Modelling and Simulation of Granuloma Formation in Visceral Leishmaniasis

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***Abstract*— Visceral leishmaniasis is a parasitic disease that can cause death. It is characterized by the formation of granuloma structures in the liver that can form at different time points after the infection. To date, the possible processes underlying granuloma formation are not fully understood. The importance of modelling in immunology is increasing, as many immunologucal phenonema are hard to study *in vivo* over periods of time, and hence modelling can provide some insight that might help deepen the understanding of the phenonema or help guide experimental work. This paper discusses initial studies into the formation of granuloma using a combination of UML like modelling and agent based simulation.**

I. Introduction

In a recent paper it has been argued that the area of Artificial Immune Systems (AIS) is widening to encompass the area of immunological modelling [1]. Along with [2] and [3] they argue for a greater interaction between computer science and the immunological community, to not only help in the development of immunological knowledge, but also bring new ideas from immunology into engineering in a more principled manner. This paper is concerned with the former: immunological modelling. The engineering implications have, to date, not been considered.

Visceral leishmaniasis is a disease which, though it does not play a role in central and northern Europe, is still a deathly threat to many people around the world, especially in the poorest countries. The disease is caused by a parasite, that can hide within specific immune cells in the liver. Those infected cells of the immune system can attract more immune cells to the site of the infection. Many immune cells together can form a larger structure called "granuloma". Our primary interest is in exactly how this granuloma can develop, and why some granulomas establish in a few days, whereas other sites of infection do not show any granuloma structures until weeks after the infection.

Joining methods from computer science and complex systems research with theories and experimental data from biology promises to elevate our understanding of biology to a new level. Biology has been very successful in gathering huge amounts of data over the past decades. But only recently systems biology has emerged as a new trend within the discipline focusing on how the many facts can be pieced together to obtain a picture of the whole system. It is generally believed that models have an important place in this endeavor. Agent-based modelling techniques are one approach that can be used to incorporate known facts into a model [4]. This model can then be used to explore the dynamics and to test hypotheses about the complex system that is instantiated by the agents and their interactions. However, to construct a model, it is necessary to initially describe the domain knowledge. The Unified Modeling Language (UML) [5] seems to be a very suitable tool to describe biological systems [3].

In this study, UML was used to produce an initial conceptual model of the process of granuloma formation within the liver, before in a second step, developing the simulation model which is a implementation of the domain model as a computer simulation. In each step, special attention was paid to the assumptions and simplification that had to be undertaken. This is of vital importance in the modelling process [6].

II. Agent Based Modelling and the Immune System

The protection provided by the immune system emerges from the interaction of the millions of immune cells. There is no central control coordinating the behavior. Cytokines released by macrophages, for example, attract other cells to the area of infection. Attracted cells find their way towards the source of infection by sensing a local gradient of cytokine concentrations. The behavior of a cell in the immune system critically depends on the environment it is in. For example, a lymphocyte binding to an antigen will become activated in the presence of co-stimulatory signals, whereas in the absence of co-stimulation binding to an antigen might lead to cell death. Furthermore, the behavior of a cell might not only depend on the presence or absence of a signal but also on its strength. The dynamics of the immune system therefore also depend on the number of cells involved. By initiating an inflammatory response that attracts many immune cells, the immune system creates the environment that is needed for the single cell of the system to function properly.

It is always tempting to assign capabilities of a system to a single agent within that system: "Macrophages ingest bacteria" or "T-cells kill infected cells". However, in an isolated system containing just that agent and its target, it is likely that exactly this would not happen. For example, if we just had a test tube that contained some blood, in which macrophages and bacteria were present, it is conceivable that they would never meet. In the body, the chance for them to meet might be much

higher because the movement of both cells is restricted by the boundaries of blood vessels. So even simple environmental constraints can greatly influence the behavior of the system. However, only few of those environmental conditions can be considered static. Blood vessels, for example, can change their permeability during an inflammatory response. Therefore, rather than asking "which cell does what?", it would be more important to address questions such as "which cells interact with each other?" and "which factors influence a certain process?". This does not mean that it is always best to try to analyse the whole system at once, but instead of focusing solely on the agents one should try to focus on the dynamics and relations as well: (or as Sauro et al. state finding functional, rather than topological, modules is one of the greatest challenges for systems biology research [7]).

