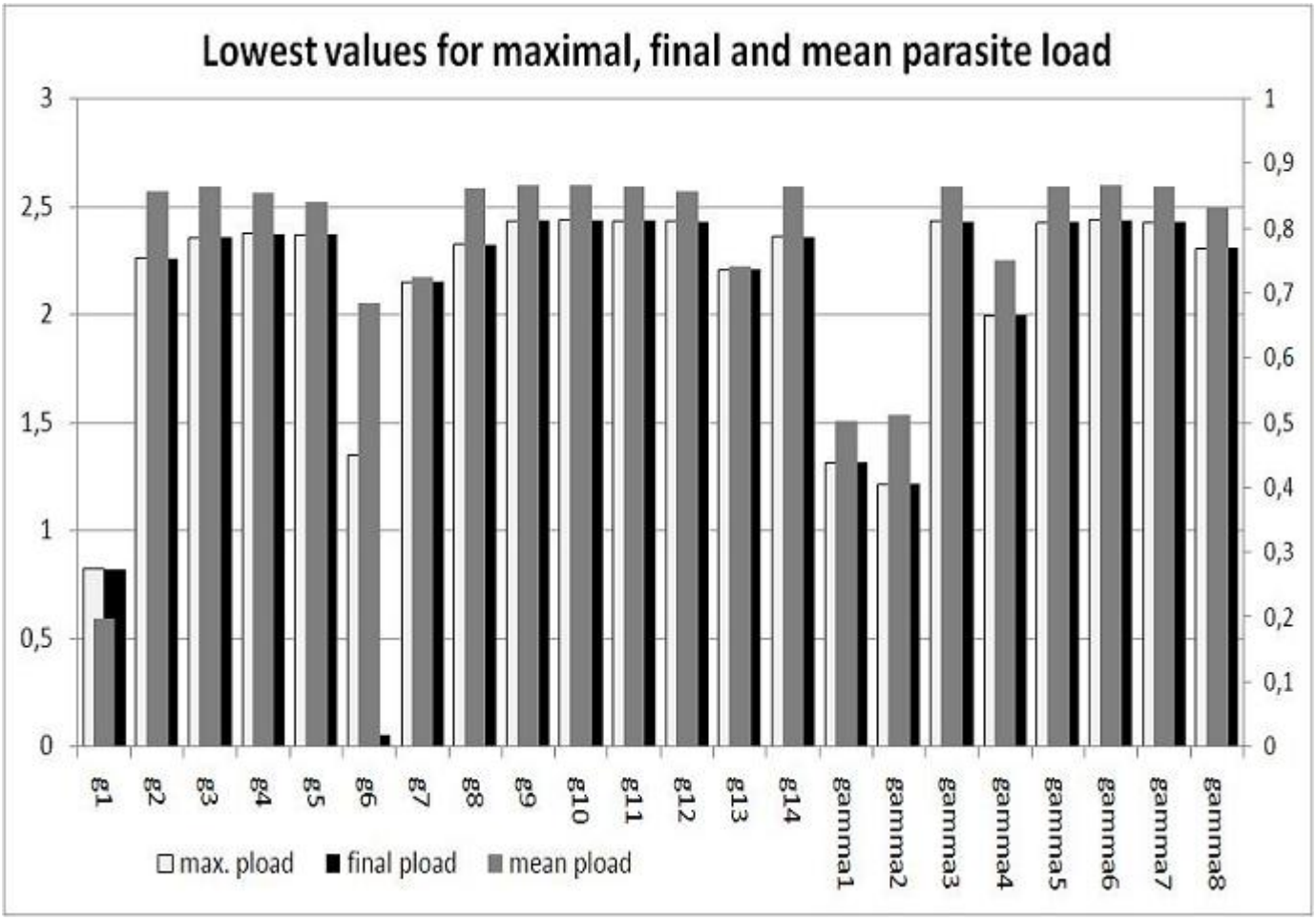

Figure 28: Lowest maximal (white, left axis), final (black, left axis) and mean (gray, right axis) parasite load for kinetic parameters g_i after multiplication with a factor $\in [0.1, \frac{3}{g_{best}}]$, whereby g_{best} denotes the value of g_i estimated in the 22-parameter-model with 1500 iterations.

parameter	factor	gradient
g_1	1.5	0.001302191
g_2	9.7	0.001440797
g_3	8.4	0.001897879
g_4	4.1	0.001907839
g_5	3.3	0.001875946
g_6	52.9	-0,004974328
g_7	49.2	0.002149254
g_8	0.3	0.001761745
g_9	0.1	0.002191625
g_{10}	22.4	0.002189017
g_{11}	1.8	0.002194415
g_{12}	1	0.002196997
g_{13}	19.8	0.002152858
g_{14}	0.2	0.001820466

Table 12: Minimal gradient of parasite load at 24 weeks (derived from the last two data points) in the 22-parameter-model and respective change factors $0.1 \cdot n$ ("minimal" refers to the minimum within iterations of genetic algorithm).

Out of all kinetic constants g_i, g_6 yields the smallest final parasite load (0.0509) and g_1 yields the lowest maximal (0.8232) and mean parasite load (0.1969). g_6 yields a maximal parasite load of 1.3493; variation in all the other kinetic constants does not yield values smaller than 2.14 for both final and maximal parasite load. The lowest values for mean parasite load vary between 0.1969 (g_1) and 0.8669 (g_9), whereby g_1, g_6, g_7 and g_{13} yield values of less than 0.8.
It has to be taken into account that the aim of a drug is to eventually clear parasite load; therefore parasite load must diminish towards the end of the time period considered i.e. the gradient of parasite load has to be negative.

Tab. 12 shows the minimal gradient for all kinetic parameters. Since g_6 is the only one yielding negative gradients, it is the only possible drug target due to the 22-parameter-model. Here simply the difference between the two last values of parasite load is taken into account. A different strategy would be to evaluate the difference between parasite load at the end and an earlier point in time.
g_6 yields negative final parasite load gradients i.e. healing when multiplied by coefficients between 48.5 and 49.7 as well as for coefficients between 50.2 and 50.8, 51.5, 51.6, 52.9, 53, 53.1, 53.2, 53.3, 53.5, 53.6. These discrete values suggest that the final gradient of parasite load has alternating signs, which means that it is almost zero. Therefore dosage of a pharmaceutical would have to be very accurate to meet a point where the gradient is negative and disease is cleared. The largest stable period for parasite load is the one between 48.5 and 49.7. However, it is assumed that in practice dosage cannot be that accurate; nevertheless an augmentation of the lymphocytes' influence on their proper production by a factor of about 50 could be tried.
Within the coefficients where the parasite load's gradient is negative (which corresponds to healing), the lowest maximal parasite load of 1.3493 appears for the coefficient of 48.5

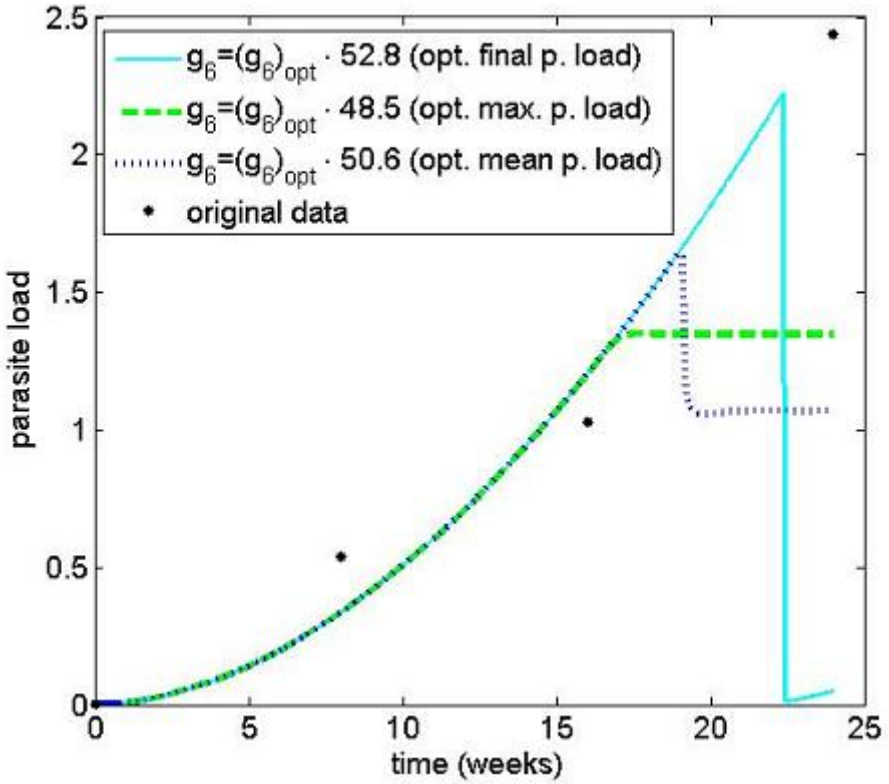

Figure 29: 22-parameter-model: Parasite load over time for original g_6 multiplied with factor=52.8 (optimal final parasite load), factor=48.5 (optimal maximal parasite load) and factor=50.6 (optimal mean parasite load).

