Università di Camerino

Obesity Paradox in Repast

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Distributed Calculus and Coordination
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The Obesity Paradox

Obesity is associated with an increased risk of developing clear cell renal cell carcinoma (ccRCC) but, paradoxically, obesity is also associated with improved oncological outcomes in this cancer.

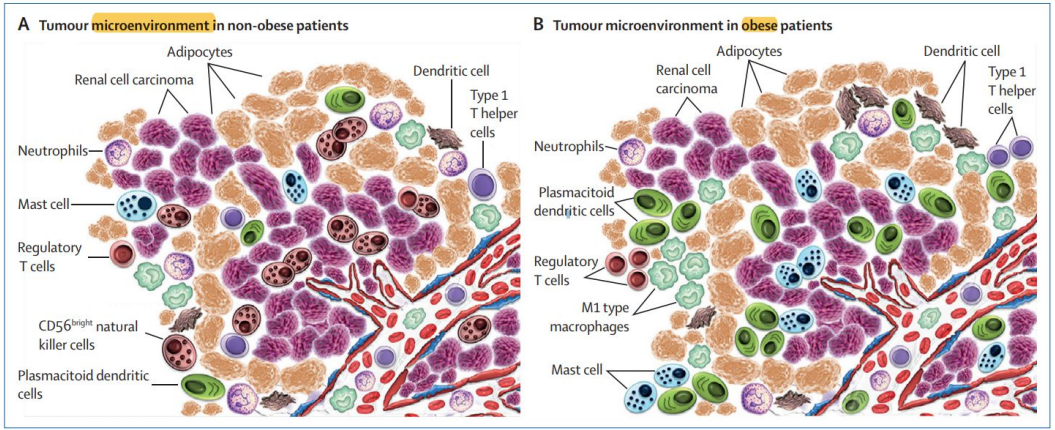


Figure 1. Perinephric tumour microenvironment in non-obese vs obese patients with renal cell carcinoma. [1]

Goals

- Use the agent-based approach with Repast
- Model Renal Cell Carcinoma microenvironment
- Model cells behaviours and their interactions
- Perform batch runs and analyse the results

Why Repast?

- First Game of Life implementation in Netlogo (user-friendly platform but not so useful as complexity increases)
- Repast Symphony:
  - Well documented
  - More flexibility
  - Access to the whole range of Java libraries

Environment - Body Mass Index (BMI)

Ratio between weight and square of the height of an individual and is used as an indicator of the state of ideal weight.

Main factor that affects the number and proportion of initial immune cells:

- More Natural Killer in lean individuals
- More Dendritic, Mast Cell and M1 Macrophage in obese individuals
- Higher CD4/CD8 ratio in obese individuals

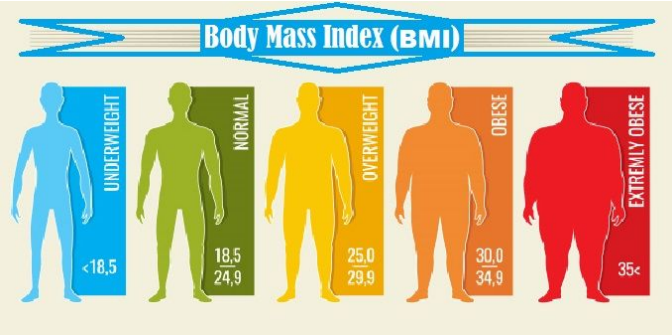


Figure 2. Body Mass Index representation.

Environment - Blood Vessel

Component of the circulatory system that transports blood cells, nutrients, and oxygen to the tissues of the body.

The blood vessel can be the target of tumour angiogenesis, in which case tumour grows in an uncontrolled way.

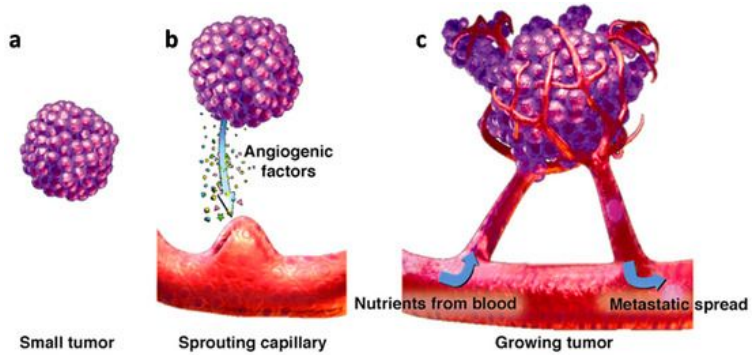


Figure 3. (**a–c**) Tumor expansion induced by the sprouting of blood vessels. From small tumor (**a**), sprouting capillary (**b**) to growing tumor (**c**). [2]

Environment - Lymph Node

Organ of the lymphatic adaptive immune system and major sites of lymphocytes that include B and T cells.

Lymph nodes are important for the proper functioning of the immune system, acting as filters for foreign particles including cancer cells, but has no detoxification function.

Once a Dendritic cell has reached the Lymph Node, carrying the antigen with it, if there is a T cell with the appropriate T cell receptor, it will be activated.

