**Diabetes: the virus that causes intolerance to insulin**

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While an increasing number of studies, mainly epidemiological, seem to show that coxsackieviruses (CVs) play a role in the appearance of autoimmune type 1 diabetes, the mechanisms of action of these viruses remain vague. As part of the European FP6 *Eurothymaide* project, Vincent Geenen and his team, in close collaboration with Lille's CHRU hospital (Didier Hober), the Centre d'Immunologie de Marseille-Luminy (Philippe Naquet) and the University of Tunis, have however discovered that infection of the thymus by the diabetogenic CVB4 virus leads to a decrease in the transcription of the IGF2 gene (Insulin-like Growth Factor 2). "And yet, this factor plays a major role in establishing the immune system's tolerance to insulin", Vincent Geenen explains. In short, the CVB4 virus infects the thymus epithelial cells, reproduces therein and decreases the production of IGF2 by these cells. It would appear that this drastic reduction in thymic IGF2 makes the body immune system intolerant to insulin, the hormone secreted by the pancreas whose deficiency causes autoimmune type 1 diabetes. The results of this study have just been published in the *Journal of Virology* (1). But Vincent Geenen's team is now focusing on the development of a new type of vaccine that would reprogram immune tolerance to the endocrine pancreas beta cells and thus contribute to both the prevention and cure of T1D.

Both characterised by chronic *hyperglycaemia*, type 1 diabetes and type 2 diabetes are nevertheless two different diseases. The first one affects children, teenagers and young adults and its origin is autoimmune. It represents approximately 10% of cases of diabetes. The second one concerns 90% of people affected by diabetes and occurs later in life. Type 2 diabetes is mainly due to a resistance to *insulin* and is associated with excess weight as well as a lack of physical exercise.

In people with type 1 diabetes, formerly known as juvenile or insulin-dependent diabetes, there is a autoimmunity selectively directed against the beta cells located in the islets of Langerhans in the pancreas. It is these cells that secrete insulin, a very important hormone in the regulation of the concentration of glucose in...
the blood. "The origin of this selective autoimmunity has never been resolved", points out Professor Vincent Geenen who runs the GIGA-R’s Centre of Immunoendocrinology at the University of Liège.

**Double control against autoimmunity**

"The thymus, or rather its dysfunction, may play a major role in the appearance of this disease because it’s here that the immune system is educated. It learns to recognise major hormone families such as insulin", explains the professor. In other words, the thymus is the safeguard of the adaptive immune system. Appearing in cartilaginous fishes (sharks and rays) about 450 million years ago, the adaptive immune system allows us to react specifically (contrary to the innate immune system which is non-specific) against any pathogen that enters our body. The thymus made its appearance at almost the same time and it is here that the T lymphocytes differentiate and reach maturity (2).

And it is also in the thymus that occurs the selection of T lymphocytes that will or will not be released into the body. Because not all of them mean well - far from it. "The thymus eliminates self-reactive T cells, also called ‘forbidden’ clones. This concerns 95% of T cells generated at random in the thymus", Vincent Geenen points out. "The thymus filters and lets through a mere 5% of cells, those that are self-tolerant. It is also capable of generating regulatory T (Treg) cells that inhibit forbidden T lymphocytes in the periphery that might have escaped the thymic filter". Thanks to this double control, the thymus prevents the adaptive immune system from turning against the body. "An increasing number of experimental arguments now reinforce this completely new concept according to which a defect in the intrathymic programming of central tolerance favours the development of autoimmunity, especially against pancreatic beta cells that secrete insulin", the professor continues.
It's a question of genes, but that's not all!

Type 1 diabetes (T1D) develops in people who are genetically predisposed to this disease. "There are more than 20 genetic susceptibility loci", Vincent Geenen explains. "But while we know that the genetic factor is important, it's not sufficient to cause the development of T1D", he continues. In identical twins, the concordance rate is only 45% for this disease. Therefore, there are other factors that favour the emergence of T1D. "In particular, environmental factors and among those different viruses including those from the family of enteroviruses, such as coxsackieviruses", Vincent Geenen stresses. "We have known since the 1980s that there are diabetogenic viruses and epidemiological studies have shown proof of a correlation between recent infections by coxsackieviruses and the incidence of T1D", the professor explains. Besides these epidemiological studies, there is a north-south gradient in the incidence of T1D. "In Scandinavian countries, the incidence of T1D is approximately 50 cases for 100 000 inhabitants compared with 8 in Belgium. The lowest incidence is observed in African countries", Vincent Geenen emphasises. The frequency of infection by enteroviruses is greater in the north than in the south of the planet which, according to some theories, could partially explain the existence of this gradient.

A disturbance in the tolerogenic function of the thymus?

While coxsackieviruses do indeed seem to play a role in the appearance of T1D, the mechanisms by which they act remain unknown. The hypothesis that Professor Geenen and Professor Didier Hober from the University of Lille have been investigating for more than 13 years, mainly as part of the Eurothymaide programme, is as follows: Coxsackieviruses infect the thymus and cause an imbalance in the tolerogenic function of the latter. "In 2002, Fabienne Brilot, a doctoral student at ULg who now teaches at the University of Sydney, demonstrated that coxsackievirus B4 (CVB4) is capable of infecting the thymus epithelial cells and of reproducing therein. Her work also helped to reveal a direct harmful effect of CVB4 on immature lymphocytes in the thymus (thymocytes) which differentiate into effector T lymphocytes", Vincent Geenen stresses.

In an article published in the Journal of Virology (1), Vincent Geenen, Didier Hober and their colleagues have recently found another answer. They have revealed that the infection of the thymus epithelial cells by CVB4 leads to a clear decrease in the transcription of the IGF2 gene (Insulin-like Growth Factor 2), a gene from the insulin family that intervenes in the programming of immune tolerance towards this entire family, especially during the foetal period. Thymic infection by CVB4 would therefore be responsible for a rupture in central immune tolerance to insulin and the beta cells that secrete this hormone.
Towards an anti-T1D vaccine

In order to check the specificity of the action of CVB4 on the expression of IGF2 in the thymic epithelial cells, scientists have increased the number of control experiments. "We looked at whether the same action of CVB4 was observed in other target cells of this virus. We analysed the effect of CVB4 on the expression of IGF2 by neuroblasts but no decrease in the expression of this gene was observed", Professor Geenen explains. "In addition, we tested to see if other viruses caused a decrease in the expression of IGF2 in thymic epithelial cells, and this wasn't the case", he continues. Moreover, these results have the advantage of unifying three of Vincent Geenen's laboratory major working hypotheses:
- Role of a dysfunction of the thymus that lies at the origin of the diabetogenic autoimmune response.
- Role of thymic IGF2 as a major tolerogenic factor of the insulin family, and
- The involvement of infection by CVB4 in the appearance of T1D.
With regards to the future stages of this research, scientists will now endeavour to reveal the mechanisms by which CVB4 induces a repression of the expression of IGF2 in the thymic epithelial cells. "It is necessary
to identify the missing links between the receptor for the CVB4 virus on the surface of these cells and the decrease in the expression of IGF2", Vincent Geenen specifies. But the professor's team is now focusing first and foremost on the development of a new type of vaccine, a 'negative or tolerogenic self-vaccine' based on IGF2 that would reprogram immune tolerance to the endocrine pancreas beta cells and thus contribute to both the prevention and cure of T1D.