i.e. for $g_6 = 48.5 \cdot (g_6)_{best} = 1.5674$, whereby $(g_6)_{best}$ is the value obtained from the parameter estimation algorithm in the 22-parameter-model.
The lowest final parasite load of 0.0509 is achieved for $g_6 = 52.8 \cdot (g_6)_{best} = 1.7063$. The lowest mean parasite load of 0.6846 is yielded using $g_6 = 50.6 \cdot (g_6)_{best} = 1.6352$. Fig. 29 shows plots of parasite load over time for these three values of g_6.
In case of $g_6 = 1.5674$ (optimal maximal parasite load), there is a stagnation of parasite load suggesting a point of equilibrium after 17 weeks. $g_6 = 1.6352$ (lowest mean parasite load) yields a sudden decrease in parasite load at week 19 which continues in stagnation at a parasite load of 1 (which corresponds to the mean over the whole time span) after 19 weeks. For $g_6 = 1.7063$ (minimal final parasite load) parasite number decreases aprubtly at week 22 after infection. This seems to be erroneous, however, the result was checked and there appeared to be an aprubt depression in parasite load for different values of g_6, the higher g_6, the closer the aprubt step is to disease outset (week 0). Based on this observation it is assumed that for small values of g_6 there is also such an aprubt decrease which does not appear in our supervision period of 24 weeks, but later.
g_6 seems to be the best pharmaceutical target since it is the only parameter yielding decreasing parasite load in the end.

g_6 has a positive sensitivity so it is supposed to be directly proportional to parasite load. Thus it would have to be increased in order to minimize parasite load which seems to be a contradiction to our results in optimization. However, sensitivity corresponds to a

parasite load	$0.1 \cdot n$	final	maximal	mean	end gradient
γ_1	0.1	0.034752979	0.034752979	0.012281210	0.000031502
γ_2	20	1.213633096	1.213633096	0.512262134	0.000719311
γ_3	1.1	2.430292748	2.430292748	0.865087334	0.002186224
γ_4	0.1	1.99411838	1.99411838	0.750846799	0.00158965
γ_5	0.1	2.429110468	2.429110468	0.864812805	0.002184278
γ_6	1	2.437046299	2.437046299	0.866898822	0.002195031
γ_7	1.3	2.426504041	2.426504041	0.864087386	0.00218213
γ_8	0.1	2.308340393	2.308340393	0.833362024	0.002015109

Table 13: Coefficients of original parameter ($0.1 \cdot n$), lowest values for final, maximal and mean parasite load and difference between the last two estimations for parasite load ("gradient"), using parameters $\gamma_i \cdot 0.1 \cdot n$ in the range [0,10] (factors in the range [0,20] for γ_2).

coefficient of the original g_6 of one, whereas the range for optimization is much wider.

Rate Constants

Concerning rate constants in the 22-parameter-model all except γ_6 lead to a lower final parasite load than that originally obtained (2.435938257).

γ_1, γ_2 and γ_4 yield a final parasite load of less than two (see Tab. 13). None of the rate constants reaches a negative final parasite load gradient which means that parasite load is increasing in the end in every case, so healing does not occur. Thus none of the rate constants is feasible as unique therapeutic target. However, like it can be seen in Fig. 30, all three parameters slow down disease progression whereby γ_1 is the most effective. This result was also obtained in the 18-parameter-model. Optimized γ_4 yields a lower parasite load along the whole interval of 24 weeks. Augmented γ_2 decelerates parasite progression at the beginning, but accelerates it afterwards: After 16 weeks, original parasite load and parasite load yielded by optimized γ_2 are equal. Between week 16 and week 24 augmented γ_2 yields lower parasite load than is obtained using the original value for γ_2.
Downregulation of γ_1 is considered the most feasible pharmaceutical interaction concerning rate constants because although it does not provoke decreasing parasite load, it slows down disease progression significantly. This is the same result as obtained from the 18-parameter-model (Section 5.2.3). In practice an increase of γ_2 means inhibiting parasite influx which pharmacologically speaking refers to prevention methods.

5.4 Optimization changing two parameters

In Sections 5.2.3 and 5.3.3 only one parameter was changed whereas the others maintained their values. Now we change two parameters at the same time and explore which pair of parameters yields minimal parasite load. With a combination of pharmaceutical agents acting on the two respective parameters we hope to erase infection. Whereas in one-dimensional optimization only change of g_6 to high values (about 50 times the original value) yielded a decrease in parasite load at 24 weeks after infection, we hope to find more therapeutic strategies yielding this behavior by two-parameter-optimization.

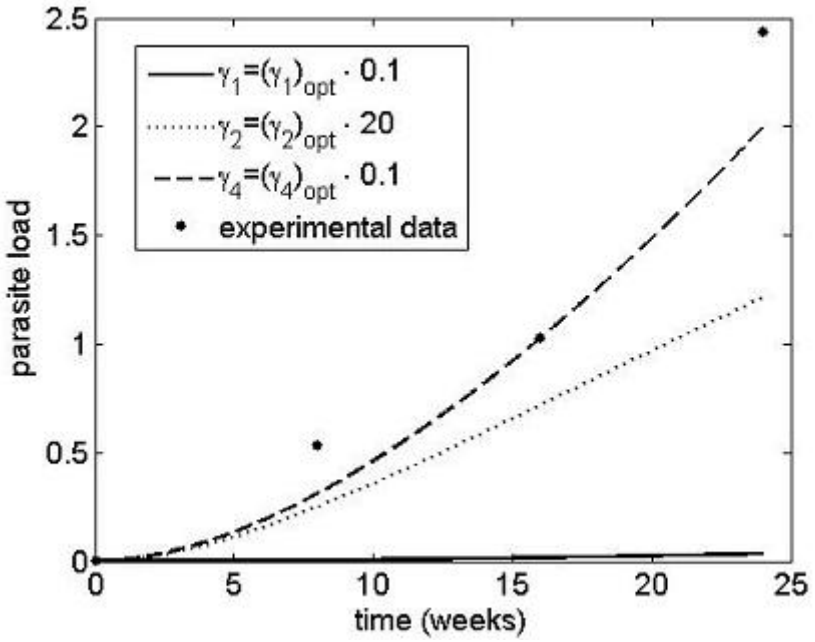

Figure 30: Parasite load over time for $\gamma_1 = 0.1 \cdot \gamma_{1,original}$ (continuous line), $\gamma_2 = 20 \cdot \gamma_{2,original}$ (dotted line) and $\gamma_4 = 0.1 \cdot \gamma_{4,original}$ (discontinuous line) and experimental data from the 10^3 parasites group (dots), 22-parameter-model.

5.4.1 Optimization Strategy

Two-dimensional optimization is performed as explained in Section 4.10.
For minimization of parasite load by change of two parameters at a time, we used the results from one-dimensional optimization (Sections 5.2.3 and 5.3.3). Parameters that yielded a final parasite load of less than 2 in at least one of the models (18-parameter-model or 22-parameter-model) are: γ_1 (both models), γ_2 (both models), γ_4 (both models), g_1 (both models), g_2 (18-parameter-model), g_3 (18-parameter-model), g_4 (18-parameter-model) and g_6 (both models). It is assumed that if minimal parasite load is yielded by up-regulation of the respective parameter in one-dimensional optimization, it is also yielded by up-regulation in the two-dimensional case. The same is assumed for down-regulation. Parameters yielding a final parasite load greater than two in one-dimensional-optimization are not considered feasible therapeutic targets.
Further it is assumed that drugs can change parameters by factors of 0.1 or 10 i.e. multiply their value with 10 or divide their value by 10. However, it is assumed that kinetic orders g_i stay in the range [0,3].

Optimization was performed changing two of the parameters mentioned above at a time for their new value (multiplication with / division by 10 within range [0,3], see Tab. 14), while maintaining the values of the rest of the parameters. There are $\binom{8}{2} = 28$ possibilities to choose a pair of parameters. If we also take into account one-dimensional optimization with these parameters we have 36 combinations in total.
Like in one-dimensional optimization, maximal, mean and final parasite load is determined. Further the end gradient of parasite load (difference between the last two estimated points) is calculated. A decrease in parasite load in the end i.e. a negative final

parameter	orig. value 18	opt. value (18)	orig. value 22	opt. value (22)
γ_1	0.168844637	0.016884464	0.127106222	1.271062222
γ_2	0.043203664	0.432036641	0.003132907	0.031329074
γ_4	6.773723979	0.677372398	7.08422542	0.708422542
g_1	0.533411348	3	0.456662477	3
g_2	1	3	0.307580207	3
g_3	0.046286528	0.462865279	0.354307548	3
g_4	0.081010111	0.810101106	0.715699797	3
g_6	0.022735632	0.227356324	0.032316599	0.323165991

Table 14: Optimization changing two parameters at a time: Original parameter values and values used for optimization

parasite load gradient, is assumed to represent healing.

5.4.2 Results for the 18-Parameter Model

Fig. 31 is a graphical representation of the results of two-dimensional optimization for the 18-parameter-model. Grey scaled squares represent final parasite load, changing the two respective parameters (row, column) in the 18-parameter-model.
Combination of g_1 with any other parameter yields the best results (considering final parasite load). Combination of γ_1 with any other parameter yields a final parasite load of less than 0.1 (i.e. 10% of the mean parasite load) in any case. Further, combined regulation of γ_2 and γ_4 yields a parasite load of less than 0.1. All the other pairs of parameters yield final parasite loads greater than 0.1. The minimal final parasite load is achieved for the pair (g_1, γ_2): $(x_1)_{final} = 2.75 \cdot 10^{-10}$. Practically this means to upregulate parasite growth rate and at the same time enhanced parasite killing.
Of further interest is the final parasite load gradient (i.e. the difference between the last two values of parasite load). A negative gradient is associated with healing. In our case, the combinations of parameters yielding a negative gradient are:

- g_1 alone or combined with $\gamma_1, \gamma_4, g_3, g_4$ or g_6
- γ_2 and γ_4, as well as
- g_4 and γ_4.