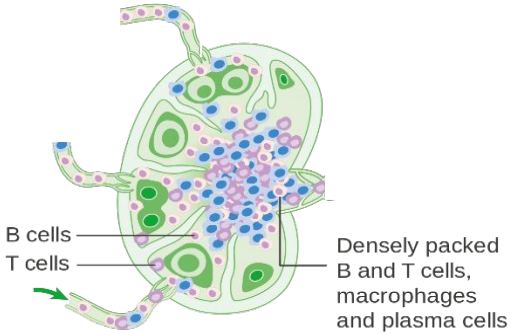


Figure 4. Lymph node representation. [9]

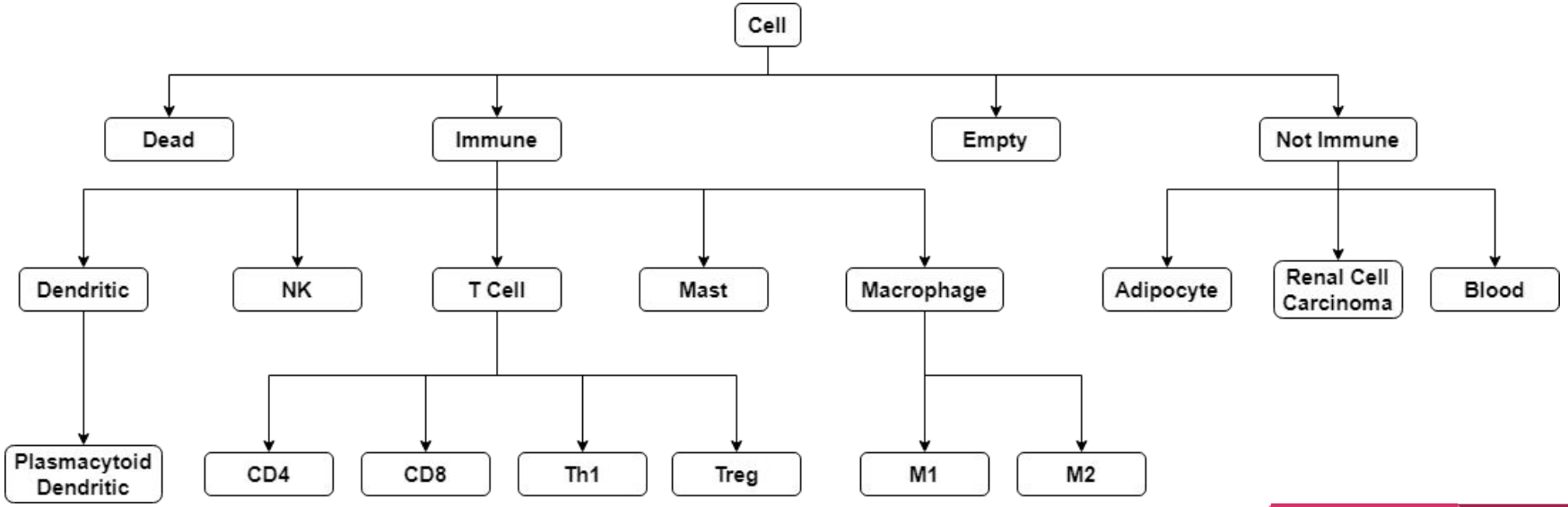
Agents

Agents are all those cells that are useful in our context and are divided in:

- Immune
- Not Immune

There are also other cells which have been useful in modeling the behavior of the immune system and which we will discuss briefly.

Agents Structure



Agent - Cell

Implementation:

Cell class is the root of all the hierarchical structure of classes.

Main features:

- lifespan
- current age and the fact that it can die
- attribute *self* to check if it is foreign or not

It could be either a Immune Cell or a Not Immune Cell.

Agent - Immune Cell

Overview:

Agent that represent all the cells that are part of the immune system.

Implementation:

Main features:

- attribute *active* to check if its effect can be activated
- random movement to a nearby empty cell by default
- can proliferate if a given percentage of the lifespan has been reached
- when it is not active, by default, it looks for a nearby tumor and eventually becomes active (*actIfNotActive* method)
- when active, each cell has to override the *actIfActive* method

Agent - Renal Cell Carcinoma

Overview:

Tumor: mass of tissue that grows in excess and uncoordinated w.r.t. normal tissues.

Tumor cells are cells whose DNA has been modified, resulting in a change of the cell's behavior.

They need a dedicated blood supply to provide the oxygen and other essential nutrients in order to grow (Angiogenesis).

A tumor cell can also implement strategies for not being recognized by the immune system's cells (hiding MHC class I molecules). In this way, cells of the immune system treat a tumor cell as a self one and no action is performed against it.

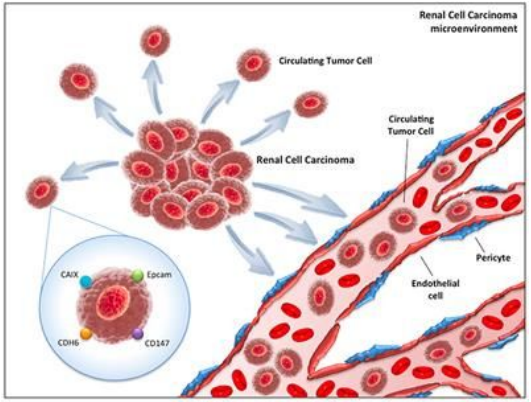


Figure 5. Circulating tumor cells (CTCs) in renal cell carcinoma microenvironment. [3]

Agent - Renal Cell Carcinoma

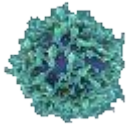
Implementation:

- **Reproduction:** *grow* method check if the time to expand has arrived (*reproTime*) or if there is a Mast Cell that is pro-tumor in its neighborhood: if one of these conditions is satisfied, the method *reproduce* is invoked to create new tumor cells, depending on *reproFactor* variable

- **Hiding MHC:** when a tumour grows it can mutate and with a certain probability it changes the *self* variable to true

- **TCell inhibition:** done by setting the TCells *active* variable to false, with a certain probability

Agent - CD4+ Naïve T Cell



Overview:

Helper T Cells (also called CD4+ T Cells) help other cells of the immune system by releasing cytokines, small protein mediators that alter the behavior of target cells that expose receptors for those cytokines.