A. Agent-based Modelling

An agent is commonly defined as an entity that is able to perceive its environment and initiate an action. An agent-based model (ABM) is a model which captures interactions between agents in their environment. Thereby it does not merely capture statistical relations between different variables, but models the agents that bring about these relations. Parunak et al. [8] explain ABMs by contrasting them with equation based models (EBMs). He states that EBMs focus on observables, while ABMs focus on individuals. Observables can be any sort of variable of interests as for example the oil price, a stock market index, and the federal funds rate in an economics model. Using this illustration, an EBM would then for example consist of three equations which mathematically describe, how the value of each of these three variables will change in the future depending on its own and the other two variables' current values. If it was a good model, those equations would predict the values of the variables with high accuracy. From analysing the equations, one might draw conclusions such as "rising oil prices lead to falling stocks", or one might even be tempted to say "rising oil prices are a *cause* for falling stocks". However, such a statement could be misleading because the oil price does not invest at the stock market. In the same scenario, an ABM, in contrast, would not model the abstract macro-level properties (i.e. oil price, stock market index, federal funds rate) and their relations, but it would for example model a collection of individual traders and their interactions. The value of the stock market index would then be derived from the individual traders making decisions regarding whether to buy or to sell shares.

1) Agent-based Models of the Immune System: Immune responses and processes such as granuloma formation involve many different types of agents and altogether, the system is very distributed with little, if any, central control. Therefore, the immune system is a prototypical candidate for being modelled by an ABM.

Many immune models are based on 2-dimensional cellular automatons or more general 2-dimensional grids, such as the IMMSIM [9], an early influential immune modelling framework that served as a basis for a number of different studies in the 1990s.

Besides pure ABMs, there have also been some so-called "hybrid" models. Examples for this kind of model are given in Simmune [10, as cited by [4]] and CyCells [11, as cited by [4]]. In these models, the immune cells are represented as agents, but molecules such as cytokines are included as concentration variables.

Segovia-Juarez et al. developed a hybrid model of the process of granuloma formation in the lungs of patients infected by tuberculosis [12]. By conducting a sensitivity analysis, the authors explored which parameters had the greatest impact on the severity of the disease. One of their findings was for example that the number of T-cells in their model was less important for the spread of the disease than the spatial distribution of the T-cells.

B. Unified Modelling Language

UML is a language that uses graphical notations and was developed to model software systems. The difference between a graphical language such as UML and ad hoc illustrations is that the symbols used in the UML are linked to a semantic. In the first half of the 1990s, many modelling languages were competing with each other. The lack of agreement on common standards hampered the acceptance of modelling tools. For this reason the Rational Software Company – a company selling software engineering tools – brought together three of the leading developers of modelling languages to join their efforts. In 1997, the first version of Booch's, Rumbaugh's, and Jacobson's unified modelling language was proposed [5]. The UML became widely accepted and the current version of the UML is the industry standard for software design.

III. Biological Phenomenon

A. Liver

The liver is important in metabolism; it is involved in the storage of sugar and in the production of bile which facilitates digestion. Additionally, the liver plays an important role in detoxification by breaking down toxic substances in the blood and by disposing red blood cells that reached the end of their life span. The liver is the largest internal organ and without the liver, a human could not survive for more than a day. However, the liver has an extraordinary capability to regenerate. Even when only less than half of the liver remains functioning, i.e. after a liver failure or an operation, it is able to regrow to a fully functional state.

The liver is made up of many 1-2 mm large lobules. Each lobule is encircled by triads of portal veins, hepatic arteries, and bile ducts. Blood enters the liver from the portal veins and hepatic arteries, whereas the bile duct transports out bile which is produced by the liver cells. The interior of the lobules is mainly made up of hepatocytes and a network of capillaries called "sinusoids" through which the blood flows from the periphery of the lobule to the central vein in the middle. Within the sinusoids resides a special kind of macrophages

called Kupffer cells. Those Kupffer cells can be a target for leishmania parasites.

B. Visceral Leishmaniasis

Visceral leishmaniasis (VL) is a disease caused by parasites of the leishmania genus. It occurs in 65 countries around the world and it is estimated that around 500.000 people get infected per year [13]. Also known under the name kala-azar or black fever, the disease is mainly prevalent in poor rural areas in tropical and subtropical regions. Ninety percent of the cases worldwide occur in the five countries Bangladesh, India, Nepal, Sudan and Brazil. The symptoms of VL are fever, weight loss, anaemia, and swelling of the spleen and the liver. If untreated the disease leads almost certainly to death. More information about the spread of the disease, strategies for controlling it, and future risk factors can be found in [13]. Although, after malaria, VL is the parasite with the second highest death-toll, the fight against the disease is lacking far behind what could be achieved. The development, clinical testing, and distribution of drugs is severely hampered by the fact that leishmaniasis is most widespread among the poorest of the poorest, rural populations of developing countries, and in regions maximally cut-off from health care systems. Recently, new drugs have been developed by a charity organisation. However, bringing the cure to the people and developing strategies to limit the new infections is still a challenge [14]. The numbers of infected people have risen over the last years, and further risk consist for example in urbanisation [15] and the co-infection with leishmania and HIV.

