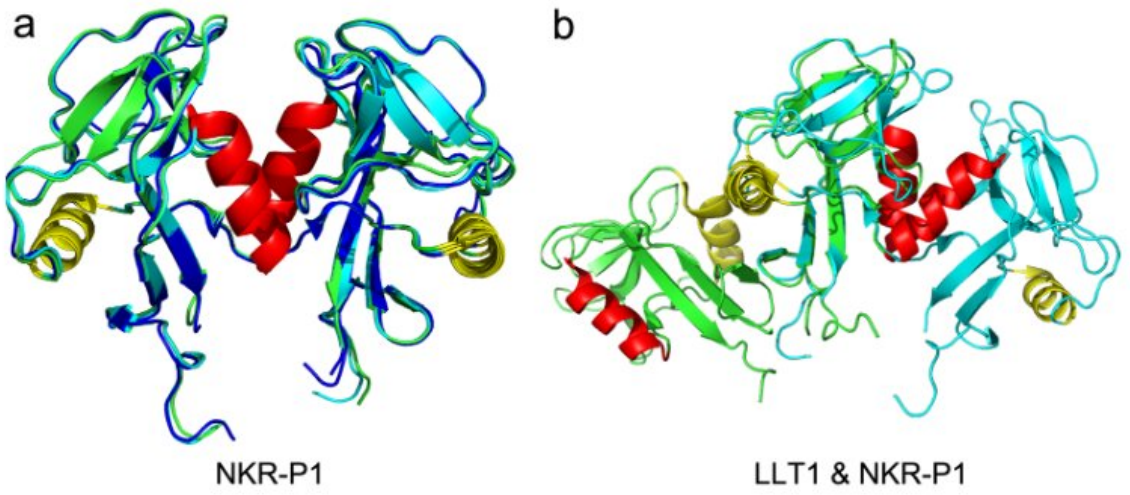
From the atom to natural killer cell: The story of an unexpected protein structure

September 20 2022



Structure of human NKR-P1 showing the unique dimerization interface. Panel (a) compares crystal structures of the NKR-P1 receptor binding domain dimers. Panel (b) shows a structural comparison of LLT1 (green) and NKR-P1 (cyan) dimers prepared by overlapping only one monomer from each dimer (middle). Although they both share a similar structure, their dimerization mode is quite the opposite. Credit: Charles University

The discovery of a peculiar protein structure and the quest to confirm it has led to the description of interacting receptor clusters on natural killer (NK) cells. The study by the research team of Dr. Ondřej Vaněk from the Department of Biochemistry, Faculty of Science, Charles University,

and his colleagues from the Institute of Biotechnology of the Czech Academy of Sciences in the center BIOCEV was recently published in the journal *Nature Communications*.

The Laboratory of Structural Biochemistry of Immune Recognition, led by Dr. Ondřej Vaněk, produced an exciting story. It all started a few years ago with observing an unexpected protein structure of a receptor, and ended with a detailed description of specific structures and interactions of the immune system.

"We are interested in how the immune system cells recognize whether other cells in our body are healthy or unhealthy," explains Dr. Vaněk. His research team focuses primarily on NK cells, which are part of innate immunity, and if they sense that another cell in the body is not healthy, can quickly eliminate it. Structural immunology here seeks to discover how receptors on the surface of immune cells recognize proteins (or other structures) on the surface of another cell. "These proteins tell the NK cell whether or not all is well. What ends up happening is not just the interaction of two proteins, but it is the interplay of a number of interactions, and ultimately either an inhibitory or an activating signal will prevail," Dr. Vaněk explains.

The study just published focuses on two proteins and their interaction. One of them is a receptor on NK cells, called NKR-P1. This receptor is interesting because it serves as one of the main surface markers by which NK cells can be defined, although its structure has been unknown until now. The NKR-P1 receptor is also found on the surface of some specific subpopulations of T lymphocytes, which are implicated in several autoimmune diseases. In this context, however, its action is not yet well characterized, probably changing from purely inhibitory to costimulatory and thus contributing to the development of these diseases.

The second protein the study focuses on is the ligand of the NKR-P1

receptor, the protein called LLT1. This protein is normally found on other cells of the immune system, and as Dr. Vaněk describes, "When cells interact and touch each other's surface, it makes them say they know about each other and everything is fine." However, the last fifteen years of research have evidenced that in many cases of cancer, the LLT1 protein is expressed on the surface of cancer cells, where it serves to inhibit the immune response. Dr. Vaněk adds, "Unfortunately, the worse the tumor type, the higher surface expression of LLT1 protein." He and his colleagues were the first to describe the structure of LLT1 in 2015.

This paper describes the two proteins and their interaction at many levels, from the atomic structure to the cellular level. The research team first produced the proteins, crystallized them, and solved the structure of their complex.

"The result was quite unexpected and interesting. One wonders at that moment whether this is just an artifact of the crystal or whether such a structure really exists on the cell surface," Dr. Vaněk observes. The next rather complex step of the research was super-resolution microscopy, and the following phases of the study were carried out on the cell surface and live NK cells isolated from donor blood. By combining several methods, the research team verified previous observations in the crystal structure of the complex of both proteins and described the resulting functional consequences—under what conditions the NKR-P1 and LLT1 proteins must meet to produce an inhibitory signal.

Both the NKR-P1 receptor and its ligand LLT1 are homodimers, i.e., they always form pairs of two identical chains on the cell surface, connected by disulfide bonds. So far, the idea has been that when the two proteins interact, one dimer of the receptor binds one dimer of the ligand. However, thanks to the crystal structure of the NKR-P1 complex with LLT1, we know that this is not true: Half of the receptor dimer interacts with half of the ligand dimer, allowing the formation of binding

clusters of these molecules on the surface of the NK cell when it interacts with the target cell.

It took several years of research to test this hypothesis from the atomic to the cellular level. The affinity of the studied proteins is very weak, and it is only through clustering that it does become strong enough for the NK cell to sense the inhibitory signal. The necessity for multiple molecules to meet is thus a kind of evolutionary protection against unnecessary or false stimuli, and thanks to the new study, we can see exactly how this interaction works at the structural level. This may help design therapeutic proteins that could desirably influence the interaction between the immune system and cancer cells.

The study was carried out by Dr. Ondřej Vaněk's team at the Faculty of Science of Charles University in collaboration with the team of Dr. Jan Dohnálek from the Institute of Biotechnology of the Czech Academy of Sciences (BIOCEV), who was involved mainly in the structural analyses. Two researchers from the University of Oxford also contributed significantly to the research, performing crystallization and X-ray diffraction measurements.

"Several generations of students from our lab have been involved in this study, and the first author, Jan Bláha, did his Ph.D. on this research. Gradually, we learned more and more methods, and the students advanced a lot. Some of them are now working at some of the best European research institutes," Dr. Vaněk explains.

Jan Bláha, the first author of the study and now a postdoctoral fellow at EMBL Hamburg, says, "The most interesting thing for me while working on this project was discovering new insights in relatively common data that led us to more complex experiments. I learned not to be afraid to follow my own crazy ideas as long as they are based on the data. I have come to understand that many of the world's experts are only human,

and the most passionate ones are playful and willing to help with any crazy scientific idea."

More information: Jan Bláha et al, Structure of the human NK cell NKR-P1:LLT1 receptor:ligand complex reveals clustering in the immune synapse, *Nature Communications* (2022). DOI: 10.1038/s41467-022-32577-6

Provided by Charles University