Upon activation they are able to differentiate themselves (via autocrine signaling) into either Th1, Th2, Regulatory T cells and other kinds of cells.

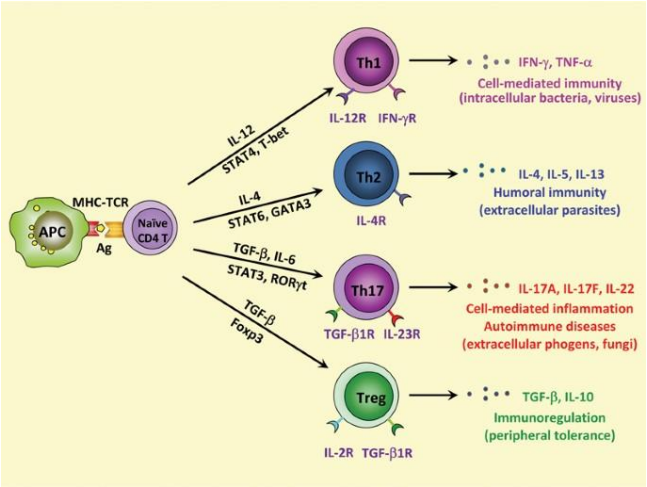
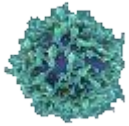


Figure 6. Differentiation of a CD4+ Naïve T Cell.

Agent - CD4+ Naïve T Cell



Implementation:

This kind of cell can be activated from any APC, such as Dendritic Cells, Macrophages, B cells and so on. Upon activation this cell can only differentiate either into Th1 or Treg.

It has a 10% probability to differentiate into Treg, otherwise it becomes a Th1.

Agent - CD4+ Helper 1 T Cell

Overview:

Th1 cells mostly produce IFN-γ, interleukin (IL)-2, and TNF-β. [6]

These cytokines are able to activate and maximize the killing efficacy of the macrophages and the proliferation of CD8+ T Cells.

This kind of Helper T Cells also enhance the production of opsonizing, and some kind of antibodies involved in antibody-dependent cell cytotoxicity.

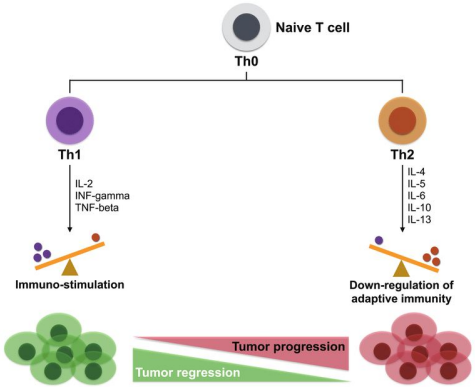


Figure 7. Th1/Th2 role in tumor microenvironment. [Lin et al. (2017)]

Agent - CD4+ Helper 1 T Cell

Implementation:

The release of IFN-γ and TNF-β has been simulated (implemented) defining 2 methods:

- **releaseIFNGamma**: simulates the release of IFN-γ by activating all the macrophages that live within a certain radius of distance from the actual Th1
- **releaseTNFBeta**: simulates the release of TNF-β by activating all the CD8+ T cells that live within a certain radius of distance from the actual Th1

After the release of the cytokines this cell is deactivated and moves randomly like any other immune cell.

Agent - CD4+ Helper 2 T Cell

Overview:

Th2 cells are responsible in helping the humoral immune response enhancing mast cell differentiation, B Cells activation and eosinophil growth and differentiation factors.

They also play an important down-regulatory role by inhibiting Th1 cells and macrophages.

Implementation:

We have not provided an implementation for this cells because their role is mostly useful when there is an infection by parasites, while Th1 cells are most important in our case because they have a role in intracellular immune response. [6]

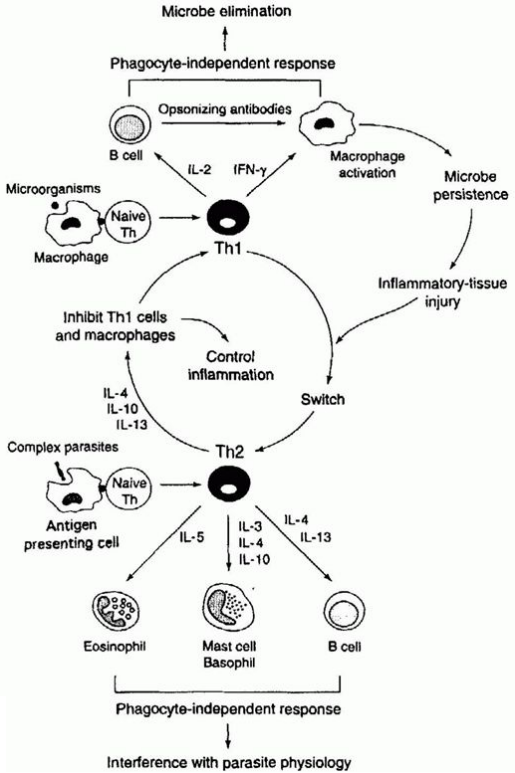


Figure 8. Th1/Th2 interactions [6]

Agent - CD4+ Regulatory T Cell

Overview:

When active, Tregs suppress activation, proliferation and cytokine production of CD4+ T cells, CD8+ T cells and other immune cells.

They do this by secreting TGF-β that suppress proliferation and differentiation, and similarly IL-10 that is able to suppress cytokine synthesis of IFN-γ, TNF-α and other cytokines, produced by cells like macrophages and some kind of T helper cell.