VL is transmitted by sandflies. When sucking blood from an infected animal or human, the parasite gets into the sandfly and can then be transmitted to other potential hosts. During this process, the parasite changes form – from the promastigote stage, which it takes up within the sandfly, to the amastigote stage it takes up within the host. The promastigote form is larger, has a flagellum to allow the parasite to move, and in this stage the parasite can multiply through cell division. However, once in the host, the parasites invade macrophages, loose their flagella and take control of the macrophages to prevent being destroyed by them and to use them to promote their reproduction

C. Granuloma Formation in Mice

When leishmania parasites enter an organism, they infect macrophages within the liver. Those infected macrophages then release messenger molecules called cytokines to attract other cells of the immune system. By this mechanism, clumps of immune cells can emerge that are called granulomas (see Figure 1).

The course of the disease can be studied by injecting the parasite into mice. Particularly, the influence of various factors on the course of the disease can be investigated by conducting experiments with different kinds of knock-out mice – that are genetically modified mice with certain genes deactivated. However, in order to observe the effects of the infection

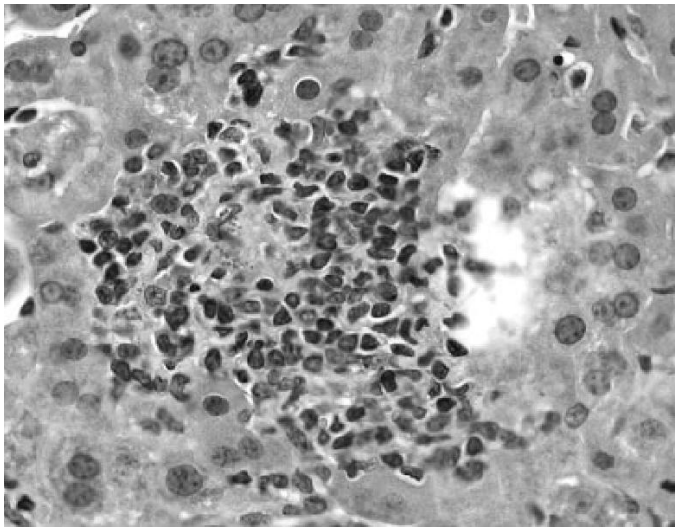

Fig. 1. Picture of a granuloma in the liver, four weeks after infection with leishmania (taken from [16]).

in the mouse, the mouse has to be killed. It is possible to record microscope videos of cells moving within the liver, but after about 20 minutes of recording, the cells will die and not be usable anymore. Yet, the formation of granuloma structures can take several weeks. Therefore, it is not possible to observe the whole processes of granuloma formation from the beginning to the end. It is possible to take snapshots in different mice at different time points, but not to monitor the progress of a specific granuloma over the entire period.

In experiments it has been shown that after one week, formation of granulomas had taken place at around 40% of the infected macrophages, a week later 60% of the infected macrophages were coated by a granuloma, and after three weeks 80%. It is still unclear why there are such differences in the time it takes for the granulomas to form.

IV. Modelling Granuloma Formation

A. Aim of the Model

The aim of the model developed for this study was to investigate how granulomas establish in the liver. The processes that follow the formation of granulomas are fairly well understood but the initialisation of the process leading to their formation is still largely unclear. Key questions to ask are: Why do some granulomas form within days of infection and others some weeks later? Which are the key players in the regulation of the early events leading to the formation of granulomas? Doing experiments to answer these questions is expensive and difficult.

As argued above, experimentation can provide just snapshots and no long term study of granulomas, therefore, the aim of the computational model implemented for this study is to simulate the process of granuloma formation and thereby to enable a better understanding of what happens in-between the snapshots. By adapting the model to implement the ideas of different hypothesis, it is possible to test the plausibility of the

competing hypotheses regarding the the granuloma formation process. Comparing different versions of the computational model then allows to systematically compare the different hypotheses. The aim is to find out what assumptions must hold for the different alternatives to be true, and what parameters might provide insight into how the system works in reality. The analysis of the model might also determine the kind of data which would still be needed to falsify or support a given hypothesis. This should finally help to design meaningful experiments and guide future research.

The model is not meant to mirror the biological process in every detail or to fit all available data as good as possible; it is rather meant to qualitatively explore different possibilities. One aspect of modelling is to make our knowledge of the biological process explicit. During the modelling process, all assumptions that contribute to the model should be detailed [6]. Finally, the simulation should allow us to vary parameters and settings, thereby allowing to conduct *in silico* experiments. Aided also by a 3-dimensional real-time visualisation, the simulation aims to "engage the minds of the experimentalist with [an] intuitively understandable representation" [2, p. 234]. By allowing the manipulation of the system and literally enable us to *see* the results, the model should facilitate the understanding and communication of the mechanisms involved in granuloma formation.

