Van Loghem laureate Frans Claas:
"No field benefits more from the CyTOF revolution than immunology"

Claas describes how the number of HLA alleles went from 65 tot 16,000 different types almost overnight, thanks to sequencing. Even more importantly, new technologies not only enabled complication, but also simplification back to two hundred epitopes. The new insight has made it possible to connect transplantation and reproduction immunology in a mutually beneficial way.

When you study Biology and Biochemistry, making a career often means a straight route towards lab research. Claas: "Before I had my master's degree, it was clear to me that I would rather opt for translational research. The direct link between what you do in the lab and the patient's health was and is very appealing to me."
As it happened, there was an opening at Jon van Rood's group, who at the time was already an authority in transplantation. "There were some pretty interesting developments going on there", says Claas. As a student, he set up a testing model for skin transplants in mice. "At the time, supporting students during their research was virtually non-existent at the department. So when I filed my report at Van Rood, he asked a bit surprised: 'Did you do this all by yourself?'. When you graduate, you can come back and I will have a job for you'."
Claas didn't forget this and Van Rood kept his promise. "I got what I wanted: patient-oriented research in blood transfusion and organ transplantation. I was mainly involved in diagnostic work: what kind of HLA antibodies do we find in patients? At that time – 1976 - diagnosis was already fairly good, but there was also still an awful lot to unravel." Claas' PhD thesis turned out to become a side step: it was about the interaction between drugs and endorphins with polymorphic cell membrane antigens like HLA. Endorphin therapy seemed to be a promising tool for a sub-population of patients with schizophrenia and HLA appeared a potential parameter to identify these patients. This is very much en vogue at the moment, but not a track that he pursued later on.

65 to 16,000
"After that, I completely focused on transplantation", Claas concludes. At that time HLA antigens were defined on basis quite 'rough' reagents, which were sera derived from pregnant women who developed antibodies against the paternal HLA antigens of their children. In this way, about 65 different types of HLA were distinguished. During the last years, things have changed dramatically. Serology made way for molecular techniques and especially sequencing based typing led to an enormous increase of the number of HLA alleles i.e. at the moment more than 16,000 different types of HLA.
"Formerly, when we had a patient with HLA A2, we used to look for a HLA A2 donor match", Claas continues. "Now we know there are hundreds of A2 subtypes. Matching has become much more refined – and simultaneously much more complicated. After kidney transplantation, patients are treated life-long with immuno suppressive drugs, which makes the need for an exact HLA match less urgent, although the better the match the better the results. However, for a bone marrow or haematopoietic stem cell transplantation, you need a very detailed HLA match. To find it, we now use a worldwide donor database, Bone Marrows Woldwide, initiated by Van Rood. It contains the HLA types of more than 30 million potential donors of hemato-poietic stem cells. The Matchis Foundation, the Dutch stem cell donor bank, plays a central role in the recruitment of donors in the Netherlands and search for donors for Dutch patients. Claas holds a seat in the supervisory board of Matchis and the board of Eurotransplant and proceeds according to Van Rood's policies.

Epitopes
Complication due to the enormous number of HLA alleles has not only risen in bone marrow matching, but in kidney matching too. "Which donor should we select?", Claas asks. "In non-related donors, more often than not we'll have to settle for a number of non-matching HLA antigens. Which of the 16,000 types have grave mismatching consequences and which haven't? We've moved beyond simple HLA-A1, -A2 or -A3 type matching. Now we've mapped the

molecules, we can look in more detail at what the antibodies actually react to."
It has been discovered lately that each HLA molecule type has its own characteristic set of epitopes. Claas Explains: "The set of epitopes is unique – but the individual epitopes aren't. These about two hundred different epitopes can also be part of other HLA molecules. And when the epitopes are shared by the patient's own HLA, it is likely that such an HLA mismatch won't be a problem. So instead of looking for HLA mismatching in 16,000 HLA types, we are now looking on the molecular level for HLA epitope (mis)matches. The approach has gone from simple to complicated and now we are bringing it back to simple again."
But the present simple is not the same simple as before: the levels of accuracy and detail have risen enormously. "We've been comparing historical serology outcomes with molecular tests. It turned out that ten to twenty percent of HLA typing was erroneous. In twenty-five percent of cases where serology found an HLA match, in reality there wasn't. In those cases, not surprisingly, survival had been far worse."

