Infection immunology in Zurich

Fascinated by Complexity

Long ago, Manfred Kopf was just too busy with research to write his PhD thesis. Today he is professor at the ETH Zurich and has just published another paper in a top journal – about interleukin signalling in chronic viral infections.

Virus infections are on everyone's tongue these days. In the era of swine flu, almost no day passes without any news about the rapid spread, epidemiology and disease symptoms of infection with the responsible H1N1 virus. And stories circulate about a potential horror scenario and threat to the human population, should it evolve more pathogenic, like H5N1 avian flu that killed around 65% of the infected individuals. The public hopes for success in research bringing new therapeutics and effective vaccines.

A quite extensive goal

"No doubt, the 2009 H1N1 is an interesting influenza variant", Manfred Kopf from the Institute of Integrative Biology at the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland, says too. However, it's just one of many interesting viruses, he thinks. And those, in turn, are only one among various pathogen types the immunologist looks at. To be more exact, it isn't the pathogens themselves but rather the immune responses against them, that drive him and his research group at the ETH Molecular Biomedicine lab. Thereby, the researchers don't focus on one single specific pathogen. "We study different facets of the system to get an idea of the immune response as a whole", Kopf encompasses his research interest. To reach this quite extensive goal, the biologist uses several different infection models in mice. He studies the response to viruses such as influenza virus, vaccinia virus, and lymphocytic choriomeningitis virus as well as to parasites like the protozoan *Leishmania major* or gastrointestinal nematodes. In addition, Kopf eyes dysregulated immune responses that are the basis of allergic asthma and autoimmunity. In all these reactions, T lymphocytes are key players. "We focus on their interaction with antigen-presenting cells, especially dendritic cells, and the differentiation into functionally diverse effector T cell subsets." These include cytotoxic T cells, also called $CD8^+$ cells, on the one hand, and T helper (Th) or $CD4^+$ cells on the other, which can be separated again into Th1, Th2 and Th17 subsets, according to the production of characteristic cytokines, as interleukins, the "hormones" of the immune system. The different Th cells act in response to different types of infection: Th1 cells are pivotal for the defence against many types of bacteria, viruses, protozoan parasites and fungal infection, whereas Th2 cells are responsible, for instance, for the clearance of gastrointestinal nematodes. But there is no such thing as a free lunch: we have to pay for this defence against pathogens with tissue damage caused by Th1 cell-induced oxygen radicals or allergic asthma mediated by Th2 cells.

Th17 cells received their name because they mainly produce interleukin 17. They have only recently been described, in 2003, and are thought to be responsible for autoimmunity. Interleukins (IL) are the major regulators that drive T cell activation, proliferation, differentiation and finally their effector responses. "Using knock-out mice lacking various interleukins or interleukin receptors, we want to get insights into the molecular backgrounds of these processes, to see, which factors play which role herein", Manfred Kopf explains.

A candidate emerges

A candidate he and his team recently focused on is IL-21. "Using IL-21 receptor (IL-21R) knock-out mice, we first studied the role of this interleukin in $CD4^+$ subset development in different infection models", describes the 49-year-old. The researchers found that Th2 responses to the nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* and development of Th2 cell mediated allergic asthma was impaired in IL-21R deficient mice, whereas the Th1 action was not affected (*Blood* 2007, 109:2023-31). It has been suggested that IL-21is responsible for development of Th17 cells and associated autoimmune diseases – but this could not be confirmed by Kopf and colleagues (*Eur. J. Immunol.* 2008, 38:1833-8). As IL-21 belongs to the same cytokine family as IL-2, IL-7 and IL-15, which are known to influence the development and maintenance of antiviral T cells, the researchers then focused on its effect on cytotoxic T cells. They wanted to see, if IL-21 acts in the same direction as its relatives. "For the control of viral infections, long-term maintenance of these T effector cells is important", Manfred Kopf explains. In chronic viral infections, however, virus-specific cytotoxic T cells lose their functionality, they are exhausted. "We know that the memory response of T cells requires the help of $CD4^+$ cells." But how do the T helper cells provide their support? "To come closer to that, we infected our IL-21R deficient mice with the fast replicating strain of the lymphocytic choriomenigitis virus, the LCMV-Docile, which causes chronic infection", Kopf describes. "And we found: they were particularly susceptible compared to the wild-type mice." In acute infection, in contrast, the $CD8^+$ cells responded normally. Thus, IL-21 seems to be critical for the memory cytotoxic T cells to maintain their function and to prevent chronic disease (*Science* 2009, 324:1576-80). Indeed, Kopf and colleagues found Th cells to be the cellular source of IL-21, using it to help the $CD8^+$ cells. "So far, we cannot confine IL-21 production to a particular Th subset – and it has various activities on different types of immune cells", Manfred Kopf thinks.

