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by

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Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Bachelorarbeit selbstständig und ohne fremde Hilfe verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt bzw. die wörtlich oder sinngemäß entnommenen Stellen als solche kenntlich gemacht habe.

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2 T Cells, Calcium Concentration

Lymphocytes form a key component of the immune system. T cells are a type of lymphocyte and are responsible for responding to viruses, fungi, allergens and tumors. Different subtypes of t cells exist, that fulfill various responsibilities. They are transported throughout the body via the lymphatic system and blood.[KCF18]

Precursor cells are formed in the bone marrow. Once they are transported to the thymus they undergo maturation and selection to become t cells. Each cell forms receptors, called t cell receptors (TCR), that respond to one perticular out of many ($10^6 - 10^9$) possible short pieces of proteins, called peptides. These peptides are attached to the major histocompatibility complex (MHC) present on antigens and antigen presenting cells (APC). Important aspects of the selection are ensuring that the t cells react to foreign peptides, but not to those present on the body's own cells.[AH24]

In positive selection cells in the thymus present peptides on their MHC. If a t cell is unable to bind, it will undergo apoptosis, a type of cell death. T cells which were able to bind recieve survival signals. Negative selection verifies that t cells will not attack the body's own cells. This is done by only selecting t cells which only bind moderatly to the peptides presented, as a strong bond sugessts that these t cells would have a high likelihood of being reactive to own cells.[Hag18] If a t cell passed both the positive and negative selection it is transported to the periphery.

There are multiple types of peripheral t cells. Native t cells respond to new antigens. Cytotoxic t cells kill cells which present peptides on their MHC compatible with the t cells TCR. Helper T cells activate other parts of the immune response. Memory t cells shorten the reaction time when the same antigen is encountered again at a later point in time. Suppressor t cells moderate the immune response.[Gan97]

2.1 Components of a T Cell

T cell components relevant in activation and subsequent changes in intracellular Ca^{2+} are listed below and schematically shown in figure 2.1.

- **T cell receptor (TCR):** Receptor on the cell surface that can recognize peptides. By the simultanious triggering of the TCR and co-stimulator signaling is induced that leads to activation.
- **Co-stimulator:** A stimulation of co-stimulatory molecules is necessary in order for signaling to occur as part of activation.
- **Endoplasmic reticulum (ER):** A series of connected sacs in the cytoplasm that is attached to the nucleus. Important functions are folding, modification and transportation of proteins.[Rog24]

- **Ca^{2+} permiable ion channel on the ER:** There are several Ca^{2+} channels present on the ER. Some receptors are responsible for releasing Ca^{2+} into the cytoplasm, when the intracellular Ca^{2+} concentration is low. [SB16]
- **Ca^{2+} storage in the ER:** Ca^{2+} is stored in the ER and can be released by Ca^{2+} periable ion channels on the ER.
- **Cytoplasm:** The semifluid substance enclosed in the plasm membrane. It contains organells, ions, proteins and molecules.
- **Stromal interaction molecule (STIM):** If the Ca^{2+} storage in the ER is depleted STIM proteins cluster where the ER is in the visinity of the plasm membrane and assembles CRAC, which then leads to uptake in extracellular Ca^{2+}. [SB16]
- **Plasm membrane:** A semipermiable structure forming the wall of the cell made up of lipids and proteins. Ion channels and transport proteins allow certain substances to move through.[Gan12]
- **Ca^{2+} release activated Ca^{2+} channel (CRAC):** Opened after a decrease in ER stored Ca^{2+} is sensed by STIM, these channels intake Ca^{2+} from outside the cell.[SI13]
- **Cytoskeleton:** A system of fibers within the cell, that allows it to change shape and move.[Gan12]
- **Nucleus:** An organelle that stores most of the DNA, controls cell growth and cell division. A double membrane separates it from the cytoplasm.[CA22]

Relevant components of APC are the

- **Major histocompatibility complex (MHC)**, which can present peptides, and the
- **Co-stimulator**, which can form a bond with the co-stimulator on a t cell.

Both are present on the surface of the APC.

2.2 Activation

Activation is necessary for t cells to divide and perform their functions.[Gan97]

When a native t cell encounters an APC that is compatible, a bond is formed between the TCR on the t cell and the peptide-MHC complex on the APC. This recognition can be triggered by less than ten molecules of foreign substance and is therefore described as near perfect. Sufficiently long contact is nececcary between the APC and the t cell in order for the t cell to activate. The role of contact time in t cell activation is modelled by Morgan et.al.[ML23].

The presence of co-stimulatory molecules is needed for proper activation. The bond between the co-stimulatory molecules on the t cell and APC plays a role in signaling. Ca^{2+} signals play a vital part in t cell activation.

