In Vitro Studies of Cytotoxic Activity of Natural Killer (NK) and Lymphokine-Activated Killer (LAK) Cells Obtained from Blood of Dogs

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This is a combination of Prof. Dr. Burkhardt's synopsis, and Pat Long's notes during his presentation October 8th, 2006 in Como Italy at the Health Symposium held by the SIBB, the Societ` Italiana Bovaro del Bernese

The immune system produces cells to mount an attack against any cells seen as foreign, or antigens. Especially two different types of lymphocytes the NK or natural killer cells, and Tc or cytotoxic T-cells (- where cytotoxic means it is toxic to cells) show this capability. The immune system is complex, and mounts a number of different types of responses to antigens. One specific type of response involves T-cells (T helper cells) producing cytokines (a signaling compound for inter-cell communication formerly named interleukines or lymphokines) which then helps activate natural killer cells and cytotoxic T-cells which will destroy the cells with the specifically identified antigen.

Tumor cells have specific surface antigens which can be recognized by the immune system which will then mount an immune response. In humans (and also in animals) with compromised immune systems such as people with AIDs this response is suppressed which makes the person more susceptible to cancers that a person with a healthy immune system would not normally acquire.

In 1985 an article published in the New England Journal of Medicine 313(23):1485-92 reported cases of people with malignant tumors where standard treatments had failed, but remission of some of the tumors was achieved by an immunotherapy consisting of lymphokine activated killer (LAK) cells. The advantage of this type of therapy would be that it allows controlled manipulation of effector cells outside the body to avoid uncontrolled and possible negative side effects.

The natural killer cells are suppressed in their killing activity by an unaltered MHC (major histocopatibility complex)I molecule which is expressed on the surface of all normal nucleated cells of the body and which allows the immune system to recognize it as friendly. For use in attacking cancer cells, which often have an altered formation of this MHCI molecule, the natural killer (NK) cells mount an unspecific and immediately starting response, while the cytotoxic T-cells (Tc) mount a response against specific tumor antigens, but with a delay.

NK cells are a special subpopulation of lymphocytes (like T- and B-cells). They are a medium size cell found mainly in the blood and spleen. NK cells will attack tumors or virus infected cells without prior sensitization.

For experimentation, the NK cells can be enriched from whole blood using a centrifuge. The cell weights will force them into separate layers using a special gradient medium. The gradient of choice is 58.% density of Percoll®. The centrifuge is used to separate a whitish band of lymphocytes from a healthy dog which can then be used for testing on different cancer cell lines.

To demonstrate cytotoxic activity of the isolated cells the test described used a cancer cell line derived from a thyroid adenocarcinoma (CTAC) cell line. S. Krakawka at Ohio State University had demonstrated that CTAC is susceptible to lymphocytes. After 12 - 14 hours of incubation there was a strong killer response and

a spontaneous killer effect. Most of the cancer cells died from necrosis caused by perforin, a toxic substance within the granules of NK cells.

But cells are also programmed to die, which is apoptosis. Apoptosis is triggered by genes, but the process can be stopped if the genes are disturbed. The genes which regulate apoptosis can be affected by a withdrawal of growth hormones, or by injury from things such as radiation, toxins, or free radicals. NK cells induce apoptosis by binding to the Fas receptor of target cells. Therefore they use both of these processes, this is the "deadly kiss" of killer cells.

The methods used to measure the spontaneous cytotoxic activity of effector cells include:
- Rose Bengal Assay (RBA - red dye) - the more the dye, the less the killing effect; this measures apoptosis together with necrosis
- Chromium Release Assay - which only measures cells with damaged cell membranes from necrosis

RBA was used in his study to measure spontaneous cytotoxic activity of isolated peripheral blood lymphocytes (PBL). They demonstrated a ten-fold increase in the cytotoxic activity of PBL after cultivation in the presence of recombinant human interleukin-2 (rh IL-2) for seven days. They also demonstrated a direct binding of effector cells to the surface of the tumor targets and both, necrosis and apoptosis of the tumor cells was the result.

A comparison was also made of the spontaneous cytotoxic activity of PBL isolated from the blood of tumor bearing dogs and healthy control dogs.

They found a statistically significant decrease of spontaneous cytotoxic activity from dogs with malignant mammary adenocarcinomas, while in dogs with other types of carcinomas or benign mammary tumors no significant decrease was observed.

In the literature three possible factors for the down regulation of an immune response against tumor cells is dicussed:
1. Soluble blocking factors secreted by tumor cells
2. A cytokine sink effect due to consumption of cytokines during the activation of an immune response against tumor cells
3. Activation of regulatory suppressor T-cells causing the immune system to accept the tumor cells as ‘self’ and thus stopping the immune reaction.

These effects may be counteracted by reactivating the effector cells in vitro by IL-2 and IL-12 outside the patient’s body, thus generating lymphokine activated killer cells LAK and reperfusing them into the patient’s body (adoptive immunotherapy).

Cytokines are small protein molecules which regulate the immune response. They are used for short distance response and for a short duration. Interleukin is a type of cytokine. IL-2 induces proliferation in activated T- and B-cells. It also activates NK cells, IL-12 leads to proliferation of these cells. IL-2 is produced by helper T-cells. From humans, recombinant IL-2 is commercially available.

A study measuring the IL-2 activity level found that IL-2 or IL-12 had to be added every 3 days. Lack of IL-2 leads to cell death.

Currently they are studying means to improve the production of LAK cells. They have generated a cell line which continuously secretes IL-2 by transfection of the gene encoding for canine IL-2 into BHK cells (baby hamster kidney cells - it is a special mesenchymal, commercially available cell line). Similar experiments are desired to achieve the permanent secretion of canine IL-12.

Conclusion: In the dog there is spontaneous cytotoxic activity against tumor cells by NK cells which can be activated and induced to proliferate by IL-2 secreting cells and enhancement of their killing activity by additional incubation with IL-12.