Reversing the approach
The latest refinement, we are working on, is a qualitative approach to epitopes (mis)matching. "We already know that some epitope mismatches hardly cause serious trouble and others do. That is vital information, especialy to children who received a new kidney at young age and will need a second one around age twenty or thirty. If there are already many antibodies, incited by serious mismatches of the first transplant, selection of a second donor will be very difficult or sometimes impossible.
Other populations with many antibodies – against almost any donor - are women who have been pregnant and people who have received blood transfusion. In the past, these sub groups remained on the Eurotransplant waiting list for fifteen years or longer. Considering the high mortality n the waitig list, there was a need for an alternative approach. Claas: "We developed a strategy to enhance transplantation of these so called highly sensitized patients by testing against which non-self HLA antigens they did **not** make antibodies. If a donor with an HLA type consisting of the combination of the patients own HLA antigens and these so called acceptable HLA mismatches becomes available, the kidney is shipped with the highest priority to this patient. In this way we have bee able to transplant more than thousand of these patients, who otherwise would not have got any chance to be transplanted.
There stills remains a group for which it proves hard to find proper matches: non-western imigrants and their descendants. "Their HLA profiles differ from that of our donor populations. That is why we are trying to link with the transplant organizations in other countries, where the HLA phenotypes are different than ere, like for instance Turkey. Simulation studies have shown that this is feasible, if only we can find funding."

Reproductive immunology
A second line in Claas' research is reproductive immunology. Something completely different – or is it? "An interesting question in this respect is: how come that pregnant women not in all cases reject the child? What local immune regulation prevents that? It turns out that T-cells and immune cells in the placenta are not instructed to destroy, but to regulate. What can you learn from that for transplantation? What if the same mechanism could be used there?" A relevant finding was, that male semen liquid influences the woman's immune response. So the question is: what mechanism does this semen liquid induce? Claas: "The liquid instructs for instance the woman's dendritic cells to induce immune regulation instead of destruction of the fetus. However, semnal fluid also contains other factors, which affect the B cell response, and soluble HLA molecules of the potential father. A possinle effect of the latter is laid down in my most frequently downloaded article which suggests that oral sex helps preventing complications during pregnancy."
What goes differently in cases of multiple miscarriages? And what happens in the nicest model for transplantation, oocyte donation pregnancies, where there are more miscarriages than average? "For the answers, the kind or number of HLA antigen (mis)matches probably plays a role", says Claas.

This means, that HLA matching of an oocyte donor might make sense for a successful oocyte donation pregnancy. In a way, pregnancy and transplantation indeed are two sides of the same medal. It makes sense to connect both fields. And both fields have this same feature: you directly work for and with the patient. That is both fascinating and rewarding."
Claas is also intrigued by what happens early in the placenta. "To find that out, we try to gather material from miscarriages as well as from abortions in order to compare the immune parameters. Most interesting are the miscarriages which cannot be explained by fetal deviations. The deposition of complement factors we found in miscarriages resembles a destructive immune reaction."

Great and worrisome developments
For our recent studies, both in transplantation and pregnancy, we are now using CyTOF analysis, Claas comments. "The possibilities are overwhelming. One can characterize the different immune cells within a transplant or a placenta and study their interactions with eachother and the environment. No field will benefit more from this technological revolution as immunology. Of course this comes with its own challenges. The bottleneck is result analysis, for which we rely on scarce bio-informaticians." This trend has changed the face of science a lot. "The isolated scientist working alone in his room or his lab is close to extinction. Modern science relies on multidisciplinary teams of immunologists, bio-informaticians and chemists closely collaborating. You need a broader scope to be successful and that is a great development."
Claas is convinced that this broader scope should also include collaboration with corporate researchers. "However", he says, "I am not a fan of the current race towards patents. In many cases this slows down progress and creates all kinds of problems, often financial by nature. We've had the chance to file patents many times, but never did it. Everybody should have the chance to use the results of publicly funded research. The main focus should lie on the patient's welbeing. When idealism gets replaced by business models, that is worrisome."

Leendert van der Ent

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