Simultaneous change in g_1 and g_2 yields a final parasite load gradient of zero.
Combining these criteria, parameter pairs yielding both a final parasite load of less than 0.1 and a negative final parasite load gradient are:

- g_1 alone or combined with $\gamma_1, \gamma_4, g_3, g_4$ or g_6 or
- γ_2 and γ_4.

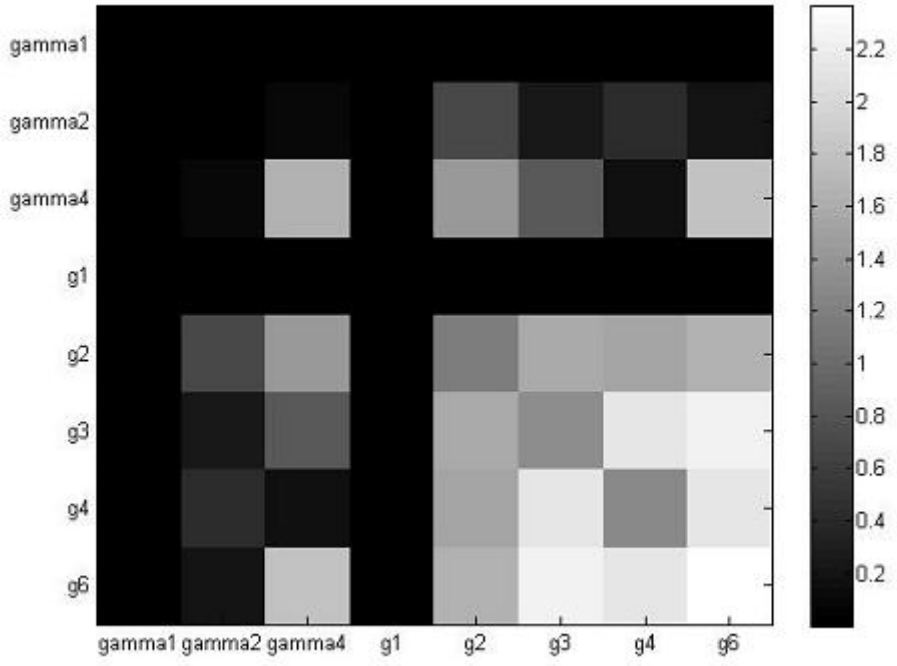

Figure 31: Optimization changing two parameters simultaneously, 18-parameter-model. The gray scale value represents final parasite load yielded by regulation of the respective pair of parameters.

These are considered the most feasible strategies when changing two parameters at a time. Practically they mean upregulating parasite growth rate, possibly combined with prevention of parasite influx as far as possible, inhibition of lymphocyte death, stimulation of the lymphocytes' or IgG2a's influence on parasite degradation or enhancement of lymphocyte reproduction rate. Change in the pair (γ_2, γ_4) corresponds to simultaneous enhancement of parasite degradation and inhibition of lymphocyte death.

5.4.3 Results for the 22-Parameter Model

Final parasite load for two-dimensional optimization in the 22-parameter-model is represented in Fig. 32. In this model a final parasite load of less than 0.1 is obtained from combination of γ_1 with any other model parameter as well as for the pairs $(g_1, g_2), (g_1, g_3)$ and (g_1, g_4). The absolute minimum of final parasite load is achieved by optimization upregulating g_1 and g_4 (final parasite load $= 8.8 \cdot 10^{-7}$.
Considering the difference between the last two values of parasite load, only the pairs (γ_1, γ_2) and (g_4, γ_4) yield a negative gradient.
Summing up, the pair (γ_1, γ_2) is considered the most feasible therapeutic targets i.e. directly influencing generation and degradation of parasites is considered the most effective pharmaceutical strategy.

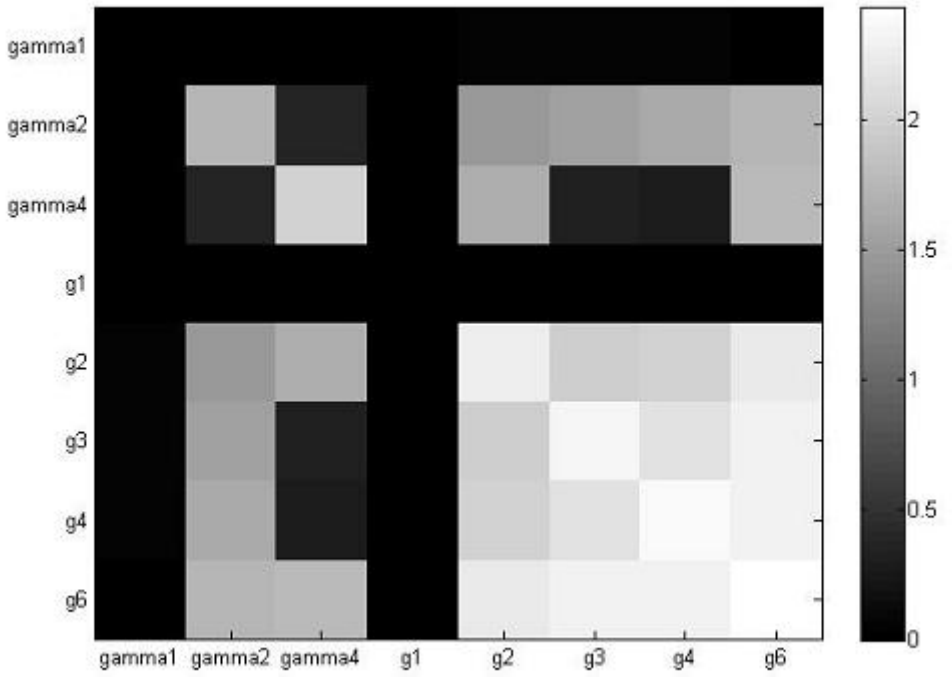

Figure 32: Optimization changing two parameters simultaneously, 22-parameter-model. The gray scale value represents final parasite load yielded by regulation of the respective pair of parameters.

5.4.4 Conclusion

The only strategy yielding a decrease of parasite load in the end (i.e. after 24 weeks) in both the 18- and the 22-parameter-model is upregulation of g_4 (augmenting the influence of IgG2a on parasite degradation) combined with downregulation of γ_4 (inhibiting degradation of lymphocytes).
Parameter pairs yielding a low final parasite load but possibly increasing or stagnating parasite load after 24 weeks which corresponds to no healing in this period of time are:

- a decrease in γ_1 (parasite influx) only, or combined with regulation of any other parameter and
- augmentation of g_1 (influence of parasite number on parasite production) combined with upregulation of g_2 (influence of parasite load on parasite degradation), g_3 (influence of the population of lymphocytes on parasite degradation) or g_4 (influence of immune globuline IgG2a on the degradation of parasites).

Though counterintuitive, the feasibility of upregulation of g_1 as a pharmaceutical target already obtained from one-dimensional optimization is further stressed by 2-dimensional-optimization.
In any case, a better result may be achieved by longer treatment (>24 weeks).

5.4.5 Comparison to Systematic Two-Dimensional Optimization

B. Wimmer [54] performed a systematic minimization of parasite load changing two parameters at a time, without using the results from one-dimensional optimization, both in the 18- and the 22-parameter-model. Here, a brief summary of her results is given.
In systematic optimization, both up- and downregulation of all parameters is tried. If we consider the majority of cases (e.g. a parameter upregulated in combination with three parameters and downregulated in combination with the remaining 19 parameters is considered to be downregulated), all the parameters upregulated in one-dimensional optimization were also upregulated in two-dimensional optimization and the same is true for downregulation. Only g_{13} (negative influence of IgG1 on IgG2a production) was downregulated in combination with 14 out of 22 parameters though upregulated in one-dimensional optimization.
Wimmer used smaller factors for optimization. They range from 0.67 to 1.8 in the 18-parameter-model; for the 22-parameter-model their range is $[0.8, 1.5]$.
In Wimmer's analysis none of the parameter pairs changed yielded a negative final parasite load gradient. However, in the 18-parameter-model parasite burden could be reduced by 99.9% resulting in a final value of $6 \cdot 10^{-4}$. In the 22-parameter-model parasite load could be reduced by up to 82%.

Fig. 33 is a graphical representation of final parasite values for optimization changing two parameters at a time in the 18-parameter-model. The gray scale value of a square indicates final parasite load changing the parameters corresponding to the row and column (compare to Fig. 31). The lowest final parasite load was achieved for upregulation of g_1 in combination with downregulation of g_2. (Applying our analysis, the lowest parasite load was yielded for combined regulation of g_1 and γ_2.) Further, combination of g_1 with any of the other parameters yields a final parasite load of less than 0.1, indeed even of less than $1.77 \cdot 10^{-3}$. Feasibility of g_1-upregulation corresponds to our results. Downregulation of γ_1 combined with upregulation of γ_2 yields a final parasite load of 0.9870. All the other parameter pairs yield final parasite loads greater than 1. In non-systematic 2D-optimization (Section 5.4.2), γ_1 yielded significantly lower parasite load combined with any of the other parameters considered, not only with γ_2.
Furthermore, Wimmer states changes in γ_6, g_3 and g_{14} as feasible therapeutic strategies since these parameters already yield a significant reduction in parasite load when changed less than 10% of their value.
Excluding the parameters directly influencing parasite load ($g_1, \gamma_1, g_2, \gamma_2$), Wimmer states that all remaining pairs of parameters yield similar results. This does not correspond to the results in Section 5.4.2 where γ_4 combined with g_3, g_4 or g_6 yields significantly lower parasite load than the other combinations.