The aim of this study was to start an iterative process of model building and experimentation – a process in which new question may arise – rather than to give any definite answers. The present study can only be understood as a first step in this venture.

B. Conceptual Model

The first stage of our work was to develop a conceptual model. The purpose of a conceptual model is to describe the biological entities and make clear our understanding. This can then be taken to form the basis of a computer simulation, if needed. In the following, the structure of the system will first be described. Then, the properties of the agents in the model will be detailed, before their behaviour will be discussed.

Throughout this section, simplifications of the biological reality had to be made. This is discussed further in Section IV-D.

1) Structure: Figure 2 visualizes the relevant parts of the liver. The blood circulation brings a continuous influx of T-cells which migrate to the diverging sinusoids. The sinusoids are small blood vessels coated by endothelial cells in which the macrophages reside. In addition to macrophages, other types of cells can also be found in the sinusoids. One such other cell type is for example the natural killer T (NKT) cell, a subtype of T cells that also expresses markers normally found in natural killer cells. NKT cells have the capacity to stimulate the macrophages. However, other cells might also be contributing to the process. Therefore, in the following these additional cell types are collectively referred to as innate liver cells (ILCs).

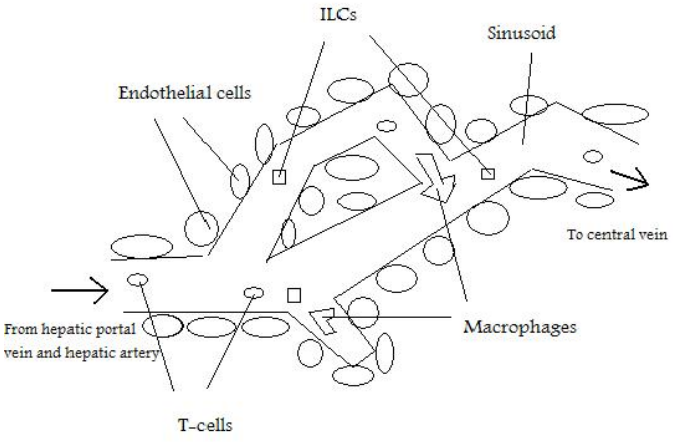

Fig. 2. Sketch of the entities involved in granuloma formation in the liver.

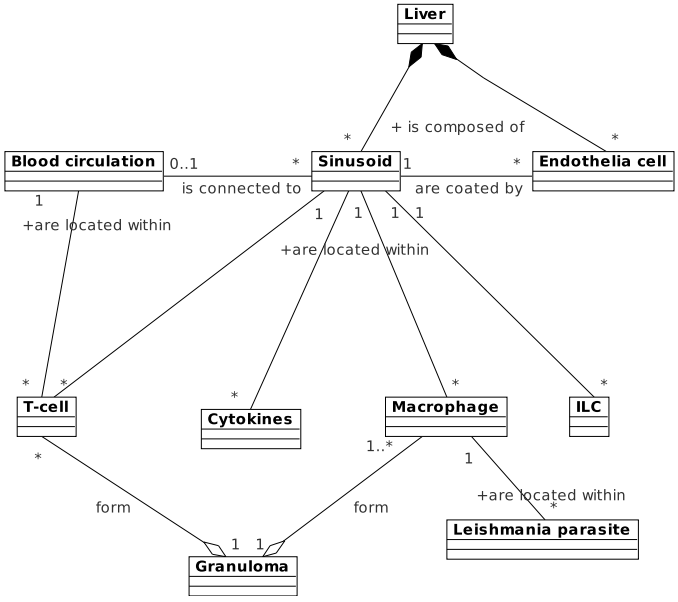

Fig. 3. UML class diagram of the basic types of entities involved in granuloma formation, and their spatial relations. The liver consists of multiple sinusoids, and endothelial cells which coat the sinusoids. The sinusoids can be connected to the blood circulation. Possibly infected macrophages, innate liver cells (ILCs), and cytokines are located within the sinusoids. T-cells are located both in the sinusoids and the blood circulation. Multiple T-cells which clump together around at least one macrophage together form a granuloma structure.

If many T-cells move towards one macrophage, they will start clumping together around that macrophage and form a bigger structure called a granuloma. Figure 3 shows the main types of entities involved in the process and their spatial relations to each other in form of an UML class diagram.