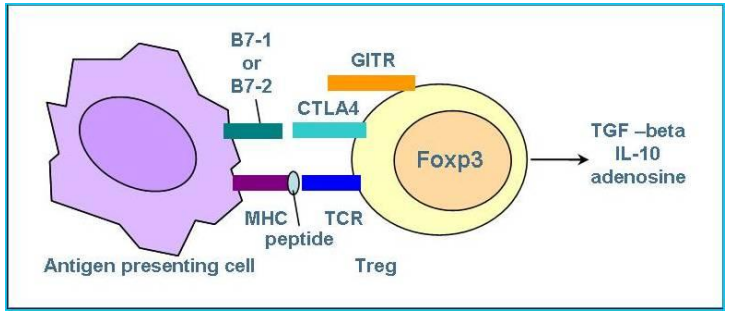


Figure 9. Activation of a Regulatory T Cell. [5]

Agent - CD4+ Regulatory T Cell

Implementation:

The suppressive role of Regulatory T Cells has been simulated (implemented) defining 2 methods:

- **releaseTGFbeta**: deactivates and decreases the growth factor of every T Cell that live within a certain radius of distance from the actual Treg Cell. In the same way it also deactivates M2 cells
- **releaseIL10**: deactivates every M1, M2, Th1, Dendritic and Plasmacytoid Dendritic Cell that live within a certain radius of distance from the actual Treg Cell

After the release of the cytokines this cell is deactivated and moves randomly like any other immune cell.

Agent - CD8+ T Cell

Overview:

A CD8+ Naïve T Cell can be activated by an APC, such as a Dendritic cell, a Macrophage, and so on. When active it differentiate into a Cytotoxic T Cell.

When a CD8+ Naïve T cell recognises its antigen and becomes activated, it has three major mechanisms to kill infected or malignant cells:

- **Secretion of Cytokines:** The first is secretion of cytokines, primarily TNF-α and IFN-γ, where the latter activates macrophages
- **Secretion of Cytotoxic Granules:** The second major function is the production and release of cytotoxic granules. Through this granules they trigger the caspase cascade, resulting in apoptosis of the target cell

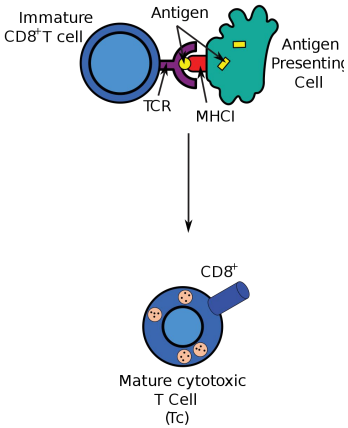


Figure 10. Activation of a CD8+ Naïve T Cell. [4]

Agent - CD8+ T Cell

Overview:

- **Destruction via Fas/FasL interactions:** The third major function of CD8+ T cell destruction of infected cells is via Fas/FasL interactions. Activated CD8+ T cells express FasL on the cell surface, which binds to its receptor, Fas, on the surface of the target cell. This binding causes the activation of a series of interactions that trigger the caspase cascade, which also results in apoptosis of the target cell

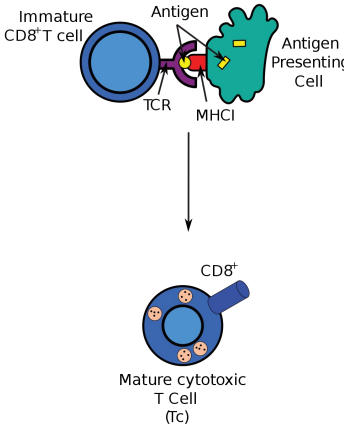


Figure 10. Activation of a CD8+ Naïve T Cell. [4]

Agent - CD8+ T Cell

Implementation:

Like all other immune cells, this cell wander within the grid until either it is activated by an APC cell or until it bumps into an RCC cell.

Upon activation it moves towards the nearest RCC cell that it is able to detect (not self).

When it reaches the RCC cell it has a certain probability (starting from 80%) to destroy the malignant cell.

In this way we implemented the second mechanism of destruction.

Agent - Natural Killer Cell

Overview:

NK are cells of the innate immune system and best known for protecting against disease and killing virally infected cells, and detecting and controlling early signs of cancer.

NK cells kill tumour cells without any priming or prior activation.
Normal healthy cells express MHC I receptors which mark these cells as self, cancer cells often lose their MHC I, leaving them vulnerable to NK cell killing.

Additionally they secrete cytokines which act on other immune cells like Macrophage and Dendritic cells to enhance the immune response.

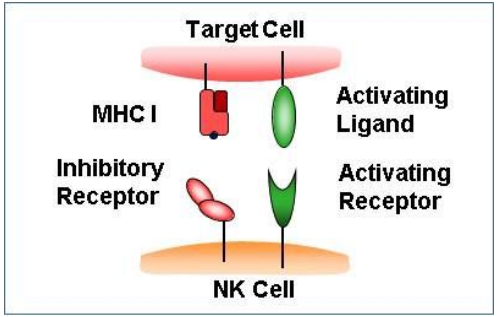


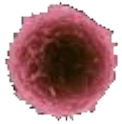
Figure 11. Activation of a NK Cell. [7]

Agent - Natural Killer Cell

Implementation:

- **Movement:** if this innate immune cell is not active, it moves randomly by default in search of a tumor cell.
- **Kill:** when a natural killer cell encounters a tumor it becomes active and tries to kill it with a certain probability (*killProb*), regardless of the presence of the MHC molecule.
- **Cell stimulation:** they also activate macrophages and dendritic cells in their neighbors by setting the *active* variable to true, simulating the release of IFN-γ and TNF-α.

Agent - Dendritic Cell



Overview:

Responsible for the initiation of adaptive immune responses, they are the most potent type of antigen-presenting cells.