Fig. 34 shows Wimmer's results for optimization in the 22-parameter-model (compare to Fig. 32). Interestingly, in the 22-parameter-model minimal final parasite values are much higher: The minimum parasite load of 0.5683 is achieved by simultaneous upregulation of g_1 and downregulation of γ_1. The higher parasite load (compared to the 18-parameter-model) is possibly due to the smaller range of $[0.8, 1.5]$ instead of $[0.67, 1.8]$ for regulation factors. Simultaneous regulation of g_1 in combination with any of the other factors yields a final parasite load of less than 1. Regulation of all other pairs of parameters yields a final

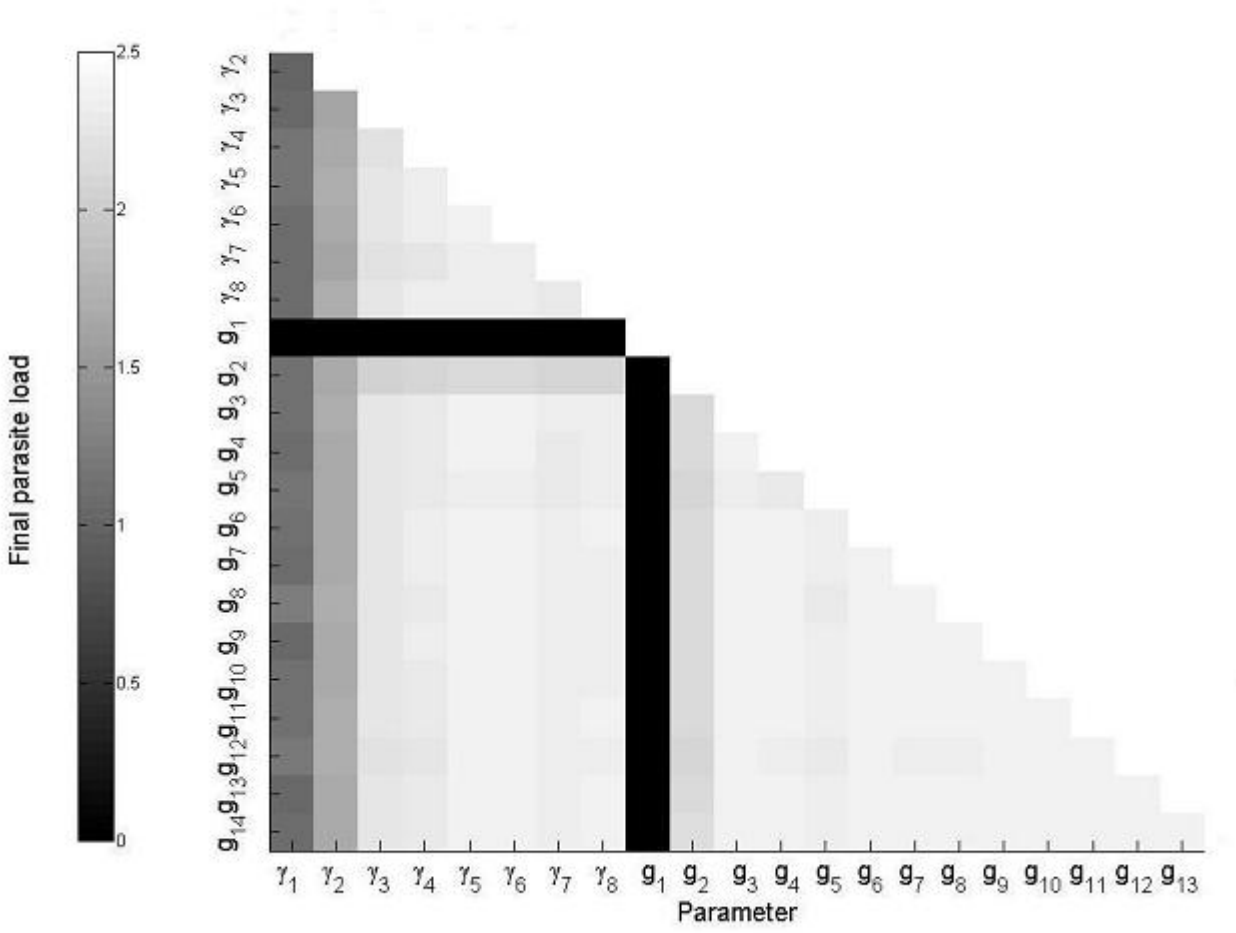

Figure 33: Results from B. Wimmer ([54], Fig. 4): Systematic optimization changing two parameters simultaneously, 18-parameter-model. The gray scale value represents final parasite load gradient yielded by regulation of the respective pair of parameters.

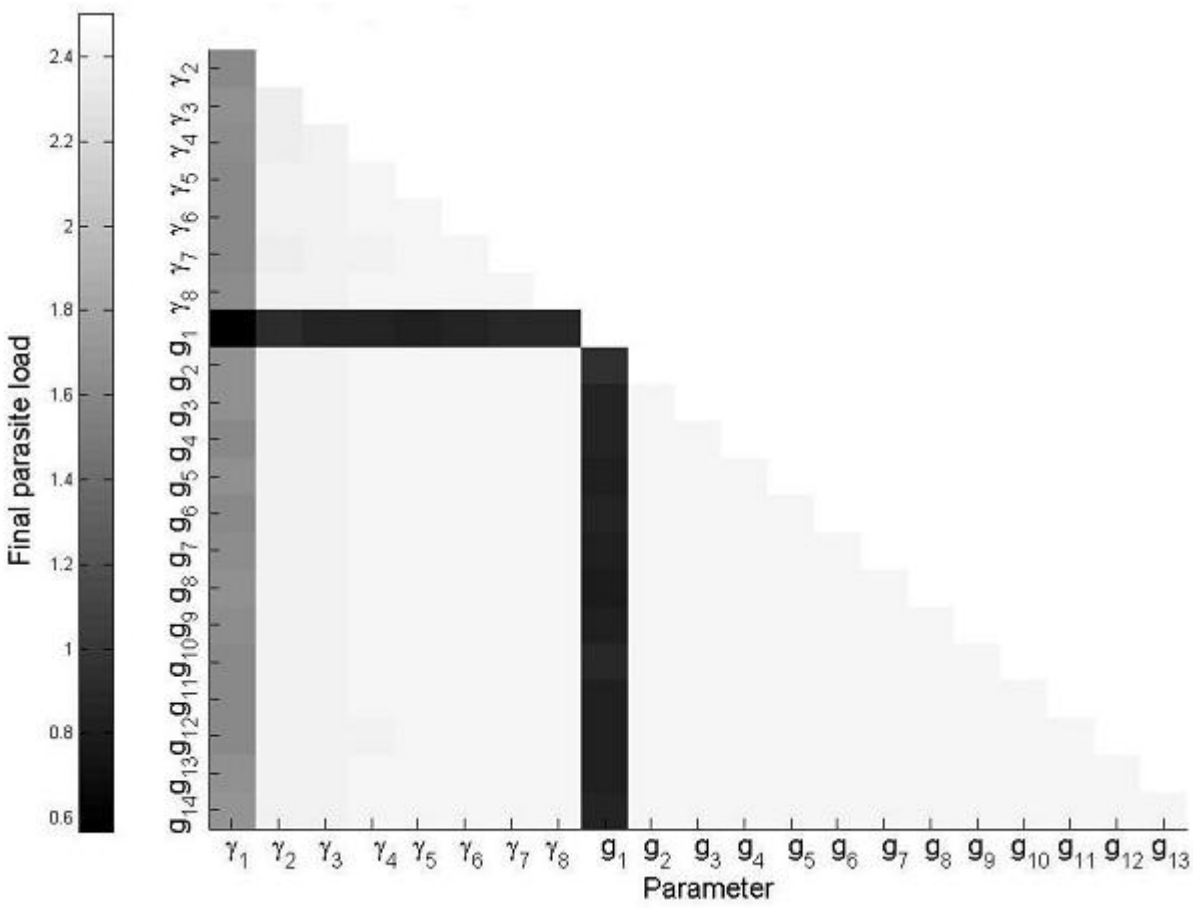

Figure 34: Results from B. Wimmer ([54], Fig. 5): Systematic optimization changing two parameters simultaneously, 22-parameter-model. The gray scale value represents final parasite load gradient yielded by regulation of the respective pair of parameters.

parasite load of between 2.3 and 2.5. Again, Wimmer's analysis does not yield feasibility of γ_4 as a therapeutic target as it is obtained in Section 5.4.3. However, it stresses the feasibility of an upregulation of g_1 (parasite growth rate) which corresponds to the results in this work. According to Wimmer, γ_1 only is effective when combined with g_1 whereas our results yield effectiveness of γ_1 regulation combined with other parameter changes as well.
Parameters that are considered possible therapeutic targets as well since they already yield significantly lower parasite load when changed less than 5% are g_5, g_{12} and γ_4. In the 22-parameter-model, no significant change in parasite load was obtained for the pair (γ_1, γ_2) by Wimmer although in our analysis regulation of this pair yielded the least final parasite load.

