the Fair Journal

Catestatin controls immune cell travelling in inflammation

by Elke M. Muntjewerff[i], Kristel Parv[i], Gustaf Christoffersson[i,ii], Geert van den Bogaart[iii]

Original title
The anti-inflammatory peptide Catestatin blocks chemotaxis.[1]

Introduction
To fight infections caused by outside invaders (such as viruses and bacteria) or repair internal damages (broken tissue e.g. blood vessels) the body starts a process called "inflammation" to recover the affected part of the body (Fig.1). During this process the inflamed body part sends special messages called "chemoattractants" to the tissue and the blood stream to attract white blood cells such as monocytes, macrophages and neutrophils. As soon as a white blood cell receives the message it will move to the inflamed body part. Upon arrival, the white blood cell attacks the invader (for example a bacteria or virus) and aids in repair of the damaged tissue. In a healthy body these cells resolve the inflammation and help to bring the tissue back to a normal state. However, in the body of a patient with chronic inflammation the white blood cells will keep on coming without the possibility to repair the damage. These patients suffer from complications due to the chronic inflammation, and as a result, patients with chronic inflammatory diseases (e.g. Rheumatoid arthritis, Inflammatory bowel disease) need lifelong treatment.

Previous research has shown that a very small protein (or in other words "a peptide") called "catestatin" has a beneficial effect on ending the inflammation. Catestatin can be released by specialized cells in the local tissue (for example in the gut or pancreas) and even via white blood cells. However, currently we do not know much about the effect of catestatin on white blood cell movement inside the body. Therefore, we took a closer look at the properties of catestatin in attracting or blocking white blood cells' movement towards inflamed tissue.

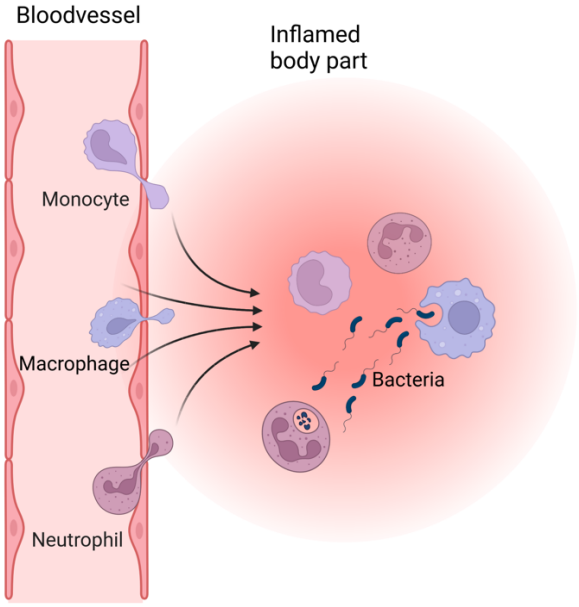

Fig. 1: Inflammation.
Figure was created with Biorender.

Findings
To be able to study the specific role of catestatin in a physiological setting, we used various models to follow the white blood cell movement directly both inside and outside of a mouse. The advantage of studying the cells outside a living organism is that it is possible to control conditions such as travelling distance for the cells or inflammatory messengers ("chemoattractants"). Additionally, this is currently also the only way to study white blood cell movement with human cells. By using both mouse and human cells, we could therefore create specific

[i] Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
[ii] Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[iii] Department of Molecular Immunology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, the Netherlands.

situations necessary to study the role of catestatin on white blood cell (monocyte, granulocyte, macrophage) movement in inflammation.

First, human white blood cells were isolated from the blood of healthy volunteers. After isolation, specific white blood cells called monocytes were isolated using magnetic beads that bind to the monocytes and pull them out from the rest of the cells. After breaking the connection with the bead, only the monocytes remain. These monocytes were placed in a special environment, called a "Gradientech chamber", where they were free to move either towards the left or the right side (Fig. 2). The cell movement is visible using a specialized microscope that takes a picture of several locations every 5 minutes. To study the cell movement, the left side was filled with a solution containing the inflammatory messenger called "Chemokine Ligand 2" (in short "CCL2"), while the right side contained only the normal liquid (without any inflammatory messengers for the cells) (Fig. 2). As expected, the cells started to move towards the messenger. Next, the left side was filled with catestatin, while the right side still only contained the normal liquid. Interestingly, again most cells choose to move towards the left side containing catestatin. This means that monocytes are attracted by catestatin in a similar way as the known messenger CCL2.

