Killer cells

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A team of researchers from ULg is studying "Natural Killer" (NK) cells. These cells, which are still relatively unknown, are lymphocytes capable of spontaneously killing certain cells that are infected by a virus as well as some cancerous and metastasizing cells. One of the challenges of modern immunotherapy will be to better understand their functioning in order to succeed in maximizing their action.

Scientists at the Experimental Pathology Laboratory of The Infection, Immunity and Inflammation Unit of the GIGA-Research Institute of the University of Liège, under the supervision of Nathalie Jacobs, research associate at the F.R.S-FNRS, have published two papers on this fascinating subject. The first, a journal article co-signed as first author by Inge Langers (1) and Virginie Renoux, retraces the advances in understanding of atypical cells since their discovery in the 1970s and their role in the fight against tumors and metastasis as well as against virus infection. The second publication by Virginie Renoux (2), which made the cover of the European Journal of Immunology in November 2011, studies the role of NK cells against the human papillomavirus, a virus which can cause cervical cancer.

These are two promising publications which represent another step in the fight against one of the major diseases of our time and which aim to understand or at least reveal part of the major role played by those human cells that act as first-class allies and protectors against viral infections and cancers.

A bridge between two types of defense against attack

In the case of invasion by a pathogenic agent, the body defends itself in two ways.
• In an "innate" way (innate immune response): this is a quick response due to the recognition of patterns common to several pathogens or PAMP (pathogen-associated molecular patterns).
• In an "adapted" way (adaptive immune response): a process of education and adaptation enables T and B lymphocytes to recognize a particular pathogen or tumor cell due to their specific receptors. This response is slower to activate itself and requires the intervention of the innate response but it develops a memory allowing a quicker response in the case of a second aggression by the same pathogen.

So what can be said about these famous NK cells which resemble lymphocytes but which do not have the same specific recognition receptors as their well-known "cousins"? And also, what can be said about their ability to spontaneously kill cancerous cells or cells infected by a virus and, above all, how do they manage to recognize and lyse them? All these questions that remained unanswered for a long time at first led to the NK cells being given the somewhat unglamorous name of "null lymphocyte", because they did not have any known receptor and were therefore supposed to be without any sophisticated recognition system or memory.

"Today, some studies suggest that these cells can also undergo education processes and develop a form of memory. In order to recognize a cancerous cell, they also have receptors which function differently from those of the T and B lymphocytes. Therefore we can say that by their nature, they are a bridge between the two different types of immunity. They also interact with the innate immune cells to enable a better activation of the adaptive immune cells”, explains Nathalie Jacobs. It can therefore be supposed that the NK lymphocytes share characteristics of the two types of immune response.

**A Balance between inhibiting and activating receptors**

In order to destroy a tumor cell or a cell infected by a virus, a cytotoxic T lymphocyte must express a specific receptor on its surface which will recognize a tumor or viral antigen shown by a major histocompatibility molecule (MHC) (figure 1). However the proportion of cytotoxic T lymphocytes possessing a specific receptor for a virus or a tumor cell is very small and the lymphocyte must first proliferate due to signals given by the innate immune response in order to set off an effective response.

On the other hand an NK cell, as its name indicates, can "naturally" kill rapidly. The question is why and how?

Firstly because they already possess the molecules able to kill target cells while the T lymphocytes still have to produce them (figure 1). Theses molecules are contained in cytotoxic granules which release granzymes and perforins after interacting with the tumor cell. Perforins are **proteins** which, as their name indicates, perforate the membrane of the target cell. The granzymes are cytotoxic proteins which penetrate the target cell and break down its **DNA** to result in **apoptosis** or programmed cell death.
All NK cells possess receptors that make it possible to potentially distinguish tumor cells (figure 1) or virally-infected cells from a normal cell.
The recognition of the abnormal cell by the NK cell is made possible due to a balance between the signals induced by inhibiting and activating receptors:

A : Inhibiting receptors (discovered by Klas Kärre) recognize MHC class I molecules present on the surface of all normal cells (with the exception of spermatozoids). They enable "self" recognition by the immune system and present antigens to cytotoxic T lymphocytes. The inhibiting receptors block the lysing process independently of the antigen presented and therefore protect the normal cells from destruction by NK cells.

B : The activating receptors bind to molecules which resemble MHC class I molecules but which are expressed after cellular stress such as tumor transformation or infection. These receptors activate the lysing process if this signal is sufficiently strong to remove the inhibition caused by the inhibiting receptors.

C : In order to avoid recognition by the T lymphocytes, tumor or infected cells do not express any more (or less) MHC class I molecules, but they are equally sensitive to attack by NK cells which will no longer be inhibited by their inhibiting receptors.

These class I MHC molecules could also be behind a form of NK cell education. "For example, studies with mice deficient for some MHC showed that NK lymphocytes were not able to respond to tumor cells, suggesting that without a "self" recognition precondition, NK cells can not be activated " explains Nathalie Jacobs

Killers of cancer and a lot more besides

Although NK cells have been studied for a shorter time than T lymphocytes, their efficiency against tumor cells no longer needs to be proven. For example, it has been shown that people whose NK cells were less efficient at destroying tumor cells in a laboratory test had a higher risk of developing cancer. In addition to their role of defense against tumor cells, NK cells are also likely to attack cells infected by a virus. "Several studies have suggested an important role played by NK cells in the control of HIV infection (editor’s note: the virus responsible for AIDS). For example, people called 'elite controllers' (infected by the virus but not
having developed the disease) had NK cells with a greater cytotoxic activity than people who were not able to cope with HIV infection". Finally, NK cells also support the adaptive immune response by stimulating antigen presenting cells and by secreting **cytokines** which activate T lymphocytes.

