A gene named Adam

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Asthma is a disease of civilisation par excellence. Its increasing incidence is cause for concern and several teams working around the world are attempting to untangle the complexity of its causes. The University of Liège has made a small step towards this understanding by identifying a protease whose role may prove to be very interesting ...

Asthma may be defined as a reversible obstruction of the bronchial tubes, resulting in inflammation. The cause of this is most often allergen exposure. Although the general public is aware of the classic image of an asthma attack - wheezing, breathlessness and respiratory distress - researchers are more interested in what happens between attacks. This is because they consider asthma as a chronic illness, with the attack being a particularly acute - and distressing - manifestation of a more global pathological process taking place in the depths of the bronchial tree.

Asthma's characteristic narrowing of the internal diameter of the bronchial tubes results from the combination of hyperactivity of the smooth muscles that surround the airways and the hypersecretion of mucous by the glands coating the inner walls. These two elements are caused by a persistent inflammatory reaction. It is the cause of this reaction that is of interest to researchers. It is probably due to environmental factors which may themselves be allergens or may be simply irritating agents (atmospheric pollution for example). However, not everyone becomes asthmatic even when people are allergic or breathe polluted air. And in contrast, in asthmatic families, some individuals
suffer from the illness while others don’t. There is therefore an individual predisposition involved, which is genetic in nature.

‘The problem is that this is not a mutation of a specific gene as in the case of cystic fibrosis, says Professor Didier Cataldo, lung specialist at the Sart Tilman CHU and lecturer at ULg. In asthma, there are probably at least two dozen genes involved. This explains why people can be asthmatic for very different reasons. Classically, an asthmatic child generally has an allergic profile that may have started with cradle cap at two months, followed by eczema in infancy. The first signs of asthma often appear during infections by minor respiratory viruses which are inevitable in children, and some young asthmatics have steroid inhalers from a young age. Later, the symptoms may abate or may last throughout their lives. However, there are also people who develop asthma following acute exposure to chlorine gas, in the working environment, for example. This purely chemical, irritative asthma, can be found in swimming pool technicians. Other forms, often fairly severe, develop later; patients for whom classical treatment generally is not effective. Asthma is therefore a highly polymorphic syndrome that covers a range of what are probably different molecular and physio-pathological mechanisms. Obviously, this explains why it is difficult to find ‘the’ gene. We thus feel disempowered, because we can’t offer patients a genetic analysis to determine their likelihood of developing asthma.’ (see also the articles Asthma, a very mysterious disease and Turning asthma on its head)

The ADAM family

Didier Cataldo and his team at GIGA-research, have for several years focussed on certain genes involved in asthma, notably those coding for enzymatic proteins in the ADAMs family (A Disintegrin And Metalloproteinases) and ADAMTS (the same, with particular repeated motifs referred to as "thrombospondines-like"). To cut a long story short, these metalloproteinases consisting of a zinc ion are closely related to proteases in snake venom, which forms another part of the family of adamalysines. This strange detail has no connection to asthma, but highlights the extent to which ADAMs and ADAMTS are ubiquitous in the animal kingdom. Around forty of them have been identified to date, 25 of which are present in the human species and 35 in mice. They play a crucial role in processes ranging from membrane fusion during fertilisation of an egg by a sperm, activation of specific growth factors during embryonic brain development, amyloid protein deposits in Alzheimer’s disease, tumoral aggression in breast cancer, the degeneration of cartilage in arthrosis, and the release of cytokines and other inflammatory factors in asthma. Hence our current position.
The protease which Geneviève Paulissen, post-doctoral student in Didier Cataldo’s team, is working upon goes by the acronym of ADAMTS-12. The first publication establishing a potential link between this protease and asthma dates back to 2006 (1); a study showing that the gene from this enzyme had been localised in the genome in the region associated with vulnerability to asthma. At the time, Geneviève Paulissen was working on her final dissertation in Biomedical Sciences, with Professor Cataldo as supervisor. The dissertation focused on ADAMs in asthmatic pathology. 'Following this publication, we thought it might be interesting to study ADAMTS-12 in an experimental model' she says, 'because we had mice available which were deficient in this gene. We wanted to verify and confirm the role of this protease in asthma with an in vivo experimental model.' It should be noted that there had been unfortunate precedents, notably ADAM-33, another protease whose gene had been clearly recognised in 2002 as being a gene also likely to indicate susceptibility to asthma. However the suppression of this gene in knock out mice provoked no particular signs of asthma in animals! The challenge was therefore to succeed in identifying evidence of differences of asthma phenotype in animals without the ADAMTS-12 protease and then to be able through these signs to decipher the role of the protease in the pathogenesis of the disease.