The next step is to clearly identify each agent that is involved in the process. Due to space, we can not detail all the information about each agent here. However, known facts such as sizes and speed of agents, total number of cells, flow rates, etc. where compiled and added to the model

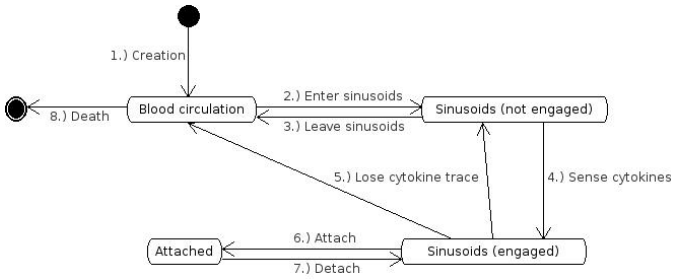

Fig. 4. State diagram of the behaviour of a T-cell.

2) Behaviour: To model agent behaviour in a similar vein to [3] we make use of UML like *state diagrams* which allow us to capture key states and transitions between states of an agent. In the context of our work, after a macrophage becomes infected, it begins to secrete low amounts of cytokines, which can attract both nearby ILCs and other macrophages. If multiple macrophages come together, they can fuse to a multi-cellular conglomerate. Those multi-cell formations are larger, they stop the macrophage movements and can secrete higher amounts of cytokines; otherwise, they show the same behaviour as a single macrophage. When an ILC reaches a macrophage or multi-macrophage conglomerate, it activates it. This leads to a strong increase in cytokine production and thereby in the engagement of T-cells within a wider area. By attracting those T-cells, the initial granuloma is formed.

a) T-cells: Figure 4 shows the low-level behaviour of individual T-cells. At any point in time, T-cells are supposed to be in one of four possible states:

Blood circulation: T-cells can enter and leave the sinusoids through the general blood circulatory system. In the simple model implemented in this study, it is assumed that all T-cells modelled in the system are identical and antigen specific. For a more refined model, it is also conceivable to introduce a second type of non-antigen specific T-cell which could be modelled as less excitable by the cytokine signal. The blood circulation is not spatially represented but just acts as a reservoir of T-cells, that sources the sinusoids with a certain number of T-cells per time unit.

Sinusoids (not engaged): The sinusoid environment is spatially represented. After entering the sinusoids, the T-cells drift with the blood flow and make random movements. Except for being able to block some space from being taken up by another T-cell, T-cells do not interact with each other.

Sinusoids (engaged): Once T-cells sense a cytokine trace, they become engaged and begin moving in the direction of the cytokine gradient. Only when they are in this state, they can attach themselves to a granuloma structure. There is a possibility for them to lose the cytokine trace and move away from the influence of the cytokines back to a non-engaged state, or to leave the sinusoid and re-enter the blood circulation.

Attached: This is the state of a T-cell which attached itself to an activated macrophage or larger granuloma structure. In our current model, there is no change in behaviour between

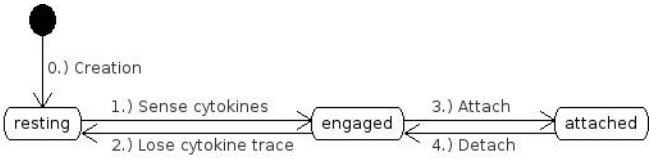

Fig. 5. State diagram of the behaviour of an innate liver cell (ILC).

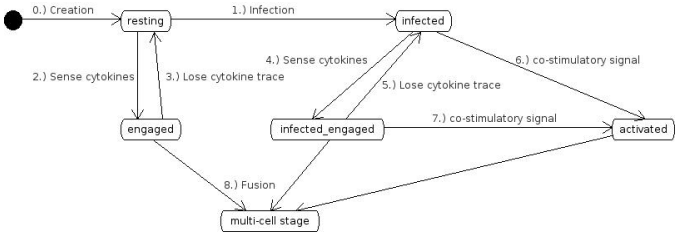

Fig. 6. State diagram of the behaviour of a macrophage.

engaged and attached T-cells, i.e. the fact that the T-cells are staying close together in a granuloma structure is only explained by the cytokine gradient that continuously forces them in the right direction. However, additional biological detail could be added to the model at this point, for example to take into account local interactions between macrophages and T-cells. Also, the cytokine production of the macrophage can be modulated by the number of attached T-cells, and finally the number of attached T-cells could be used as a measure for the successful establishment of a granuloma.

b) Innate liver cells (ILC): Figure 5 shows the relation of the three possible states an ILC can be in.

Resting: ILCs are initially created in a resting state; during the run-time of the simulation the ILC population stays constant. Resting cells do not move.

Engaged: This state is identical to the state "Sinusoids (engaged)" of the T-cells, except that an ILC cannot leave the sinusoids to enter the blood circulation.

Attached: Again this state is similar to the respective state of the T-cell, apart from the fact that ILCs that get attached to a macrophage trigger its activation.

c) Macrophages: Figure 6 shows the states that a resident macrophage within the liver can be in according to the model.