All in all, B. Wimmer's results from 2-dimensional optimization stress the feasibility of g_1 as a pharmaceutical target. Counterintuitively, the influence of parasite load on parasite production should be augmented in order to reduce final parasite load. Furthermore, prevention i.e. inhibition of parasite influx (γ_1) is considered an effective strategy but only in combination with augmenting parasite degradation (γ_2) or parasite growth rate (g_1). According to Wimmer, inhibition of lymphocyte degradation (γ_4) is not an effective strategy.

6 Discussion

This Section compares the results of our model to a previous leishmania model derived by Dancik et al. [15, 16]. Moreover, it gives an overview about anti-Leishmania agents and discusses pharmaceutical strategies using the results from our model.
Quantification of the effectiveness of drugs is difficult. It can be determined to what extent a drug is able to reduce parasite load in the body (Nakayama et al. report a reduction of up to 98% in [35]), but experimentally quantifying generation velocities, potentials and influences, which correspond to our model parameters, is practically impossible.

6.1 Anti-Leishmaniasis Drugs

This Section overviews existing anti-Leishmania drugs as well as possible therapeutic targets, without claiming completeness.
Standard treatment uses chemotherapy whereas recent investigation is focused on immunotherapy and immunochemotherapy. The term "immunotherapy" means the treatment of a disease by inducing, enhancing or suppressing an immune response [56]. Immunochemotherapy is a combination of immunotherapy and chemotherapy.
In case of leishmaniasis, immunotherapy aims at accelerating the specific immune response. In terms of our model (Fig. 6), immunotherapy means changing parameters $\gamma_3, ..., \gamma_8, g_3, g_4, g_6, g_8, g_9, ..., g_{14}$; whereas most chemotherapeutic agents directly target parasite destruction (γ_2) or inhibition of its proliferation (g_1).

6.1.1 Standard Treatment

The first drugs against leishmaniasis, pentavalent antimonials, were discovered in the 1940s. There are two types: sodium stibogluconate and meglumine antimoniate. Sodium stibogluconate is known as Pentostam and is slowly injected intravenously. Meglumine antimoniate, known as Glucantim, is injected intramuscularly or intravenously. Conventional monotherapies need continuous treatment during 21-30 days [21].
Another important drug is amphotericin B. It is given by intravenous injection, too. Paromomycin is injected intramuscularly. In the late 1980s a study proved lower mortality and fewer complications using combined therapy with sodium stibogluconate and paromomycin.
Miltefosine represents an oral drug. Combination of paromomycin and miltefosine yields the most economic result [21]. This combination therapy reduces duration of infection as well as cost and burden on the health system [21]. Economic considerations may seem secondary, however, the total cost of visceral leishmaniasis treatment was 1.2 times the annual per-head income in Bangladesh, 1.3 times in India and 1.1 times in Nepal [21].
Drug resistance mainly depends on the parasite burden, the probability of spontaneous resistance mutations and the fitness cost due to these mutations [21].
Combination therapy has not yet been tested on a large timescale; it is promising, but possible side effects have to be taken into account.

Tab. 15 provides a list of standard anti-Leishmaniasis drugs including their effects and lists a possible significance of the respective drug in terms of our model parameters. As

name	**effect**	**interpretation**
amphotericin B	inhibits completeness of parasite membrane leads to parasite death	$\gamma_2 \uparrow$
aminoglycosides	alter parasite's messenger RNA	$g_1 \downarrow, \gamma_2 \uparrow$
antimonials	inhibit glycolysis and oxidation of fatty acids, induce decrease of energy biosynthesis of the amastigote	$g_1 \downarrow$ $\gamma_2 \uparrow$
imidazole and itraconazole	inhibit desmethylation of membrane, inhibit parasite biosynthesis	$g_1 \downarrow$ $\gamma_2 \uparrow$
interferon	augments macrophages' capacity to reduce parasite load	$\gamma_2 \uparrow$
pentamidine	inhibits polyamine and DNA synthesis in the parasite	$g_1 \downarrow$ $\gamma_2 \uparrow$
pyrazolopyrimidines	block protein synthesis and destroy parasite RNA	$g_1 \downarrow$ $\gamma_2 \uparrow$

Table 15: Anti-leishmanial drugs and their effects (source: Dr. Cristina Pou, Universidad de La Laguna) and possible interpretation in terms of our model

can be seen in Tab. 15, most drugs are toxic i.e. directly targeted on the destruction of parasites. In our model this means increasing γ_2 (parasite destruction). This is effective due to the results obtained from optimization in Sections 5.2.3 and 5.3.3.
Moreover, many drugs inhibit parasite proliferation which means downregulation of g_1 in our model. According to our results which suggest increase of g_1 in order to inhibit parasite load this may be counterproductive. Although our result may seem counterintuitive, it could be due to the fact that if parasites replicate fast, the immune system can recognize them more easily [16]. Parasites use mechanisms like inhibition of antigen presentation to escape immune response, however, a high growth rate induces massive macrophage recruitment [16]. An explication could also be that the parasites produce a certain molecule that stimulates an immune response of the body. Investigating a model of tuberculosis infection, Segovia-Juarez et al. [47] also found that the partial rank correlation between growth rate and extracellular bacteria is negative in a certain time interval.
Dancik et al., 2010, received the same result analyzing a model of *Leishmania major* infection: Depending on the stage of the disease, a decrease or an increase in parasite load leads to minimal parasite load [16].

Roberts, 2006 [42] further mentions flucanzole and imiquimod as important drugs against cutaneous leishmaniasis, immunotherapy as effective against mucocutaneous leishmaniasis, paromomycine against visceral leishmaniasis and miltefosine against both cutaneous and visceral leishmaniasis.

6.1.2 Other Anti-Leishmanial Drugs

Besides the well-proved leishmaniasis drugs mentioned in Section 6.1.1 there are many other substances under investigation. The problem is that on the one hand, our model is an enormous simplification and does not include all the factors necessary to describe the

name	**effect**	**interpretation**
bednets	reduce biting rates	$\gamma_1 \downarrow$
betle leaves extract	reduce viability of promastigotes	$\gamma_2 \uparrow$
deltamethrin	impregnated dog collars protect dogs	$\gamma_1 \downarrow$
dendritic cells	activate T, B and NK cells capture antigens, transfer them to lymph nodes, activate specific Th1-response	$g_3 \uparrow$
gp63	specific CD8+ and CD4+ T cell immune response	$g_3 \uparrow$
IL-12	stimulates Th1	$\gamma_2 \uparrow$
IL-10R blockade	less antimony required to kill liver amastigotes	$\gamma_2 \uparrow$
imidazol and itraconazol	inhibit desmethylation of membrane, inhibit parasite biosynthesis	$g_1 \downarrow$ $\gamma_2 \uparrow$
insecticides e.g. DDT	inhibit biting	$\gamma_1 \downarrow$
interferon	augments macrophages' capacity to reduce parasite load	$\gamma_2 \uparrow$
KY62	interaction with membrane sterols resulting in disruption of the cell membrane with leakage of intracellular components ($\rightarrow$ parasite death)	$\gamma_2 \uparrow$
rLmSTI1	elevates specific IgG activity with a predominant IgG2a titer	$g_4 \uparrow$
saponin adjuvant (leishmune)	dog vaccine	$g_3, g_4 \uparrow \gamma_2 \uparrow$
vaccines	complete parasite killing by induction of live, attenuated or killed vaccines	$\gamma_2 \uparrow$ $g_3, g_4 \uparrow$

Table 16: Other therapeutic agents against leishmaniasis, their effects and possible interpretation in terms of our model parameters

interaction mechanism of the drug in the body, on the other hand, often this interaction mechanism is not even known thus it is not exactly known which of our model parameters are influenced by this drug. In pharmacological studies, the main interest is put on effectiveness of the drug i.e. the percentage of people healed by the drug. Tab. 16 shows some anti-leishmanian agents of which the mechanism of interaction is known, together with their effects and possible significance in our model scheme.
Prevention against sandfly bites or infection (bednets: reduction up to 64%-100% of sandfly bites [49], deltamethrin-impregnated dog collars: 86% protection [29], insecticides as well as human or dog vaccination) inhibit the influx of parasites into the body represented by γ_1 in the model. Most of the mentioned drugs are toxic which means they aim to kill parasites and thus augment γ_2. Vaccination can be done by induction of live, attenuated or killed parasites, or injected DNA yielding protein expression [42].
However, dog therapies are not recommended by the WHO since usage of the same vaccines for human and canine treatment increases resistance of parasites [51].
Identification of the genome of *Leishmania major* yielded 8500 identified genes as potential vaccine candidates [24].