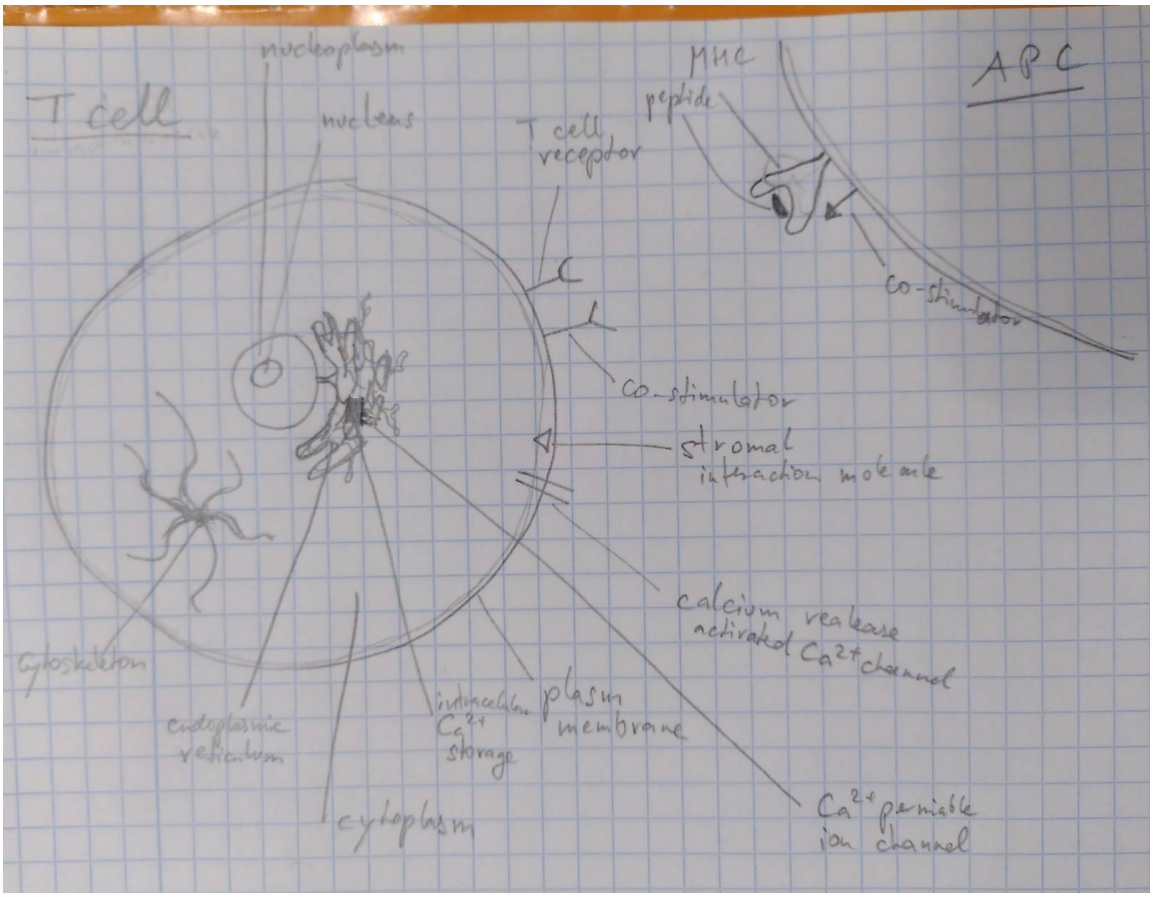

Figure 2.1: Skematic view of a t cell and antigen presenting cell, with all relevant components.

An increase of Ca^{2+} in t cells during activation is caused by the stimulation of Ca^{2+} permiable ion channel receptors on the ER membrane. Ca^{2+} is released from the ER into the cytoplasm. Additionally this decrease in Ca^{2+} is sensed by STIM, which leads to an influx of Ca^{2+} through plasma membrane CRAC channels.[SKJ09]

As the intracellular Ca^{2+} concentration is dependent on the interaction between Ca^{2+} sources and sinks, a variety of different forms in Ca^{2+} concentration have been observed. Examples are infrequent spikes, sustained oscilations and plateaus.[Lew01]

Intercellular Ca^{2+} increase together with other signals lead to a redistribution of receptors, signaling molecules and organelles.[JRB14]

3 Data

calcium concentration shows activatedness of t cells (reference chapter t cells), relativly easy to measure

3.1 Structure of Data

what format is the data in? which columns are present + datatypes

Name	Data Type	Description
x	float64	Position of cell in pixels along the horizontal axis
y	float64	Position of cell in pixels along the vertical axis
frame	int32	Number of frame, with frame rate of 1 frame per second
mass short	float64	Brightness of cell in 340nm channel
bg short	float64	Background in 340nm channel
mass long	float64	Brightness of cell in 380nm channel
bg long	float64	Background in 380nm channel
ratio	float64	Calculated as mass short divided by mass long
particle	int32	Identification for each particle

Table 3.1: Description and data type of all columns present in the data matrix.

3.2 How it was generated

exprimental setup, what types of t cells where used?, apc layer, explain steps in experiment

- Date: 18/12/23
- Cells: Jurkat wt labelled with Fura-2
- Sample: PDMS coated with OKT3 (positive control)
- Imaging: SDT3, ratiometric Ca imaging, 340nm & 380 nm, Total cycle time 1000ms (-¿ 1 frame per sec in sum/ratio image)
- pixel size: 1.6 um / px

3.2.1 Measuring Calcium Concentration

how is the calcium concentration measured? different wavelengths and then ratio between them, show example video frame

3.2.2 Processing

tracking of particles (in sum of two images), numbering them, removing bad ones (too out of focus, too short)

4 Optimization Algorithm

objective, mathematical formulation of problem

4.1 Algorithm Name

algorithm description
  pseudo code for algorithm
  [proof of convergence, if applicable]

5 Results

6 Conclusion

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