

Tumour immunology in Lausanne

Delicate Balances

The Columbian-born physician Pedro Romero is trying to develop cancer vaccines. A sensitive field in which he repeatedly ran into problems. One of these, for example, was caused by the restrictive behaviour of a pharma company.

Tumour biology and immunobiology are often cross-linked disciplines. The immune system plays a pivotal role in defence against pathogens as well as abnormal tissue growth as it happens during tumour development – this is nothing new. Therefore, approaches to cancer therapies include more and more immune-based methods aimed at yoking the body's natural weapons to combat the tumour.

Pedro Romero from the Division of Clinical Onco-Immunology at the Ludwig Institute for Cancer Research in Lausanne, Switzerland, pursues the same idea. Together with his group he works on the development of cancer vaccines, especially for melanoma patients. "In the recognition and destruction of tumour cells – as of intracellularly infected cells, T lymphocytes and among them the cytotoxic T lymphocytes, CTLs or also called CD8 T cells, play a central role", explains the physician. Thus, these are the players the researchers want to induce by vaccination with tumour antigens. The idea is that cancer-specific T cells should selectively destroy the tumour, with no or very low damage in the circumventing tissue.

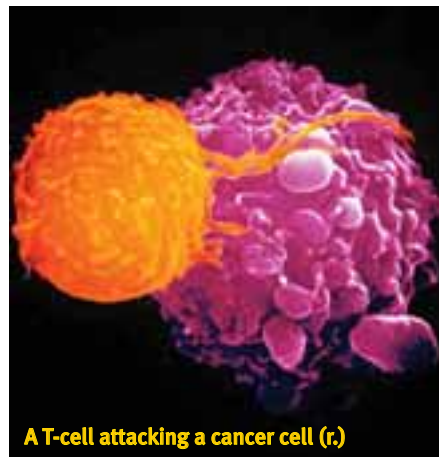
From malaria to cancer

The Colombian, Pedro Romero, has been living and working in Lausanne since 1989. He obtained his medical doctor at the National University of Colombia in Bogota and researched there at the Institute of Immunology on malaria vaccine development. He continued this work as a postdoctoral fellow at the New York University. "At that time, we collaborated with the Ludwig Institute in Lausanne to clone malaria-specific T cells", Romero describes his first direct contact to Switzerland. As a result, he joined the Lausanne branch. "Here, I first studied the molecular aspects of malaria parasite antigen recognition by cytotoxic T lymphocytes." In 1993 he shifted to human tumour immunology to search for cancer vaccines. Defining the optimal peptide based vaccines and adjuvants was and still is the goal. "A very important first step was, that we were able to monitor tumour

antigen specific T cells", says Pedro Romero. Using "tetramers", soluble fluorescent complexes of peptide, and the antigen-presenting molecule MHC class I, the researchers could identify tumour antigen specific CD8 T cells in ex vivo preparations of metastatic melanoma tumours (*J Exp Med*, 1998, 188, p. 1641).

Stimulating toll-like receptors

"This in hand, we were able to start with phase I clinical trials, testing molecularly defined cancer vaccines", remembers Pedro Romero. The outcome, i.e. the T cell response, could be displayed. The scientists vaccinate patients suffering from metastatic melanoma. "For vaccination we use a peptide from the melanoma differentiation antigen called Melan-A or MART-1 which is presented by the MHC class I molecule HLA-A2", explains Pedro Romero. As the genes of MHC molecules are polymorphic, the scientists have to make sure that the patients they are treating carry the HLA-



A T-cell attacking a cancer cell (r)

A2 allele. "And, of course, their tumours have to express Melan-A!" This melanoma-specific antigen is also present on "normal" melanocytes, which explains a potential side effect of the vaccine treatment, depigmentation or vitiligo. But compared to the possible benefit of the application, the patients would be prepared to put up with this, Romero considers.

When vaccinating with the antigen alone, the researchers did not see a detectable effect. "Not surprising, as peptides in saline are very poor immunogens", knows Pedro Romero. But for safety rules, it had to be shown that the antigen itself is not toxic.

Then, Romero and his team emulsified the antigen with the mineral oil Montanide™ as adjuvant which indeed led to an increased response of specific T cells, but only in about half of the patients.

"Well, how do we improve our vaccine?" Pedro Romero and Co. asked. And they had an idea. "What about adding toll-like-receptor (TLR) agonists to the emulsion?" TLRs play a central role in the stimulation of the immune system. About 10 different types are known today. Pedro Romero and colleagues added to the vaccine formulation a synthetic agonistic ligand for TLR-9, an intracellularly located and DNA-recognising receptor. CpG, a 23 bp based oligodeoxynucleotide rich in deoxycytidyl-deoxyguanosine dinucleotides, which is a characteristic motif for bacterial DNA, was the candidate they used. "We collaborated with the biotech company Coley Pharmaceutical Group who had developed this TLR agonist", describes Romero.