Another group of anti-Leishmania drugs are those interacting with the immune system (immunotherapy). After infection with Leishmania there are three reaction mechanisms

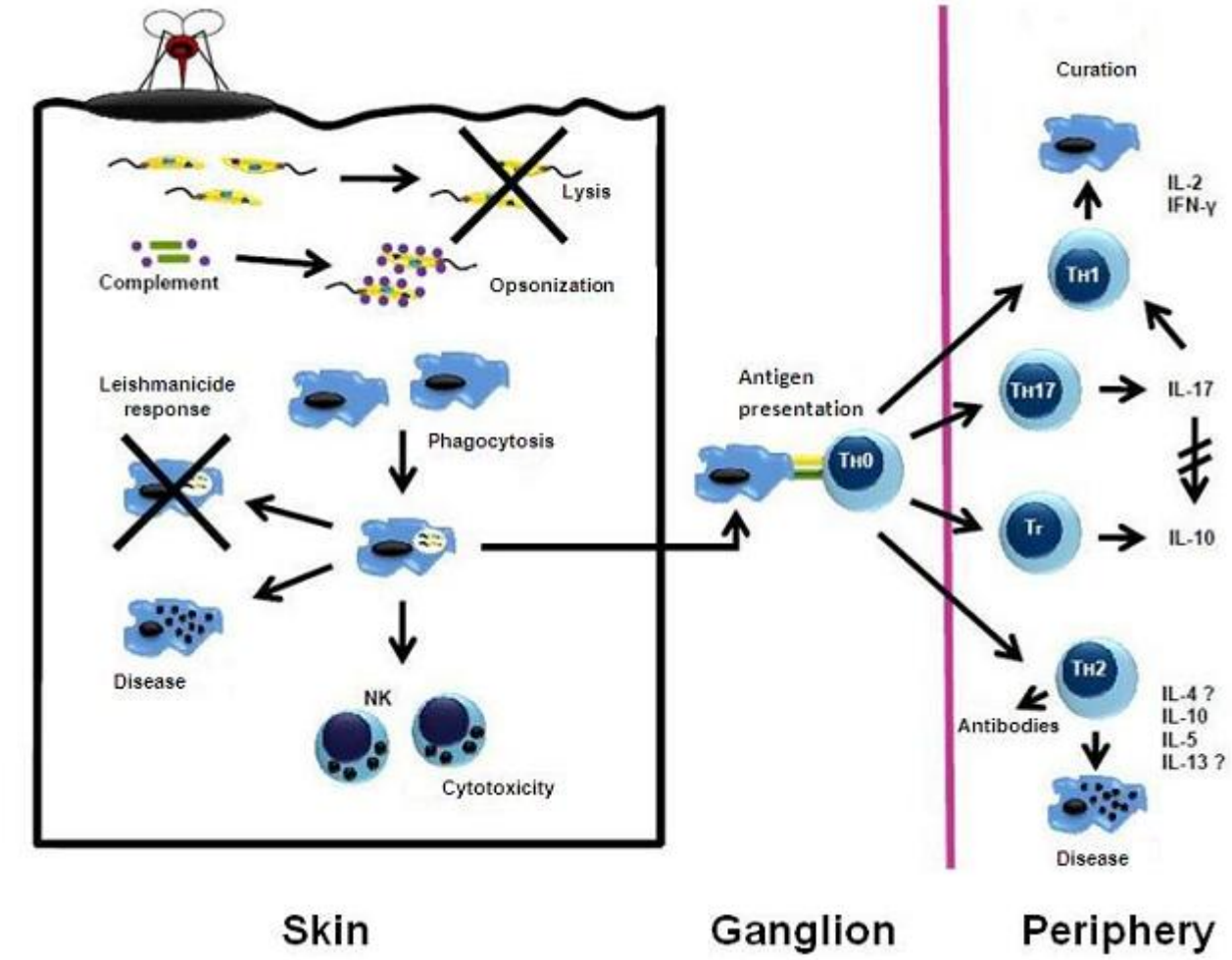

Figure 35: Immune response mechanisms after Leishmania infection (Fig. 3 in [1]). Infection or cure depends on the type of innate response as well as on the type of secondary immune response.

of the body:

- innate immune response (at the inoculation point)
- Th1 response (related with cure) and
- Th2 response (related with infection).

The last two points refer to the body's specific immune response.

Fig. 35 shows a scheme of the body's immune response to Leishmania infection. Parasites enter via the skin and produce an innate immune response: Leucocytes are recruted at the inoculation site, migrate to tissues and initiate their functions by release of substances, phagocytosis or destruction of microorganisms (leishmanicide activity) or modifying neighbor cells by production of cytokines or antigen presentation. The innate immune response is non-specific and results in local tissue inflammation [1]. The non-specific immune response can influence the later appearing specific immune response which determines if cure (Th1-response) or disease (Th2-response) is established [2].

Cells involved in the innate immune response are macrophages, neutrophils, eosinophils, natural killer cells and mastocytes, among others [1]. Regarding specific immune response, granulocytes are the first population that migrates to the infection site [34]. A few days later, natural killer cells and macrophages come into action [37]. After one or two weeks specific T lymphocytes migrate to infected tissue [6, 46].

Most drugs augment parasite degradation γ_2. Some of them, like CD4+ and CD8+ lymphocytes, can have adverse effects - different experiments showed that they are able to augment [38] or diminish [45] γ_2.
Dendritic cells and gp63 activate lymphocytes and other immune cells for specific response. Activation of T cells leads to production of IFN-γ which is related to Th1 response that yields healing. In our model they are assumed to augment the parameter g_3 which is the potential of lymphocytes to destruct parasites.
Leishmania major stress-inducible protein 1 (rLmSTI1) which is encapsulated in liposomes activates IgG2a. Since the action of IgG2a to diminish parasite load is represented by the parameter g_4 in our model, this parameter is assumed to be elevated by rLmSTI1.

According to the results from optimization in both models, the most feasible therapeutic strategies are to increase g_1, g_6 or γ_2 or to decrease γ_1 or γ_4. From Tab. 16 the following agents may provide changes in the mentioned parameters:

- prophylactic agents like bednets, impregnated dog collars and insecticides (diminish γ_1)
- betle leaves extract, IL-12, interferon, KY62 and vaccines (increase γ_2)

None of the considered drugs is assumed to have an effect on g_6 or γ_4 or to yield augmentation of g_1.

6.1.3 New Targets

The main target of this work is to provide new ideas that may enable a more effective search of effective anti-leishmaniasis drugs. This chapter provides a list of components involved in the body's immune response which could be used as future therapeutic strategies.

Tab. 17 and 18 show agents involved in immune response against leishmaniasis as well as their mechanisms of interaction and the model parameters that are influenced. It has to be mentioned that if therapeutic agents have an influence that is not represented by any of our model parameters but has to do with the in- or outflux of a model variable, we resume this by change in the respective rate constant γ_i which resumes all influences that are not caused by g_i.
Of special interest are the factors increasing the influence of parasites on their proper proliferation (g_1), because this factor is considered to be crucial according to our model results, and until now no pharmaceuticals increasing g_1 have been tested against Leishmania. Insuline-like growth factor 1, interferon-γ and possibly TNF-α are considered to stimulate parasite replication inside macrophages, and it would be interesting to test their

anti-leishmanial effectiveness experimentally. However, insuline-like growth factor also increases the number of parasites and reduces parasite-toxic production of nitric oxide.
There are a lot of immune response components in charge of parasite killing, namely chemokines, the complement system, granulocytes, interferon-γ, leukotrienes, activated macrophages, natural killer lymphocytes, the platelet-activation factor, prostaglandin E2, TNF-α and toll-like receptors. TNF-α can augment γ_2 (parasite degradation) and γ_7 (generation of IgG2a), two targets of which γ_2 is considered a feasible therapeutic target according to our optimization analysis (Sections 5.2.3 and 5.3.3). Toll-like receptors interact via augmentation of γ_2 and decreasing IgG1 production (γ_5) or enhancing IgG1 degradation (g_{11}). CD4+ and CD8+ lymphocytes are able to cause parasite death, however, in other stages of the disease they increase parasite load. Dendritic cells and immunoglobulins kill parasites on the one hand, but they also elevate the level of IgG1, although this should be decreased according to our model.
Hipoxy-induced factor 1, interleukin-4 and interleukin-10 inhibit γ_2 among other parasite-favorable regulations (downregulation of γ_7 and g_3), so blocking of these agents could also be a feasible therapeutic strategy. Effectiveness of IL-10R blockade, showing enhanced parasite killing and accelerated resolution of infection in IL-10 knockout mice is proved experimentally in [33, 32, 8, 25]. Combination of anti-IL-10R with antimony increases parasite killing [33].
Tab. 17 contains no immune response-related agents for regulation of g_6 and γ_4 which are considered to possibly be effective pharmaceutical targets by our analysis.

6.2 Existing Models

This section presents a short summary of the model developed by Dancik et al., 2006 [15], further analyzed 2010 [16], and compares their model and findings to our results.

6.2.1 The Agent-Based Model of Dancik et al. [15, 16]

G. M. Dancik, D. E. Jones and K. S. Dorman from the Iowa State University developed an agent-based model representing the immune response to an infection with *Leishmania major* [15, 16]. Agent-based models describe the dynamics of a complex system whose properties depend on the behavior of interaction components.
Like we do, Dancik et al. model the immune response to Leishmania. However, their experimental settings are different. [15] is based on experiments of Vanloubbeeck et al., 2004 [52] who investigate *Leishmania major* promastigotes in C3HeB/FeJ mice. The inoculation site is the footpad. The simulation period is 2-71 days after infection. The delayed starting point of simulations 2 days after infection avoids problems with missing initial values.
[16] uses the same model structure, but experimental data from Belkaid et al., 2000 [7] who investigated *Leishmania major* in C57BL/C mice. The simulation period is 3.5 to 8 weeks after infection, since after eight weeks the infection was healed.
In contrast to [15] and [16] our model investigates *Leishmania amazonensis* promastigotes in BALB/C mice with a simulation period of 0-24 weeks after infection. The inoculation site is the hind paw.
The model of Dancik et al. is of stochastic type i.e. simulation with the same data can

produce different results. The authors model a footpad cross-section of 2mm x 2mm as 100 x 100 grid of squared micro-compartments. The compartments are toroidal which means that an element leaving them on the one side re-enters at the other side. The models contain 25 parameters of which 11 [15], respectively 6 [16], are varied whereas the rest are constants taken from literature.
Initially, 105 macrophages and 50 parasites are placed randomly on the lattice. The lifespan of macrophages is considered as well as their maximal carrying capacity. When the number of intracellular parasites reaches a threshold ("transfer threshold"), the macrophage enters a dying state, in which it distributes the contained parasites to neighbor macrophages within a certain range of neighborhood. Thereby, activated macrophages kill the parasites they receive. After distribution of all its parasites, the dying macrophage is removed from the system. Cell movement is described by a probability based on the amount of one generic chemokine. Further, the model considers T cell recruitment using different compartments representing blood vessels. T cell movement is also described by probabilities. When T cell recruitment begins, all uninfected resident (i.e. not activated) macrophages are turned into inflammatory macrophages which move to the draining lymph node and have a reduced lifespan. T cells, more accurately speaking: Th1 CD4 cells, activate macrophages with a specified probability.