During pathogen invasion, immature DCs detect intruders via pattern recognition receptor, capture antigens and quickly leave the tissue. They crawl through and migrate to the draining lymph nodes (LN).
During their migration from the peripheral tissues, DCs undergo phenotypical and functional maturation.

After reaching the LN, DCs move to T-cell zones, present the antigen to TCells and activate them. For instance only one mature DC is required to stimulate 100–3000 TCells.

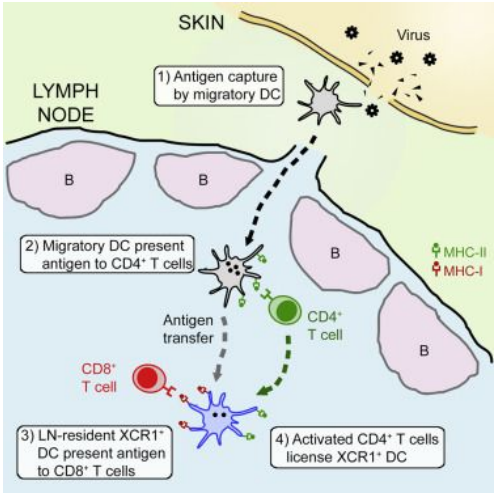
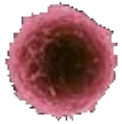


Figure 12. Behaviour of an activated dendritic cell. [8]

Agent - Dendritic Cell



Implementation:

- **Not mature:** randomly moves in search of a tumor cell
- **Mature:** when a tumor is discovered it moves in the direction of the lymph node (outside the grid, simulating the antigen presentation). On the way, if it encounters a TCell it is also able to activate its effect. When it is on the edge of the grid, a number of CD8 and CD4 are introduced into the grid (depending on their ratio). Then it become not mature

Agent - Plasmacytoid Dendritic Cell

Overview:

It is a rare type of dendritic immune cell that are known to secrete large quantities of type 1 interferon (IFNs) in response to a viral infection.

For example, the secretion of type 1 interferon α triggers Natural Killer cells to produce IFNγ.

In addition, they can produce cytokines as well, helping to recruit other immune cells to the site of infection and serve as a bridge between innate and adaptive immunity.

Implementation:

Same behavior as Dendritic cells but, once they have reached the LN, they also introduce into the grid some Natural Killer cells.

Agent - Macrophage

Overview:

Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms.

The way they interact with the immune system depends on their polarization:

- **M1**-polarized macrophages promote inflammation and immune response (they are activated by the release of IFN-γ and once active release IFN-γ and TNF-α)
- **M2**-polarized macrophages suppress inflammation and immune response (they are activated by the release of IL-10 and TGF-β and once active release IL-10, TGF-β, and TNF-α).

Agent - M1 and M2

M1 Implementation:

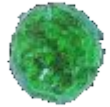
- **Immune response activation:** stimulate the activation of NK cell, Dendritic cell and other M1 by setting their *active* variable to true (simulating the release of IFN-γ and TNF-α)

M2 Implementation:

- **Immune response suppression:** inhibit the activation of TCell, Dendritic and M1 by setting *active* to false and stimulate other M2 (simulating the release of IL-10 and TGF-β)

Phagocytosis: both of them randomly move looking for Dead Cell to ingest

Agent - Mast Cell



Overview:

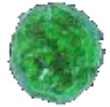
Mast Cells are best known for their activity in allergic reactions, but they have been involved in different conditions, like in renal cell carcinoma where they can promote angiogenesis.

It is a migrant cell of connective tissue that contains many granules.

During degranulation, they release a large variety of mediators that could act in an unpredictable manner depending on the context:

- ProTumor effect promoting tumor growth and progression
- AntiTumor effect promoting TCell, APC and other cells activation

Agent - Mast Cell



Implementation:

- **Movement:** randomly move in search of cancer cells

- **Effect:** when it is close to a tumor cell, it can have either a pro-tumor or anti-tumor effect (50%). If it is *ProTumor* it promotes tumor growth, otherwise it can activate the effect of nearby Tcells (set *active* variable to true), to simulate the release of mediators in its granules

Agent - Blood Cell

Overview:

Agent used to represent the blood and in particular the tumor angiogenesis effect.

Implementation:

This Class it is used to represent a single blood vessel (the other ones are supposed to be outside the grid) and it can be placed on top / bottom / left / right of the grid (as input parameter).

When a Blood cell has a certain number of tumor cells (or more) in its neighbors, it can be the target of tumour angiogenesis, in which case tumour growth is increased and a new Blood cell is created.

Agent - Adipocyte

Overview:

Adipocytes, also known as lipocytes or fat cells, are the cells that primarily compose adipose tissue, specialized in storing energy as fat.

In obese patients these cells are present in greater quantities.

Implementation:

Adipocyte class is a dummy agent, just a placeholder for cells (fat) that composes the kidney itself and they do not perform any action.

Agent - Dead Cell

Overview:

Agent used to represent a Dead cell.

Implementation:

A Dead cell is a dummy agent and can not perform any action.

A cell reaches the dead state if it has been killed by an immune cell or it has exceeded its maximum age in order to simulate apoptosis effect.

Agent - Empty Cell

Overview:

Agent used to represent a Empty cell.

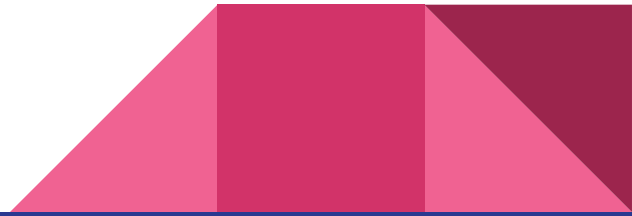
Implementation:

Empty cell is a dummy agent simply implemented to simulate empty spaces and to allow cells to move around.

