DNA, a protagonist in vaccination

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Most vaccines contain adjuvants which boost their effectiveness by stimulating the immune system. The mode of action of these adjuvants however remained misunderstood. Researchers at ULg discovered how alum, the most frequent adjuvant, acts during vaccination. A process where our own DNA plays a key role.

It would seem that the first form of vaccination (read Vaccines in action) was introduced in China centuries ago to protect people from smallpox. This practice, called variolation, involved putting the person to be immunised in contact with the content of vesicles of an ill person with the hope that this person was affected by a non-virulent form of smallpox... Since then vaccines have changed and are no longer based on luck!

Since the end of the 19th century we have thus seen the emergence of vaccines against a large number of illnesses such as polio, hepatitis B, diphtheria or tetanus. In particular vaccination allowed smallpox to be completely eradicated. There remain, however, infectious illnesses such as AIDS or malaria that are also real scourges for society and against which, to date, no effective vaccine is available. Aside from studying these
illnesses, the in-depth understanding of the mechanisms of vaccination is essential in order to develop new vaccines.

A vaccine contains a killed or weakened form, certain components of or a synthetic substitute of an infectious disease-causing agent. In contact with these antigens, the immune system is stimulated and prepares to defend itself against the enemy. When our organism is genuinely attacked by this infectious agent, the immune system can directly use adequate ammunition and strikes down the attacker without it having the time to provoke the illness for which it is responsible.

An adjuvant that is a well-kept secret!

Although the basic principle of vaccination is well known, the mode of action of current adjuvants, which are added to many vaccines to increase their immunogenicity and their effectiveness, is less so. Aluminium salts, often collectively referred to as alum, are the most frequently used type of additives. "Adjuvants boost the responsiveness of the immune system in the face of the antigen but also direct this response towards a certain type of immunity. They allow an increase in the production of antibodies directed against the elements of the vaccine», explains Christophe Desmet, FNRS Researcher at the Laboratory of Cellular and Molecular Physiology at the GIGA. «Alum has been used since the 1930s. Once injected, the aluminium salt forms spongy-looking macroscopic deposits. Several hypotheses linked to this spongy form have been put forward to explain why alum might increase the effectiveness of vaccines», continues the researcher.

The simplest models suggest that "the sponge" of alum gradually releases elements of the vaccine and allows the prolonged action of the vaccine in the organism. Another hypothesis relating to the alum's mode of action holds that the spongy deposit that it forms is a sort of reserve in which the cells presenting antigens come to take antigens to present them to the lymphocyte T-cell. "In the beginning scientists thought that deposit of alum was immunologically inert but, in 1985, a team discovered that it could induce the production of inflammatory cytokines, thus indicating that alum itself stimulates the immune system", specifies Christophe Desmet. However the task of discovering how alum actively stimulates the immune system proved to be arduous. In the 2000s, following the discovery of so-called Toll-like receptors, receptors that play a key role in the activation of the innate immune system, a new hypothesis was put forward: might alum activate Toll-like receptors? This hypothesis was quickly invalidated. More recently, it was discovered that alum activates another family of innate immunity receptors, inflammasomes, and that this is how it triggers the production of inflammatory cytokines. The hypothesis that alum plays its adjuvant role by activating the inflammasomes was thus put forward. However, it still faces problems of experimental validation. Until now, the secret behind alum’s mode of action remained safe!

From research into asthma to the key to the mystery of alum

The laboratory of cellular and molecular physiology, directed by Professors Fabrice Bureau and Pierre Lekeux, is specialised in the study of mechanisms involved in respiratory illnesses, particularly in the appearance of allergic asthma. For their research, scientists use murine models (experimental models using mice). "We use aluminium, more specifically aluminium hydroxydes, as an adjuvant to induce a TH2 type response when mice are vaccinated against ovalbumin, an egg white protein. These mice then develop an inflammatory illness similar to allergic asthma when they are exposed to aerosols of ovalbumin", says Christophe Desmet. The TH2 response designates the predominant immunological mechanism during the allergic reaction. "This model has been used for a long time to study transcription factors, proteins that control
the expression of our genes, involved in this illness”, he specifies. IRF3 (interferon response factor 3) is a transcription factors involved in the response of the immune system against viruses and bacterial endotoxins and plays a very significant role in innate immunity. “Because innate immunity plays a significant role in triggering respiratory allergy, we asked ourselves whether IRF3 might be implicated in respiratory allergy”, continues Christophe Desmet. “To test our hypothesis we used mice genetically engineered to lack IRF3, which we vaccinated against ovalbumin with aluminium hydroxide. We then noticed that mice that did not have IRF3 were not effectively vaccinated against ovalbumin», explains the researcher.

Following these observations, the researchers decided to use another model to test the involvement of IRF3 in respiratory allergy. "We made mice lacking IRF3 inhale house dust mites and we noticed that IRF3 was indeed playing a part in the occurrence of airway allergies", continues Christophe Desmet. Indeed, these mice, in comparison with "normal" mice, had a greatly weakened form of asthmatic allergy when they were in contact with this type of allergen. This work on the role of IRF3 in breathing allergies to acarins was the subject of an article in the Journal of Allergy and Clinical Immunology (1).

These results allowed researchers to draw a dual conclusion: IRF 3 plays a significant role in respiratory illnesses but also in vaccination. "Alum needs IRF3 to provoke a large part of the TH2 response as without IRF3 the mice did not develop this response and were not vaccinated", summarises Christophe Desmet.
DNA, a key immunostimulant

With their curiosity pushing them still a little further, the scientists then started to ask themselves what allowed IRF3 to be activated during alum vaccination. "The list of molecules that are known to activate IRF3 is relatively short, as it essentially responds to bacterial endotoxin and extranuclear nucleic acids. However this type of molecule is not present in alum. This led us naturally to think of nucleic acids that might come from the body itself. And the most abundant form is DNA", says the post-doctoral fellow. Eagerly looking more closely, in vivo, at the surroundings of alum deposits, the researchers discovered that it was covered in DNA. "There were also dead cells and a lot of free DNA in the interstitial liquid near the alum.", specifies Christophe Desmet. Thus, it would seem that alum leads certain cells in the body to release their own DNA! Could it be that it is this that goes on to boost the immune system to favour the response to the vaccine? "We knew that DNA can be a "danger" signal when it is in a free form in which it does not exist in normal conditions. It can, however, be freed by cells, particularly in the event of bacterial infection where it plays a part as an antibacterial safety net".

To answer this question, Christophe Desmet and his colleagues conducted two types of experiment. The first involved replacing the dose of alum with the quantity of DNA that the cells released in the presence of this adjuvant. "We were able to observe that the intensity of the humoral response was practically the same as when the vaccine contains alum. This means that the DNA itself boosts the immune system significantly", explains the researcher. The purpose of the second experiment was to observe the effect of the removal of free DNA on the effectiveness of the vaccine. In order to do this the scientists injected mice with alum but also...
DNase, an *enzyme* which damages DNA when it is found outside cells. Result: "Then we lost the immune response! ", reveals Christophe Desmet.

This work, carried out in partnership with Japanese researchers of the University of Osaka, is the subject of an article in the journal *Nature Medicine* (2). The discovery of this unexpected protagonist in vaccination, DNA, allows us to, on the one hand, better understand how existing vaccines work. On the other hand it should help to create new adjuvants with very targeted and effective action for the vaccines of the future.