Dancik et al. use a technique called Gaussian process. Gaussian process approximation means fitting a multivariate normal distribution to data based on a given parameter vector. Computer model output at a new parameter vector is predicted using standard multivariate normal distribution theory.
The Gaussian process enables sensitivity analysis i.e. to determine how the model parameter vector $\theta = (\theta_1, ..., \theta_p)$ which consists of parameters taken from literature and parameters fitted to field data, influences computer model output. The main effect of parameter θ_k is the expected computer model output $E[z(\theta)|z_{known}, \theta_k]$. Importance of that effect can be measured by the percentage of total variance it represents. The latter can be determined by a FANOVA decomposition.

Comparison of Results

Out of the 26 parameters considered in the model of Dancik et al., 2006 and 2010 [15, 16], only the following were found to also appear in our model: initial number of parasites $X_1(0)$, intracellular parasite growth rate (γ_1 or g_1) and the probability a T cell will activate a macrophage which corresponds to the influence of lymphocytes on parasite degradation (g_3) in our model, since the lymphocytes fighting Leishmaniosis are T cells and an activated macrophage destroys the parasite. Furthermore, the amount of necrotic tissue released following macrophage activation is represented by γ_2 in our model, and T cell recruitment rate can be considered to correspond to γ_3 (lymphocyte influx).
The rest of the parameters used by Dancik et al. refer to chemokines, macrophages, T cells as well as recruitment, threshold and time scale parameters. All of these factors are not considered in our model and therefore do not have corresponding parameters.

Dancik et al. perform sensitivity analysis resulting in "main effect graphs" which plot the expected computer model output (y) depending on the value of the parameter (x), illustrating the effect of one parameter on model output. They also consider two-way interaction effects, however, the results provided in [16] only consider pairs of parameters

of which at least one does not appear in our model; thus these results are not comparable.

A comparison of the results of Dancik et al. [15, 16] is daring, since the experimental data they use originate from experimental settings which are different to ours: The mouse species, the site of infection, the inoculation amount and the parasite type differ from those of C. Pou's experiments [1]. So the result is expected to differ from ours, too. A big difference between ours and their model is that infection is healed in their model, especially because C3HeB/FeJ mice used in [15] are resistant to *Leishmania major* due to a Th1 immune response which yields activation of macrophages resulting in parasite death. Thereby, healing means the persistence of a low level of parasite load in the body which causes immunity to re-infection. T cells are included in the model in order to model this parasite persistence. Pathogens survive in a number of 1 to 30 macrophages at the site of infection [16].
The results of Dancik et al. yield the strength of the Th1 response, the speed of resting macrophages and the parasite carrying capacity of macrophages as crucial for the time until the infection is healed.

Results from [15] indicate that the most sensitive parameters with respect to final parasite load (i.e. parasite load after 10 weeks in their case because at that time infection was healed) are parameters not considered in our model, namely the speed of resting macrophages, the threshold at which macrophages enter dying state and the lifespan of infected macrophages. The forth-most important parameter is the probability of T cell recruitment corresponding to γ_3 in our model. Thus, due to different experimental settings, Dancik et al. receive results which are different from ours.

Dancik et al., 2010 [16] consider the intracellular pathogen growth rate (γ_1, g_1), the transfer threshold of infected macrophages, the macrophage recruitment hyperparameter, the probability of T cell recruitment (γ_3) as well as the amount of total pathogen that triggers the T cell response (corresponds to the level of X_1 that leads to a sudden increase in g_3) as most important parameters. These six parameters explain over 99% of the variation in output (FANOVA). Out of these, the parasite growth rate is assumed to yield the highest effect on final parasite load, whereas the effect of the probability of T cell recruitment (corresponding to γ_3) yields an effect 22 times lower than that of the parasite growth rate. Corresponding to our results, the pathogen growth rate which corresponds to γ_1 or g_1 in our model was also found to be the most influential according to Dancik et al. despite the different model conditions. The authors report that a higher parasite growth rate yields a higher increase in pathogen load in the beginning but also a higher decrease afterwards, so that, all in all, parasites are erased earlier if the growth rate is higher. The counterintuitive result that a higher growth rate decreases the amount of parasites was also achieved by our analysis. However, in the model of Dancik et al. infection was cleared after eight weeks in any case which does not correspond to our results where parasite number is constantly increasing (maximal = final parasite load, both in the 18- and in the 22-parameter-model). Thus the main analysis done by Dancik et al., namely finding a relation between parasite growth rate and the peak (i.e. maximum) parasite load is not reflected in our model due to different experimental settings and thus cannot be compared.

As a reason for the counterintuitive behavior Dancik et al. state that a pathogen that pro-

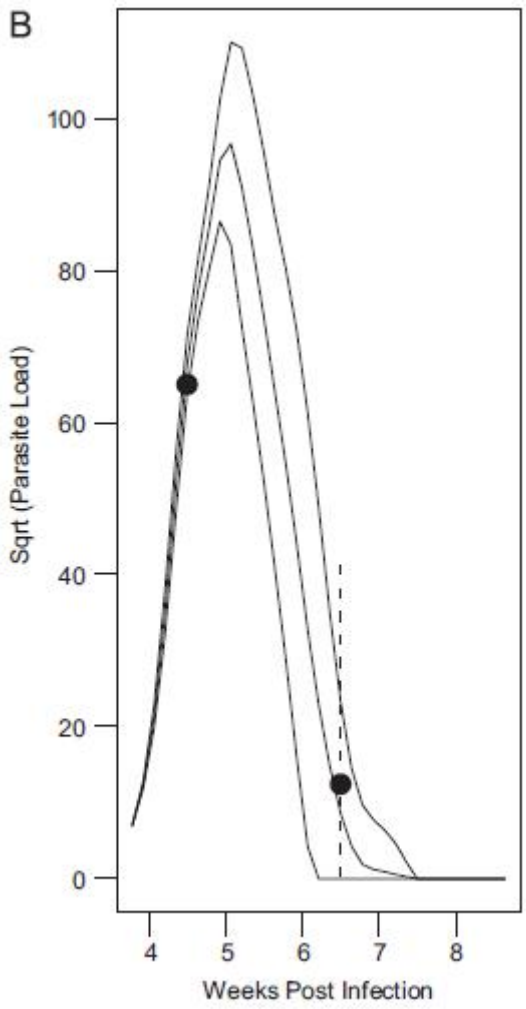

Figure 36: Square-root of parasite load in the model of Dancik et al. ([16], Fig. 8B)

liferates fast is more likely to be detected by the immune system. Therefore pathogen load decreases as growth rate increases, and slowly replicating pathogens persist longer than fast growing pathogens. They state: *"A fast-growing pathogen peaks early and quickly disappears, while a slow-growing pathogen peaks late and typically lower."* [16].

Tab. 19 tries to represent a comparison of parameter values between our 18- and 22-parameter-model and the model of Dancik et al. It has to be taken into account that our model is normalized which can cause major difference in model parameters. The rest of the parameters used by Dancik et al. refers to interactions of other components that do not appear in our model like chemokines, macrophages, the amount of total pathogen that triggers T cell response or T cell speed. Since our model does not take into account these components, these parameter values are not comparable.

It is interesting that Dancik et al. come to the same principal conclusion as we do, despite the different experimental conditions. Fig. 36 shows parasite load field data and model approximation in their model. Compared to Figures 11 and 24 parasite load shows a different behavior: After a sharp increase it decreases, already within the first seven weeks, whereas in our model parasite load steadily increases. Here the different experimental conditions lead to different results in Dancik's versus our model.

6.2.2 An immune response model of Nelson and Velasco-Hernández [36]

P. Nelson (University of Michigan) and J. X. Velasco-Hernández (UAM Iztapalapa) present a model of the innate immune response to an infection with *Leishmania* [36], showing that this primary response has a high effect on the secondary or specific immune response.
The model consists of three compartments (variables): resident macrophages, activated macrophages and a parasite. Furthermore, they consider nine parameters, seven of them refer to macrophages, the remaining two are the parasite growth rate and the kinetic rate constant for parasite death.
Out of the three variables, our model only takes into account parasite load (X_1); out of the parameters, the parasite growth rate (γ_1, g_1) and the parasite death rate (γ_2) appear in our model. In contrast to our model, Nelson and Velasco-Hernández developed a general model without using field data, considering A initial macrophages and B parasites. Subsequently, they perform a steady-state analysis resulting in two steady-states, namely:

- an unstable parasite-free steady-state corresponding to healing and
- a stable state of chronic disease corresponding to the capability of macrophages to control the disease.