Second, to confirm the described cell movement in a physiological environment, the cell was followed by a live-action shot using microscopy in the muscle of a mouse. To visualize the various white blood cells, we used different-colored antibodies to visualize the monocytes in red and granulocytes in green. In a normal situation, the cells are not detected because they move too fast through the blood stream to be captured by the camera of the microscope. However, once these cells receive the messenger signal they slow down and start to roll along the wall of the blood vessel. They do this to be able to move out of the bloodstream and towards the affected tissue (Fig. 3). Again, in this model the addition of catestatin attracted the cells in the blood vessels in a similar way as the known messenger CCL2.

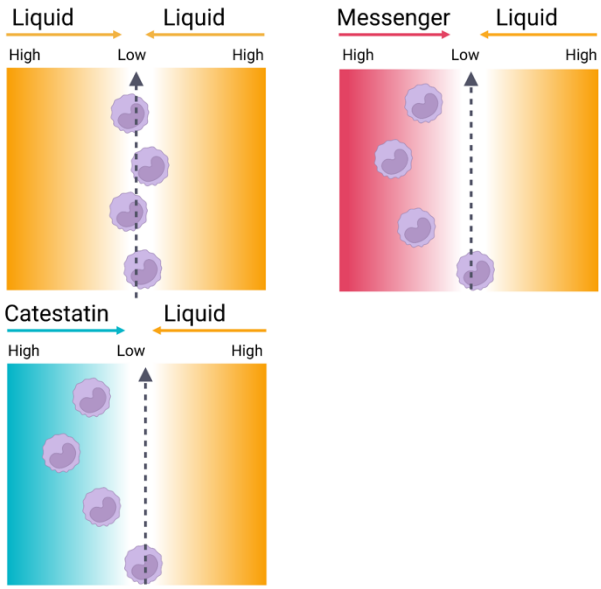

Fig. 2: Gradientech assay. Human monocytes move in the direction of the messenger (red) or catestatin (blue). *Figure was created with Biorender.*

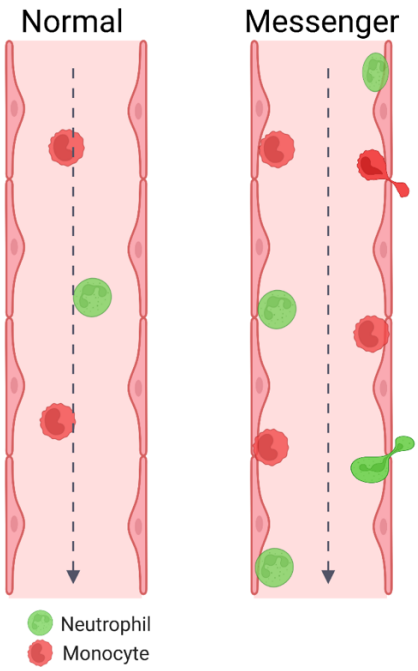

Fig. 3: Mouse model to study white blood cell travelling. Normal situation (left) and in presence of a messenger (right). *Figure was created with Biorender.*

Third, we created an environment in a plastic dish with actively growing blood vessels to mimic the environment inside the mouse, while allowing us to closely follow the cell movement and new blood vessel generation with and without messenger molecules. A tiny ring of the main mouse blood vessel (called "aorta") was removed by surgery and placed in a gel together with other cells called "pancreatic islets". In a normal situation, the pancreatic islets will produce messengers to tell the blood vessel ring that they need nutrients. As a response the blood vessel ring starts to make new blood vessels that grow towards the pancreatic islets to be able to provide them with nutrients. At the same time, macrophages (white blood cells) will start to respond to the messengers as well and travel from the blood vessel ring towards the islets using the newly made blood vessels (Fig. 4). In these experiments, the blood vessels were visualized with a microscope using a red tag and the macrophages were tagged green. Using this advanced system, we found that compared to the normal situation, the addition of catestatin led to even more new blood vessels growing towards the islets, but macrophages did not travel towards the pancreatic islets at all. This suggests that the addition of catestatin may interfere with the messengers produced by the pancreatic islets resulting in less macrophage travelling.