**Cells which remained in the shadows for a long time**

NK lymphocytes were observed for the first time in the 1970s, but they still have a lot of secrets to reveal. "Scientists were more focused on the actors of adaptive immune response such as T or B lymphocytes which generate a more 'sophisticated' immunity. In addition, there are less NK cells in the blood as they represent less than 10% of the white blood cells. Until recently, only few of the markers of these cells were known, which made them more difficult to isolate and study", explains Nathalie Jacobs.

However, the interest about these cells has been up and down. Shortly after the discovery of these cells, which are able to spontaneously attack cancer cells, the first studies created strong interest in immunotherapy laboratories. "During the 1980s, researchers tried to amplify the anti-tumor activity of these NK cells, in particular by stimulating them in the presence of a cytokine such as interleukin 2 (IL2). IL2 activated NK cells killed the tumor cells more efficiently and some very encouraging results were obtained with mice", recalls Nathalie Jacobs.

Unfortunately, the results of clinical trials based on these animal studies were disappointing. Indeed high concentrations of IL-2 needed to be administered in cancer patients in order to allow in vivo activation of NK cells, but in large doses IL-2 is toxic. Moreover IL-2 activates another type of lymphocyte, the regulatory T cells. These lymphocytes ensure regulatory function: they control the immune response in order to prevent an over reaction which would lead to auto-immune diseases. In the present case, these regulatory T cells inhibited the NK cells, the same cells that the researchers were trying to activate.

**Succeeding in activating the cells in vivo**

"Following this clinical failure, and faced with the lack of understanding concerning the mechanism of action of these cells, the interest in NK cells faded. Now that we have begun to understand how these cells work, there has been a new interest in NK cells over the last few years. In order to stimulate them without activating other cells which will inhibit them, other cytokines less toxic than interleukin 2 are being tested in order to use them in immunotherapy", explains Nathalie Jacobs. More broadly speaking, the more recent discovery that these cells play an important role in the immune response as a bridge between the innate and adaptive immune responses, has led to researchers once again taking an interest in NK cells today.

Nathalie Jacobs team has focused its research on studying the different functions of NK cells. "We are focusing our work on infections by human papillomaviruses (or HPV), those viruses which can cause cancers and, in particular, uterine cervical cancer. This model therefore offers us the possibility to study the anti-viral response directed against the virus, but also the antitumor response directed against the tumors caused by these viruses."

**A double case-study of NK cell response, the HPV model**

Nathalie Jacobs team is the first one to put forward the hypothesis that NK cells could recognize the human papillomaviruses (HPV) and respond to an infection by these viruses. HPVs which infect the mucosa are very widespread. During their lives, between 50 and 75% of women will be infected by this virus. "Having said that,
of all the women infected, less than 1% will develop uterine cervical cancer while more than 90% of them will have eliminated the virus in the two years following infection."

Women who have a deficient immune response, such as AIDS patients, develop uterine cervical cancer more often. This suggests that the immune system plays an important role in the fight against this particular cancer. However, the cells involved in this immune response are not well defined. "In the literature, an infiltration of the NK cells has been described in the lesions associated with HPV infection while these cells are very rare in the normal tissues corresponding to these lesions. We have confirmed this infiltration of the NK cells with a more specific marker for these cells and therefore we have studied the interaction between this virus and these lymphocytes."

In order to study the interaction with the virus, it was not possible to use the virus directly because it is very difficult to produce the virus in a laboratory since it requires 3-D cell cultures to grow. "We therefore carried out our experiments using virus-like particles (VLP). These pseudo particles look like a virus but they do not contain its genetic material. These same VLPs are present in the existing vaccines against uterine cervical cancer."

Thanks to these virus pseudo particles, the ULg team showed that the NK cell was capable of recognizing the virus and internalizing it via a receptor present on their surface, the CD16. Interestingly, when the NK cells interact with the virus, they produce cytokines (i.e. interferon-γ) which can amplify the immune response. As the NK cells are killer cells, their lysing activity has also been analysed. The virus pseudo particles induce the release of perforins and granzymes contained in the cytotoxic granules of NK cells. HPV+ cancer cells which express molecules recognized by NK cell activating receptors are then lysed more efficiently by NK cells. On the other hand, the normal epithelial cells which do not express molecules that can be recognized by the NK cells are not sensitive to their cytotoxic activity in the presence of VLPs. "These results strongly suggest that the NK cells have a role in the host response against HPV infection. However, we are the first to have shown the activity of these cells in this pathology and not everyone is ready to believe us yet. It is up to us to convince them by improving our knowledge about NK cell mechanisms of action". This pioneering research on the role of NK cells against uterine cervical cancer development could lead to more efficient ways of preventing disease. In any event, these cells have not yet revealed all their secrets and hold more big surprises in store.