Keeping growth rate and initial concentration constant, the authors perform simulations yielding a more severe disease reaction if the effectiveness of the immune system in killing the parasite is low, and a low parasite load which corresponds to control of the disease if the immune response is sufficient.

In the same paper, they present a second model, extending the first by two further compartments: one for IL-12 production and another for T-helper cells. For this model, they only give the model equations in [36], without an analysis of results.

name	**effect**	**interpretation**
adenosine	downregulates inflammatory process	
B lymphocytes	infection by priming CD4+ cells → cell migration to infection site	$\gamma_3 \uparrow$ $g_3 \uparrow$
CD4+ lymphocytes	may produce Th1 related cytokines, may produce Th2 related cytokines	$\gamma_3 \uparrow$ change g_3
CD8+ lymphocytes	IFN-γ, perforin; may produce Th1-related cytokines absence of IL-12 → Th2-related cytokines	$\gamma_3 \uparrow$ change g_3
chemokines	CXCL10 induces macrophages to kill parasites and delay lesion development, DCs stimulate production of IL-12, CD4+ lymphocytes stimulate production of IFN-γ; induction of NO production	$\gamma_2 \uparrow$ $g_3 \uparrow$
complement system	kills parasites	$\gamma_2 \uparrow$
dendritic cells	in resistant mice strains: produce IL-12 → Th1-response (cure), in susceptible mice strains: produce IL-4 → Th2-response (disease)	$g_3 \uparrow$
gluthatione	maybe: reduction of parasite load in lesion and drainin lymph node	
granulocytes	increases number of eosinophils → better control over infection (parasite killing by hydrogen peroxide)	$\gamma_2 \uparrow$
hipoxy induced factor I	may aid in survival of parasites (induce parasite survival)	$\gamma_2 \downarrow$
immunoglobulins	immunoglobulin-opsonized parasites can activate DCs	$\gamma_2 \uparrow, \gamma_5 \uparrow$
insulin-like growth factor-I	increases parasites numbers and intensity of inflammatory infiltrate; induces parasite proliferation inside macrophages; reduces NO production	$\gamma_1 \uparrow$, $g_1 \uparrow$ $\gamma_2 \downarrow$
interferon-γ	stimulates amastigote replication; Th1-response	$g_1 \uparrow, \gamma_2 \uparrow, g_3 \uparrow$
interleukin-4	inhibits IFN-γ and IgG2a production	$g_1 \downarrow, g_2 \downarrow, \gamma_7 \downarrow$
interleukin-10	limits Th1, immunosupression	$\gamma_2 \downarrow, g_3 \downarrow$ g_3, γ_3
interleukin-12	stimulates IFN-γ production in CD4+ T cells	$g_3 \uparrow, g_1 \downarrow, \gamma_2 \downarrow$
interleukin-5, 6, 13	promote IgE production, regulates hypersensitivity reaction	$g_3 \uparrow$
leukotrienes	LTB4 increases synthesis of NO by macrophages → enhances parasite killing	$\gamma_2 \uparrow$
macrophages	kill parasites by NO and superoxide when stimulated by cytokines or other factors	$\gamma_2 \uparrow$
MHC class II molecules	activation of CD4+ T cells, presence of COOH-terminal fragment of *L. amazonensis* cysteine proteinase B in cytoplasm of macrophages	$g_3 \uparrow$
natural killer lymphocytes	maturate and activate when phagocytose antibodies-opsonized parasites; resistant mice strains: produce IL-12 → Th1, lyse *L. amaz.*	$\gamma_2 \uparrow$ $g_3 \uparrow$

Table 17: Immune-related components and possible mechanism of interaction [45] as well as interpretation in terms of our model parameters

name	**effect**	**model**
platelet activaton factor (PAF)	enhances NO production by macrophages	$\gamma_2 \uparrow$
prostaglandin E2 (PGE2)	augments platelet response to their agonists	$\gamma_2 \uparrow$
TNF-α (cytokine)	activates macrophages to kill parasites, stimulates B lymphocytes to produce IgG2a and IFN-γ $\rightarrow$ infection cure, BUT also activates infected Langerhan cells to migrate from skin to draining lymph node	$\gamma_2 \uparrow, \gamma_7 \uparrow$ $g_1 \uparrow, g_{11} \uparrow$
toll-like receptors	stimulate IFN-γ, suppress IL-4	$\gamma_2 \uparrow \gamma_5 \downarrow$

Table 18: Immune-related components, possible mechanism of interaction [45] as well as interpretation in terms of our model parameters (continued)

parameter	Dancik	meaning	value Dancik	18-par-model	22-par-model
$X_1(0)$	P_0	initial number of parasites	50	1E-06	1E-06
g_1	α_1	parasite growth rate	(2.41,9.63)*1E-4	0.533411348	0.456662477
g_3	T_{actm}	probability a T cell will activate a macrophage	1	0.046286528	0.354307548
$\frac{1}{\gamma_3-\gamma_4}$	T_{ls}	T cell lifespan	3	1.039964861	0.747220893
γ_3	p_{Trecr}	probability of T cell recruitment	(0.0150,0.025)	0.046286528	0.354307548
γ_2	A_{necr}	amount of necrotic tissue released following macrophage activation	(0,6)	0.043203664	0.003132907

Table 19: Comparison of model parameters between the model of Dancik et al. [15] and our two models (18- and 22-parameter-model).

7 Conclusions

The foregoing sections yield the following conclusions:

1. Out of the models presented, the 22-parameter-model which is using all parameters neglecting a-priory-knowledge, has the following properties:

   - It yields the smallest error between original data and model approximation ($f_{obj} = 0.1412$).
   - Its parameters are less sensitive to infinitesimal changes than those of the 18-parameter-model i.e. the model is more robust (median of absolute sensitivities $m = 0.0198$).
   - It is the most general model avoiding errors resulting from wrong a-priori-assumptions and enabling variability due to its high number of parameters.

2. The 18-parameter-model is considered preferable due to the following reasons:

   - Due to its lower number of parameters it is simpler than the 22-parameter-model, however, the median of its absolute sensitivities is not much higher (0.0671 instead of 0.0198).
   - The use a-priori-knowledge is justifiable because it is reasonable to assume variable degradation proportional to the variable value.
   - It yields a better fit applied to different experimental data (data of the 10^6 parasites group: $f_{obj} = 0.1973$ instead of $f_{obj} = 0.2167$ for the 22-parameter model).

3. Influence of parasites on their proper proliferation (g_1) as well as influx of parasites (γ_1) are the most sensitive parameters with respect to parasite load in both models.

4. Possible pharmaceutical strategies could be:

   - Augmenting influence of parasites on their proper proliferation (g_1) as much as possible.
   - Augmenting influence of lymphocytes on their proper proliferation (g_6) by a certain factor, not higher than a threshold.
   - Decreasing parasites influx (γ_1) as much as possible.
   - Augmenting degradation of parasites (γ_2) as much as possible.

5. Optimization changing two parameters at a time yields regulation of the following parameter pairs as most feasible therapeutic targets:

   - g_1 combined with any of g_2, g_3, g_4
   - γ_1 combined with any of $\gamma_2, \gamma_4, g_1, g_2, g_3, g_4, g_6$ as well as
   - (g_4, γ_4),

whereby $\gamma_2, g_1, g_2, g_3, g_4$ and g_6 have to be augmented and γ_1 as well as γ_4 have to be decreased in each case.

These conclusions give new ideas for the design of drugs against Leishmania. It always has to be born in mind, that a simplified mathematical model of a biological system cannot give detailed solution concepts, but can serve to elucidate conceptual relationships [48]. According to this analysis, the most effective drugs may be:

- prophylactic arrangements like vaccination (active immunization), bednets or insecticides
- betle leaves extract, IL-12, interferon and KY62 and vaccines killing parasites

There are still no vaccines augmenting the parasites' or lymphocytes' reproduction rate. Future research could investigate drugs influencing these factors since they are assumed to possibly be feasible drug targets according to our analysis.

A Appendix: Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
BALB	Bagg Albino
BLCP	Brazilian Leishmaniasis Control Program
CD	Cluster of differentiation
CXCL	C-X-C motif chemokine
dev.	Deviation
DC	Dendritic cell
DDT	Dichlorodiphenyltrichloroethane
DNA	Desoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FANOVA	Functional analysis of variance
GMA	Generalized Mass Action
gp63	a Leishmania surface protease
HIV	Human Immunodeficiency Virus
IE	Índice de Estimulación (Stimulation Index)
IFN	interferon
Ig	Immunoglobulin
IL	Interleukin
KY62	a water-soluble analog of amphotericin B
LbAgS	Soluble extract of Leishmania braziliensis proteins
LbHSP	Leishmania braziliensis heat shock protein
LbL6R	Leishmania braziliensis L6R protein
LbL6R-HSP83	Fusioned LbL6R and HSP83 (chimera)
NK cells	Natural killer cells
NO	nitric oxide
PCR	Polymerase chain reaction
pload	Parasite load
rLmSTI1	Leishmania major stress-inducible protein 1
LT	Leukotriene
PAF	Platelet activation factor
PG	Prostaglandin
RNA	Ribonucleic acid
Th cell	T helper cell
TNF	Tumor-necrose-factor
TU	Technische Universität (University of Technology)
USD	US-Dollar
WHO	World Health Organization
VL	Visceral Leishmaniasis

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