Environment in Repast

The environment wasn't implemented as a Java Class but was represented only as 3 parameters that the user can choose:

- **BMI -** integer between 15 and 40. The number and percentage of initial Cells depend on this values
- **Blood Vessel -** represented as a set of Blood cells positioned in one of the possible orientations of the grid (north / south / west / east or front / back / up / down / left / right)
- **Lymph Node -** represented as a direction towards which some cells will move when active (north / south / west / east or front / back / up / down / left / right)

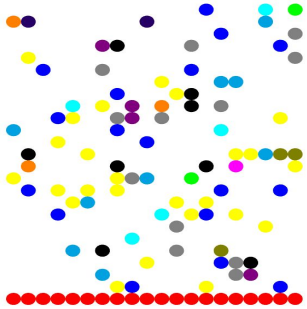


Environment - Grid 2D vs 3D

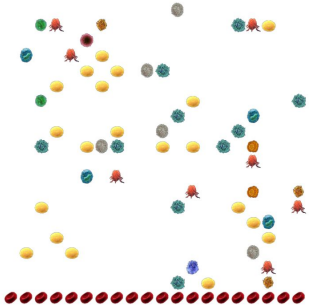
Grids are a mainstay of agent based simulation and they can be used to simulate spaces and to create highly structured relationships between agents.

Grids are a 1 or more dimensional data structure (n-dimensional matrix) that is divided up into a number of cells. These cells can be referenced by their integer coordinates.

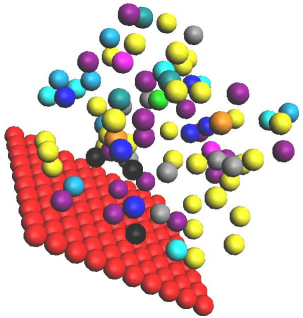
In our project we implemented both a 2-dimensional and a 3-dimensional grid in which the agents can move and interact with each other.



2D Grid. 2d representation of agents with simple circles



2D Grid. 2d representation of agents with images

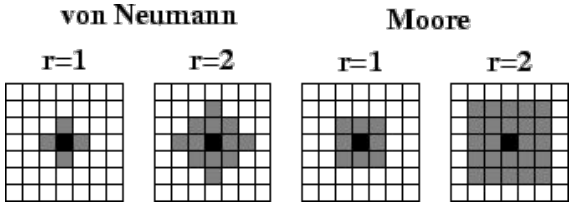


3D Grid. 3d representation of agents with spheres

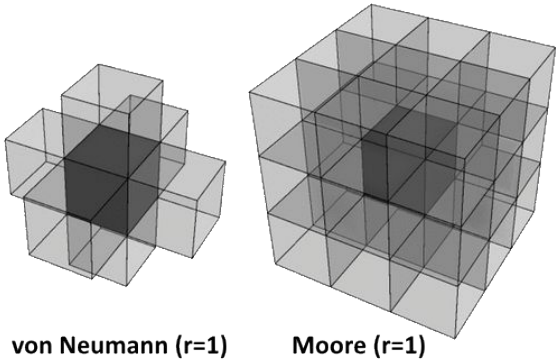
Agents - Main features

- Only one agent in each cell
- Random positioning
- An agent cannot go outside the grid
- Number of immune cells can’t be more than 10% of the grid’s size at tick 0
- Number of each immune cell depends on BMI or on cd4/cd8 ratio
- Renal cell carcinomas occupy 2% of the grid’s size
- NK and CD8 kill probability are internally set and don’t change

Agents’ Behaviour and Effects

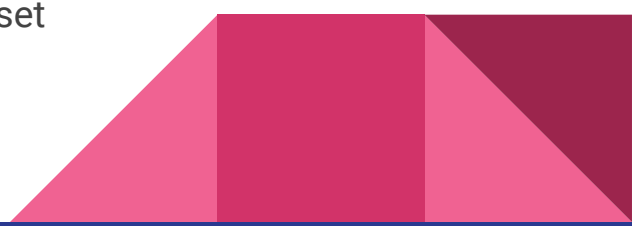


- *@ScheduledMethod* annotation to manage the priority of each action
- Interaction between agents and effects range have been implemented in one of these three ways:
  - neighborhood - an agent or an effect may influence only the nearby cells (Moore)
  - radius - an agent or an effect may influence only the cells within a certain radius
  - grid - an agent or an effect may depend on or influence the whole grid (e.g. spawn new cells, move towards the nearest tumor, etc…)
- Most actions and effects happen with a certain probability



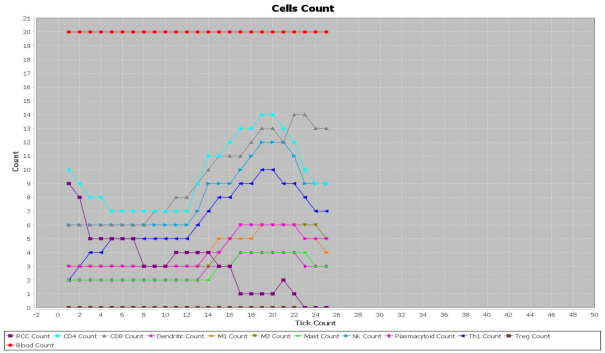
Repast settings

- **Styles and Displays**: different styling and displaying options for agent visualization:
  - 2D display with cells represented as circles - XML stylesheets
  - 2D display with an image for each cell type - a style Class for each cell
  - 3D display with cells represented as sphere - a single Class to manage all the colors
- **Scenario**: ObesityParadox.rs and ObesityParadox3D.rs folders with all the configuration files needed for the context, displays, dataset loaders, parameters initialization and so on