Manfred Kopf and .. well, *not* lab members

Since LCMV infection in mice is used as a model for chronic viral diseases in humans, the results of the Swiss have an impact on the understanding of infections

induced by viruses like HIV and hepatitis B and C virus (HBV and HCV). "Thus, researchers will investigate the role of IL-21 in those human infections in the next years", Manfred Kopf is certain. Is IL21 down-regulated in HIV patients suffering from a progressive disease in comparison to those developing symptoms later? Or is the IL-21 receptor present on $CD8^+$ cells? These will be amongst questions of interest, he presumes.

Interplays and overlapping activities

If the virus itself is able to regulate the IL-21 production to escape the immune response and persist in the organism chronically, remains unclear so far, too.

In mice, Kopf and his team want to study how IL-21 interplays with other cytokines. "IL-21 and, for instance, IL-2 have an overlapping activity that may be synergistic", the biologist imagines. When the role of both is defined more precisely and the intersection of the action is identified, the researchers hope to gain even deeper insights into how they act. "Knock-out mice for both interleukin receptors will help us here." Manfred Kopf thinks, that both cytokines could have roles at different stages in the infection, since IL-2 is produced early, whereas IL-21 not until later.

For the therapy, IL-2 was already used in the nineties, for instance, to support anti-tumour T cells in their defense against cancers. "However, this was not as effective as expected and has not come to the great breakthrough. "Combining both cytokines for treatment of viral infections or also in tumour treatment, might be more promising," Kopf thinks. "However", he continues, "this is quite speculative at the moment and only a dream of the future. As Interleukins act in picomolar concentrations in the body, very careful toxicity studies are necessary. One also has to be aware of all the manifold related players in the immune response as well as the cross-links and interaction between them. But if we understand the complex mechanisms cells use to prevent or fight against infections, we will be able to develop therapies that work against different variants of a pathogen. This would be more effective than specific vaccination against one single virus variant that changes its face over time due to selection pressure and thus escapes the treatment."

Well, complex biological systems have always been fascinating for Manfred Kopf, even as a student. He first plunged into the world of the immune system when he had a student's job at the Max Planck Institute (MPI) for Immunology in Freiburg, Germany. "The Nobel laureate Georges Köhler had just arrived at that time, which made the work there even more interesting." Thus, Kopf did his diploma and also the doctoral thesis in Köhler's lab. Like all the others in this group, Manfred Kopf started to study B cells. "When the first publication about knock-out mice came out, the technique fascinated me so much that I wanted to do it myself", he recounts. To his mentor's surprise, who saw him knock out the B cell receptor, Kopf was somehow rebellious and wanted to study interleukins, only seven of which were known at that time. Choosing IL-4 and IL-6 for knock-out, which were believed to be important for B cell development, he gave in and built a bridge to Köhler's interest. "As IL-4 plays an important role also in the immune response to infection, I got in touch with pathogens, especially *Leishmania*, at this time, too."

Group leader without a PhD

After seven years at the MPI in Freiburg, Manfred Kopf was offered a group leader position at the Basel Institute for Immunology in Switzerland, in 1995 – although he had not yet submitted his doctoral thesis. He was just too busy with research. When he finally succeeded in generating the knock-out mice, he wanted to go on and characterise them. "Getting the PhD title was not important for me at all! I wanted to understand the genes' function and thus continued my lab work!" And he wrote several papers in top-class journals, rather than his thesis.

"Many students today think – or better it has been drummed into them – that being young and getting a title as fast as possible is the most important for success", exclaims Manfred Kopf in astonishment, "but there are so many other experiences that take you further in life!" Thus, he tells his students about his biography to dissuade them from the alleged right path and to encourage them to go their own way.

"You don't have to go overseas"

Manfred Kopf eventually finalised his PhD, completing the exam within 3 weeks of moving 70 km up the River Rhine to Basle. "Collaborations with Martin Bachmann, who did his PhD in the lab of the virus researcher Rolf Zinkernagel, triggered my interest in anti-viral immune responses there." That is how, piece by piece, Manfred Kopf collected his scientific topics to-date.

Again, seven years later and, again, just about 80 km further, the biologist started at the ETH in Zurich, in 2002. "I had several opportunities including overseas in Harvard." But when he received the offer from Zurich, there was no questioning where he would go. "The research environment is top here, as it also was in Basle!" This is just another message for Kopf's students. "You don't necessarily have to go overseas for a postdoc to find great research labs and make a career in science." Of course, as an Alamannian, born in Freiburg, he somehow has an affinity to being surrounded by mountains, he admits. "The mentality of the Swiss-Germans is presumably closer to mine than that of North Germans", he laughs. And it is only a stone's throw away from his hometown – close enough for regular weekend trips. "But the major reasons to come here were clearly scientific in nature."

Now, seven years have passed again and the question arises as to whether it is time to move on. "I feel very well here at the moment", Manfred Kopf says laughing. And the ETH is currently building a new six-storey research building, including a large mouse facility, into which he and several other mouse researchers will move, once it has been finished – scheduled for the beginning of 2012. "That will bring me to a fantastic and inspiring research environment!" Let's see what transpires next. SUSANNE DORN