Resting: The resting state of a macrophage is identical to that of an ILC.

Infected: At the start of the simulation, a certain number of macrophages are infected by parasites. If infected, a macrophage secretes low-amounts of cytokines which can attract nearby ILCs, other macrophages and T-cells. The parasite load of infected macrophages could vary and have an effect on the amount of cytokines produced by a specific macrophage.

Engaged: If another macrophage near a resting macrophage starts secreting cytokines, the resting macrophage becomes engaged and starts moving towards the infected cell (just as an engaged ILC).

Infected_engaged: Infected macrophages react to a cytokine

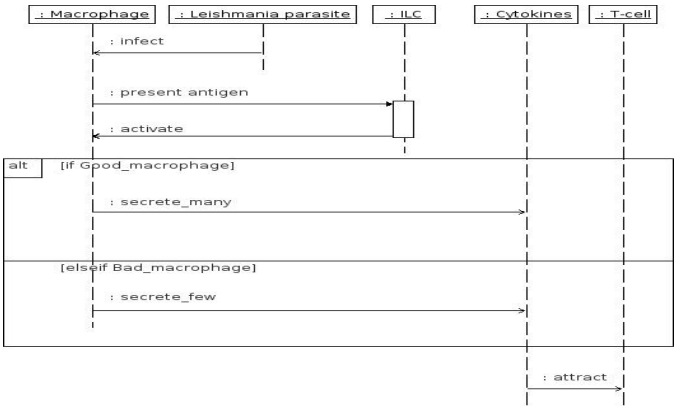

Fig. 7. UML sequence diagram showing the process that leads to granuloma formation for the hypothesis of having two subtypes of macrophages.

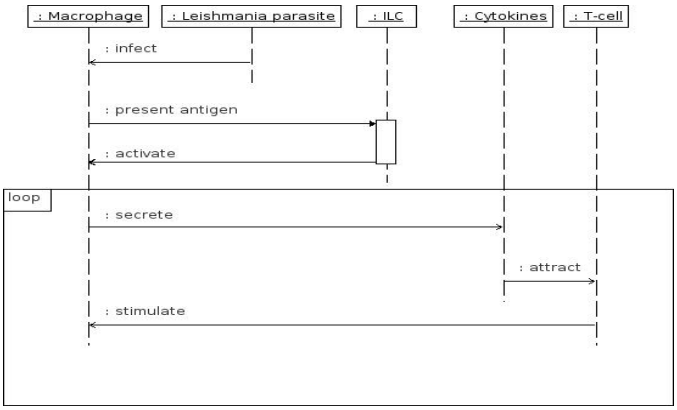

Fig. 8. UML sequence diagram showing the process that leads to granuloma formation under the hypothesis of positive feedback.

signal in the same way as non-infected macrophages.[1]

Activated: If the macrophage could attract a nearby ILC, the macrophage will be activated by the ILC and produce much higher amounts of cytokines, which in turn can attract T-cells over larger distances.

Multi-cell stage: Multiple macrophages coming together can fuse to multi-cell conglomerates which can produce large amounts of cytokines. At least one of the macrophages has to be activated for the fusion to take place

C. Competing Hypothesis

To explore why different macrophages start attracting T-cells at different points in time, two competing hypotheses are modelled.

One possibility would be that some macrophages are not activated and do not at all secrete cytokines for a certain time period. However, no mechanism is known which would explain why an infected macrophage would reside in the liver for a couple of weeks without being activated.

The most obvious explanation left is therefore that there is only a limited amount of T-cells available for which all the macrophages compete. If some macrophages were more successful in attracting T-cells, they would be more rapid in forming granulomas. Once these more successful macrophages would reach a stable state, with the size of the granuloma not increasing any more, the less successful macrophages could also start recruiting T-cells and form granulomas.

However, two very different mechanisms could explain why some macrophages might be more successful than others.

It could be the case that there are two different subtypes of macrophages, one type that secretes high amounts of cytokines and another type which secretes low amounts of cytokines. In this case, there would be a constant difference in the effectiveness of attracting T-cells between the different types of cells.

[1]If two nearby macrophages are both infected, they might both start attracting the respective other. However, this does not have to be the case because the diffusion of the cytokines does not need to be uniform in all directions. It could for example be modulated by blood flow.

A second hypothesis is that there are no significant differences between the macrophages, but that T-cells interacting with the macrophage could stimulate it to secrete even higher amounts of cytokines. Therefore, a macrophage that has just a slight advantage over another macrophage could be rewarded by a positive feedback mechanism which would in turn increase the difference between the two macrophages.