Finally, to learn more about the halt in immune cell movement, the previous models were used to study the effect on immune cell movement when the cells are exposed to both catestatin and the inflammatory messenger CCL2 at the same time. In the controlled environment of the Gradientech chamber from section one, the exposure of both catestatin and the messenger CCL2 resulted in a total stoppage of human immune cell movement. Meaning that in this model the immune cells no longer moved towards the right (catestatin) or the left (CCL2) side. In line with this, the physiological model from section two showed a similar stoppage of immune cell attraction. Here, cells in the mouse blood vessel no longer slowed down to roll on the wall or migrate out of the vessel after addition of both catestatin and CCL2. Altogether, these experiments show that catestatin in combination with other inflammatory messengers results in a stoppage of immune cell travelling.

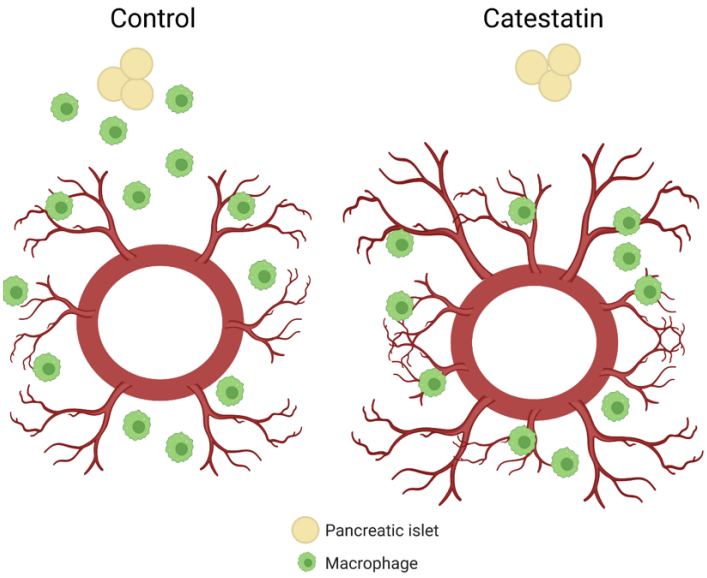

Fig. 4: Aortic ring model. Aortic ring model to study blood vessels growth (red) and macrophage (green) movement towards the pancreatic islets (yellow) in a normal situation (left) and in presence of catestatin (right). *Figure was created with Biorender.*

Conclusions

Altogether, the peptide catestatin plays an important role in controlling white blood cell movement in inflammation. On its own, the presence of catestatin results in white blood cells (specifically macrophages, monocytes and neutrophils) travelling towards the inflamed part, which in a normal situation helps to eliminate invaders or restore the tissue. However, catestatin in combination with other messengers blocks the travelling of white blood cells towards the inflamed part. In chronic inflammation this mechanism might help to reduce the constant attraction of white blood cells, that do more damage than good in this situation. Although we now know a great deal more about how catestatin controls cell movement, more

research is still necessary to define the exact working mechanism in patients with inflammatory diseases.

Article info
Submitted by: Elke M. Muntjewerff[i], Kristel Parv[i], Gustaf Christoffersson[i,ii], , Geert van den Bogaart[iii]. Reviewed by Jonas N. Søndergaard (editor at the Fair Journal). ID: 2022.01.28.000013. Please refer to the original article[1] for more technical details.

Author affiliations
[i]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
[ii]Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[iii]Department of Molecular Immunology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, the Netherlands.

References

1. Muntjewerff, EM, Parv, K, Mahata, SK, *et al*. *The anti-inflammatory peptide Catestatin blocks chemotaxis*. J Leukoc Biol. 2021; 00 1– 6.