Thwarted by a pharma giant

"We tested the mixture of antigen, adjuvant and CpG in the clinic and indeed, the results were great", says Pedro Romero enthusing, meaning that a brisk T cell response in 100% of the patients could be detected. In addition, the responses to come were much faster and the magnitude of the response was ten times higher than those observed with the vaccine without the CpG (*J Clin Invest*, 2005, 115, p.1467).

Very promising results, one would say. "Yes, but we have two major problems", counters Pedro Romero. "The first illustrates how sad the things could go!" Coley pharmaceutical sold the license for using CpG in oncology to Pfizer. "They want to push CpG as monotherapy together with chemotherapy to use it as a drug," says Romero, knowing that the company is con-

ducting a randomised phase III trial in lung cancer patients at the moment. Pfizer put a moratorium on CpG for all other uses. "This means we no longer have access to CpG, we can not continue with our research!" Pedro Romero sighs. If Pfizer is able to commercialise CpG as a drug, it would be possible to obtain it, thinks Romero. "But in any case, it will take years 'til CpG comes onto the market!"



Pedro Romero (right photo) and his team

This pushes the scientist to look for other possibilities, other TLR agonists that might be as efficient. A bit frustrating to start from the very beginning again, feels Pedro Romero. "But everybody working in that field is doing so."

The second major problem, Romero mentions is, "Although these vaccines seem to be more and more efficient in inducing the desired T cell response, practically they are not in inducing tumour regression." The vaccines give objective tumour responses in about 3-10% of the treated melanoma patients. "Some say, this is already something". But Romero disagrees. "If you are a pessimist – or a realist – you see that this is no effect!"

Effect and memory

The scientists see reasons for that. "First of all, we need to keep on trying to optimise the vaccines", of this Pedro Romero is sure. This might be reached with testing different peptides and several peptides at the same time, as the scientists are trying at the moment.

Additionally, one has to be aware that T cell responses are complex things. "Of course, you need to induce effector T cells, which are active in killing target cells and production of cytokines..." But on the other hand, one has to induce a long living mem-

ory T cell response, he adds. This seems to be pretty weak or completely non-existent after Romero's vaccination. "At the moment we don't understand very well how to induce the right mixture of effector and memory T cell responses."

The population of human T cells is very heterogeneous. "You can distinguish naïve, effector and memory cells", he ex-

plains. "And you also have effector memory cells which combine characteristics of both – memory and effector T cells." This is an interesting population of CD8 T cells, thinks Pedro Romero, because understanding what they do could be essential to knowing what kind of T cells one needs to induce with vaccines.

"Shut down," signals the tumour

Recently, the researchers came a bit closer to this. They studied the nature of effector memory T cells and found that even this population consists of four sub-populations (*J Immunol*, 2007,178, p.4112). These cover a spectrum from cells that look more like resting memory cells to cells being more like active effector cells." As these cells mediate the T cell response during infection, the researchers hope they will also do so in tumour defence. "Understanding how they sustain for a very long time in the organism and how they can be very active in tumour tissues could be very important for the tumour therapy", thinks Pedro Romero. In future, the researchers want to immerse themselves deeper in the secrets of tumour specific T cell response. "We want to go from T cell populations to individual tumour responding cells and characterise them to get more insights into their function."

A third and very important aspect is that the immune system is "horrified of making damage to self tissues", as Romero says. And this is what the scientists try to trigger with their vaccines, a sort of autoimmune response. "Indeed, the tumour is tissue growing in an uncontrolled fashion but from its antigen pattern it looks very similar to 'normal' tissue." The immune system is designed to be tolerant to self antigens, unless there is a very good reason to react with a strong response, e.g. an infection. But there are important differences between the immune response induced by an acute infection and that after vaccination, says Pedro Romero. He explains, "Superficially speaking, during infection you have inflammation triggering the innate immune system." In contrast, a very "clean" vaccine preparation does not have any microbial molecules telling the immune system that now is the time to react strongly. "If the lymphocytes go to the tumour they will encounter the microenvironment in the tissue that is very 'tolerogenic', meaning there are a lot of signals that tell the T cells to shut down", explains Romero. "But we advance in understanding these inactivating signals in the tumour! Hence, the researchers generate the idea to design improved vaccines by adding signals that counteract the peripheral tolerance". They want to combine the vaccines with monoclonal antibodies that block, for example, the inhibitory molecules CTLA-4 or PD-1. Recently, the latter has been shown to mediate anergy, a sort of dormancy or tolerance on the part of T cells in chronic viral infection. "T cell function can be partially restored after blocking PD-1", Romero explains. "Probably it also works with tumour-specific T cells and we can thus improve our vaccines", the physician predicts.

Acting in concert

As in research on human beings, the available material is limited, the experiments are tedious, long and expensive; Pedro Romero places emphasis on cooperation with other groups. "On the one hand, we collaborate with clinicians at the University Hospital of Lausanne." However, he also underlines that just as important for him is the cooperation on the bench side and the tearing down of frontiers between groups, inside or outside the Ludwig Institute for Cancer Research, because he feels confident, "The strategy to act in concert with other group leaders was and will be productive."

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