Model and visualization

- **Chart**: other than visualization styles and displays, Repast Simphony allows for the creation of Time Series Chart and more. In this case we created only one chart containing all the cells in the grid at each tick, to better see how the environment evolves, but the possibilities are endless
- **Data Set**: is the model data in memory, whose content is, however, not in a human-readable format and gets deleted when Repast is closed. To avoid that, we can send the Data Set content to a Text Sink
- **Text Sink**: is used to send data to either the Eclipse program console or to an output file; since we wanted to have the model available for later analysis, we dumped the model via a File Sink, that is used to write data to a file



Time Series Chart. A chart showing the evolution of the system

	A	B	C	D	E	F	G	H	I	J	K	
1	run	tick	BMI	RCC Count	CD4 Count	CD8 Count	Dendritic Count	M1 Count	M2 Count	Mast Count	NK Count	Pla
2	1	1.0	15.0	8	9	6	2	1	3	1	6	
3	1	2.0	15.0	7	9	6	2	1	3	1	6	
4	1	3.0	15.0	6	9	6	2	1	3	1	6	
5	1	4.0	15.0	6	9	6	2	1	3	1	6	
6	1	5.0	15.0	6	9	6	2	1	3	1	6	
7	1	6.0	15.0	6	9	6	2	1	3	1	6	
8	1	7.0	15.0	6	9	6	2	1	3	1	6	
9	1	8.0	15.0	6	9	6	2	1	3	1	6	
10	1	9.0	15.0	5	8	6	2	1	3	1	6	
11	1	10.0	15.0	5	7	6	2	1	3	1	6	
12	1	11.0	15.0	10	6	6	2	1	3	1	6	
13	1	12.0	15.0	10	6	6	2	1	3	1	6	
14	1	13.0	15.0	9	6	7	2	1	3	1	7	
15	1	14.0	15.0	7	8	8	2	1	4	2	7	
16	1	15.0	15.0	6	8	8	2	1	5	2	9	

File Sink. The model output as a CSV file

Launchers and Batch parameters

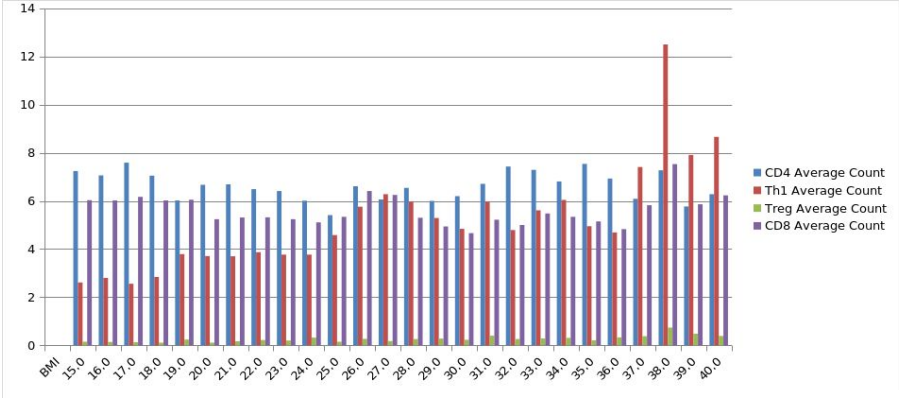
- **Parameters**: XML file used to configure other parameters:
  - Width, Height (and Depth) of the grid
  - Tumor mutation percentage, reproduction time and factor, percentage of disabling TCells
  - Immune cell growth percentage
- **Batch Parameters**: also configured through an XML file. They have the same names of the parameters, but the values may be different or even range from a starting value to an ending value with a user defined step increment. The numbers of runs can also be defined here
- **Launchers**: the Eclipse launch configuration files that are used to run the Repast model. To simplify the development and the testing phases, we created a launcher for each configuration, both 2D and 3D

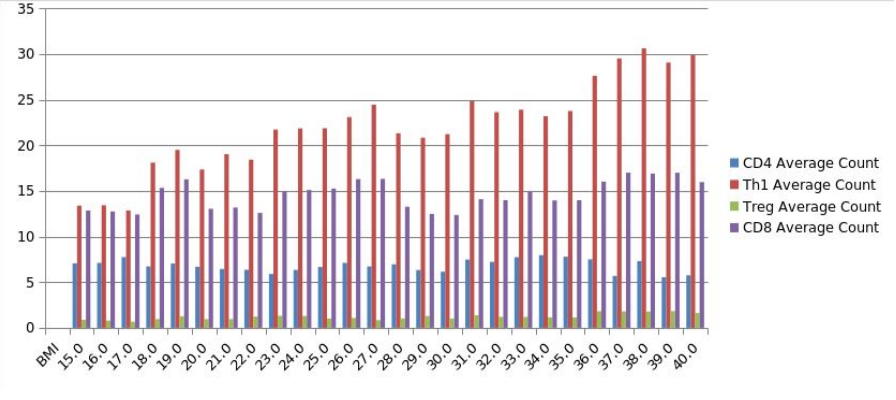
Batch runs

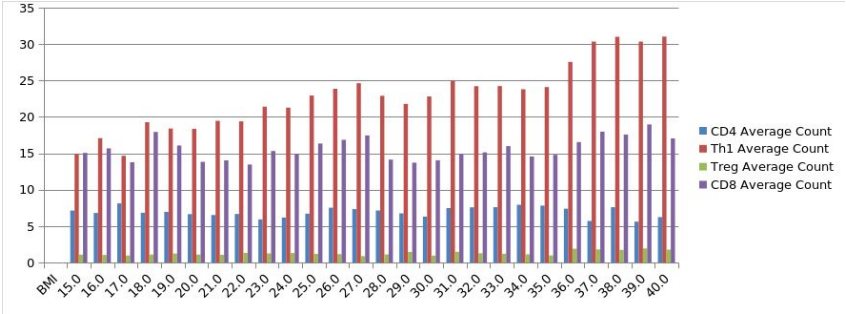
Allow to run the model several times (in order to minimize the impact of potential outliers) with a different set of parameters each time.