The slight advantage of one macrophage over the other at the beginning of the process could be explained by a variety of reason. For example, one macrophage might become infected slightly earlier, one might get the co-stimulatory signal from the ILC earlier, one might be located closer to the blood supply, or multiple macrophages in one sinusoid might add up their effects.

D. Validation of Conceptual Model

For several reason, modelling requires us to make abstractions and simplifications of the reality. First, we are not able to build models as complex as reality. And, secondly understanding also involves separating important from secondary. A model that would be as complicated as reality might not be easier to understood than reality itself.

Because simplifications are crucial in modelling, a core part of this project is to document and discuss the simplifications made. We undertook this process, and used this to inform the development of our agent based simulation. Space prevents us from detailing all the assumptions, but these are available on request.

V. Implementation Model and Simulation

The purpose of the implementation model is to translate the domain knowledge from the conceptual model into a model that suits the implementation in a concrete programming environment. We undertook a similar process in terms of modelling approaches and developed UML like diagrams to capture agent and environmental behaviour. We then implemented the model in an agent based simulation tool known as MASON [2].

[2]http://cs.gmu.edu/∼eclab/projects/mason/

A. Test Cases

Three different test cases were implemented:

- The standard behaviour with just one kind of macrophage and no stimulatory effect of T-cells.
- The hypothesis that the differences between different granulomas are caused by the fact, that some macrophages secrete more cytokines than others.
- The hypothesis that some granulomas establish faster than others because of an initially small difference that is enforced by a positive feedback mechanism.

In all three cases, there was one central sinusoid, through which there was a flow of blood (and T-cells), and which had two dead-end sinusoids branching of one either side. In both branching sinusoids, there was a macrophage and a ILC at identical distances to the central sinusoid.

In the first case, both branches were completely identical. In the second case, throughout the whole run, one macrophage secreted double the amount of cytokines that the other would have secreted on the same activation level. In the last case, one ILC was hindered to do any movements for the first 500 time steps. Thus, it was not able to stimulate the macrophage in that branch. This gave the other side an advantage because it could already attract the first T-cells. In addition, T-cells were able to stimulate macrophages in this case, just as ILCs could do as well; the effects of multiple cells stimulating a macrophage were added up.

VI. Results and Discussion

A. Behavioural Results

In all three test cases, it was possible to observe granuloma formation. At first, T-cells flowed through the central sinusoid, and left the liver environment at the other end. The macrophages started by producing only a very low amount of cytokines which were not enough to attract the T-cells. Therefore, T-cells did not get distracted from the central sinusoid. However, the amount of cytokines was enough to attract the ILCs to approach the macrophages, and once they reached the macrophage, through the ILC stimulation, the amount of cytokines released by the macrophages was greatly increased. From this stage on, the macrophages competed for attracting the T-cells in their direction.

In the first test case, both macrophages had the same chance of attracting T-cells, and though sometimes, by chance, one attracted a few more than the other, a granuloma generally formed around both macrophages at about the same time (see Figure 9).

In the second test case, a single T-cell sometimes migrated towards the macrophage that produced the lower amount of cytokines, but the majority was clearly attracted by the macrophage which produced larger amounts of cytokines. A granuloma first formed around this macrophage (see Figure 10). Once this granuloma reached a certain size, it sometimes happened that the T-cells pushed away the ILC from the close proximity of the macrophage, such that the macrophage was not any longer stimulated. Then, the other macrophage had the chance to attract some T-cells and after a considerable delay, a granuloma formed around this macrophage as well. Although this result resembles the observation from biology, it remains open whether the process by which it evolved also resembles the natural processes.

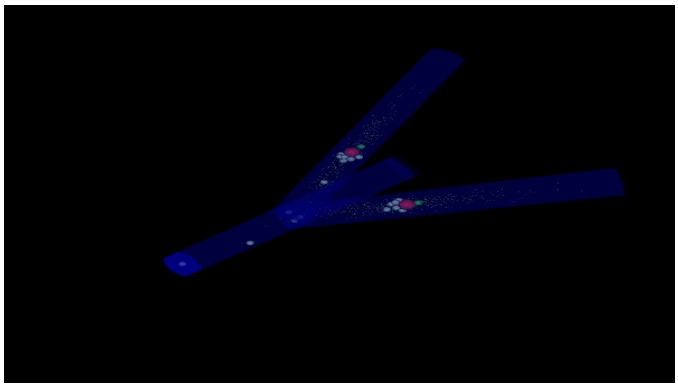

Fig. 9. Typical result after 1000 steps in the first test case. Macrophages are shown as large red spheres, ILCs as medium-sized green spheres, T-cells in white, and cytokines as small yellow particles.

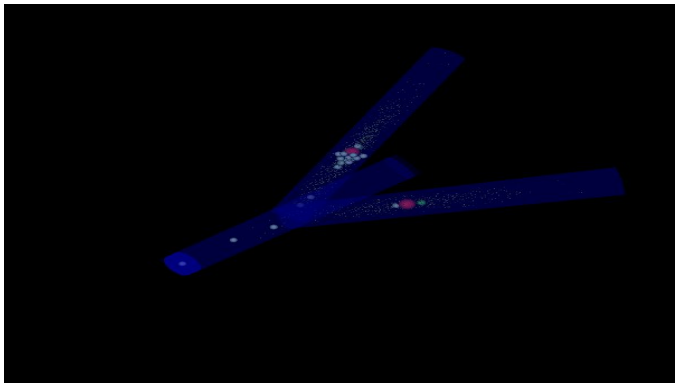

Fig. 10. Typical result after 1000 steps in the second test case. Macrophages are shown as large red spheres, ILCs as medium-sized green spheres, T-cells in white, and cytokines as small yellow particles.

In the third test case, sometimes a single T-cell wandered towards the disadvantaged side before the T-cells which went the other way stimulated the macrophage on their side and the cytokines had time to diffuse towards the central sinusoids. Once the cytokine gradient was established, the rest of the T-cells all migrated towards, the side that had the advantage. As there was no negative feedback mechanism balancing the positive feedback loop, the difference between the two sides continuously increased, and there was no chance for the second macrophage to form a granuloma at a later point (see Figure 11). Therefore, based on this hypothesis, the current model could explain why certain granulomas form earlier, but not how the formation of the later granulomas is triggered. However, this result is not very surprising, and it is not suitable to disprove the positive feedback hypothesis in general. But instead, it points to the question of which negative feedback mechanisms could balance the positive feedback; in nature, unbounded processes rarely exist.

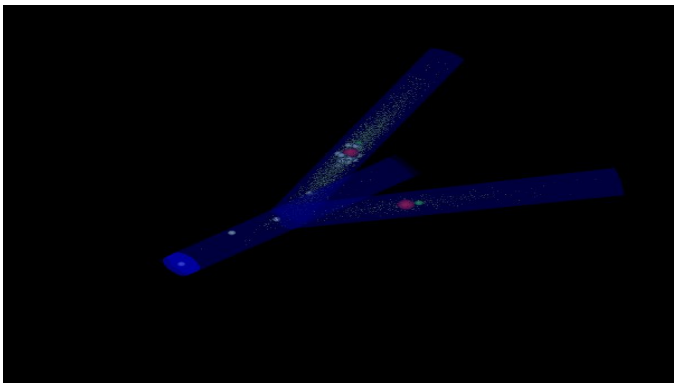

Fig. 11. Typical result after 1000 steps in the third test case. Macrophages are shown as large red spheres, ILCs as medium-sized green spheres, T-cells in white, and cytokines as small yellow particles.

VII. Summary

The current stage of the project presented here is still too early for the model to answer any specific biological question. However, it was already possible and very encouraging to see how presenting a different view on a biological system immediately provoked us to come up with new ideas for experimentation. Of the early conceptual diagrams, especially the sequence diagrams stimulated us to see things in a new light. We readily came to the conclusion that in reality, everything would probably be much more complicated. However, we still thought that it would be an interesting experiment to deplete mice from those ILCs that are supposed to initially stimulate the macrophages. Simple class diagrams showing the structural relations of the entities can probably not provide any new insight or deeper understanding to someone how already has domain knowledge.

The choice of a 3-dimensional visualisation was crucial for the present study as it allowed us to think in a more 3-dimensionall manner think about how immune cells interact within liver sinusoids. This reinforces Cohen's [2] claim stating that an appealing visual presentation is an important property of a model to help to get an intuitive understanding of the processes. But additionally, it might also point to a further role a good model should fulfil; it should compensate for the specific weaknesses of the available experimental methods. For example a weakness of microscopy might be that it only provides a 2-dimensional view on the microscopic slide.

A further result of the modelling process was an increased awareness of the amount of biological information that is not yet available. For example, it is not clear how many granulomas exist on average at a specific time point after the infection in the liver. Similarly, we do not know whether those macrophages around which granulomas form earlier, are on average closer to the entrance from the blood circulation. These gaps of knowledge suggest new scientifically worthwhile experiments. Another experiment that could be conducted and that was also inspired by the model would be to track the macrophages within the first hours of the infection in order to investigate in more detail if fusion of macrophages plays a decisive role in the onset of the granuloma formation.

Acknowledgements

This work is part of the CoSMoS [3] project, funded by EPSRC grants EP/E053505/1 and EP/E049419/1. Anton Jakob Flügge was part funded by a Microsoft Research studentship and the German Academic Exchange Fund (DAAD).

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