- 100 runs
- Duration of 100 ticks
- BMI increasing each run by 1 from 15 to 40
- A total of 2600 runs
- Data was taken as snapshots at 10, 50, 100 ticks

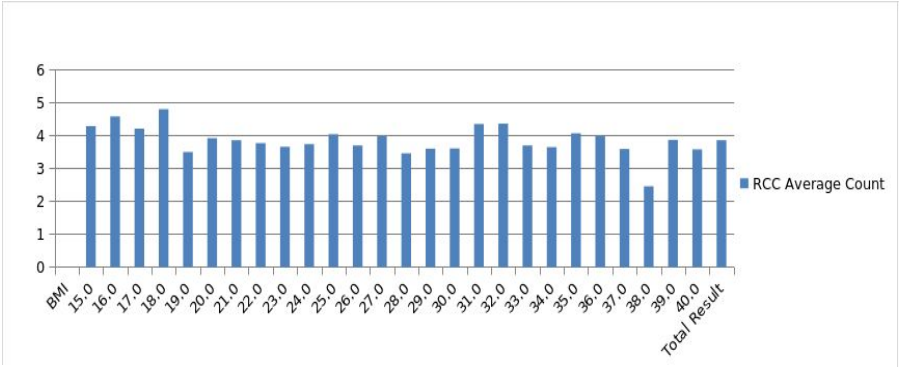
Result

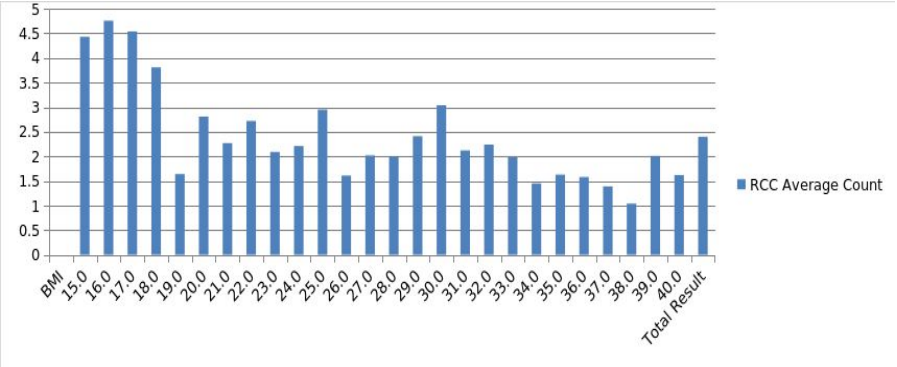

CD4 Average Count
Th1 Average Count
Treg Average Count
CD8 Average Count

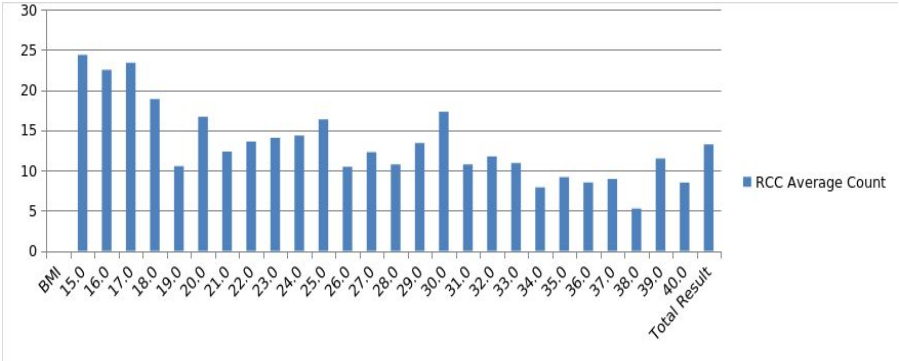

CD4 Average Count
Th1 Average Count
Treg Average Count
CD8 Average Count


CD4 Average Count
Th1 Average Count
Treg Average Count
CD8 Average Count

Result 2


6
5
4
3
2
1
0
BMI
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.0
32.0
33.0
34.0
35.0
36.0
37.0
38.0
39.0
40.0
Total Result
RCC Average Count


5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
BMI
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.0
32.0
33.0
34.0
35.0
36.0
37.0
38.0
39.0
40.0
Total Result
RCC Average Count


30
25
20
15
10
5
0
BMI
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.0
32.0
33.0
34.0
35.0
36.0
37.0
38.0
39.0
40.0
Total Result
RCC Average Count

Future works

Even though the system behaves as we expected, there are still some improvements that can be done:

- Add further cells and behaviours, such as: B Cells, Memory T Cell, Th2, opsonizing mechanism, etc…
- Add cells speed and improve activation mechanism
- Adjust cells percentage in the 3D context

Reference List

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4) Adapted from File:Antigen presentation.svg. (2020, December 16). *Wikimedia Commons, the free media repository*. Retrieved 16:12, January 16, 2021 from https://commons.wikimedia.org/w/index.php?title=File:Antigen_presentation.svg&oldid=519246997.
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9) Adapted from File:Diagram of a lymph node CRUK 022.svg. (2020, October 27). *Wikimedia Commons, the free media repository*. Retrieved 22:07, January 16, 2021 from https://commons.wikimedia.org/w/index.php?title=File:Diagram_of_a_lymph_node_CRUK_022.svg&oldid=503059642.

That's all folks!