Wild type and knock out mice

With this in mind, Geneviève Paulissen developed two experimental models exposing mice deficient in ADAMTS-12 to two allergens. Her choice focused on ovalbumine, a protein which is inert but which is capable of triggering asthma attacks after allergisation, and the House Dust Mite (HDM), which is responsible for most of human allergies. In the ovalbumine experimental model, mice were simply placed in plexiglass enclosures where they inhaled a solution containing the allergen in aerosol form for 30 minutes. In the second model, the solution containing the HDM allergen was instilled directly into the naval cavities of sleeping mice. By means of comparison, the wild type mice were subject to the same protocol as their knock out cousins for
ADAMTS-12. Bronchial responsiveness was then measured in all the mice, similar to the tests conducted on humans to diagnose asthma. 'We saw that the mice which were deficient in the ADAMTS-12 gene had bronchial reactiveness which was much higher than the wild type mice who were exposed to the same allergen. This reactiveness is the principal characteristic of asthma', explains the young researcher. The mice were then sacrificed to analyse the inflammation markers present in their lungs. 'We observed that mice which were deficient in ADAMTS-12 showed a significant increase in the two populations of key cells in asthma, polynuclear eosinophils (characteristic in allergic reactions) and mast cells (responsible for releasing histamine, the main intermediary for allergic manifestations). Then we examined the bronchial tissues under the microscope and saw that peribronchial inflammation had also increased in these mice. Delving a little deeper, we wanted to know whether the cytokines were connected with these inflammatory signals, using a cytokine-array (a procedure to identify a panel of cytokines known to be involved in inflammatory phenomena) and we then confirmed their presence using Elisa tests. Most of them were also increased.' All these increases were significant; in all cases, they had at least doubled in comparison with the wild type mice.

A protective effect

These results (2) thus clearly demonstrated, without any possible doubt, that the ADAMTS-12 protease plays a role in the expression of the asthma pathogenesis. A manifestly protective role because, when ADAMTS-12 is deficient, the asthmatic manifestations both in vivo and in vitro are more marked. 'To our knowledge, this is the first study which has shown that ADAMTS-12 has a role in an experimental asthma model' stresses Didier Cataldo. 'Because we have been able to show that deficiency in this enzyme provokes an increase in bronchial reactiveness and an increase in inflammation, we can conclude that ADAMTS-12 certainly has a protective effect, and it would be interesting to investigate the mechanism behind this. This is currently underway.' 'Our study is also interesting from a more general perspective,' he continues, 'because a few years ago, some research hypotheses had recommended investigation of protease inhibition, notably in slowing down the progress of cancer. The pharmaceutical industry therefore stepped into the breach, thinking they had discovered the goose that lays the golden egg, but without having a real understanding of this area. A multitude of unspecific metalloprotease inhibitors were thus developed ... and none of them resulted in anything. On the contrary, a number of clinical trials were interrupted, leading to millions and millions of dollars being lost. Why? Because nobody knew exactly about the biology of this family of enzymes. Once again, this demonstrates the usefulness of fundamental research! A lack of understanding of the range of protease families and cytokines with which we are working leads to unexpected and unknown interactions. Even if a beneficial result emerges, this may potentially be swamped by side effects due to lack of specificity of the approach.'
It is thus understandable that when he is asked if this discovery is likely to have implications for the future treatment of asthma, the researcher remains cautious: ‘Not yet!’ But these findings are nonetheless indispensable for the development of new therapeutic classes precisely because of the understanding of the network of molecular and cellular interactions remains paramount. ‘For the time being’, continues Didier Cataldo, provocatively, ‘we treat asthma with corticosteroids. How do they act? We don’t really know actually! The inhibit inflammation, but in doing so, they act on the expression of a very high number of different genes. We can’t therefore say that we ‘understand’ what is going on. On a mechanistic point of view, we are still at the same stage as when we used suprarenal extracts in the 19th century in our therapeutic agents. Production methods may be more sophisticated, and administration through inhalation reduces the toxicity of the drugs, but we still don’t know exactly what we are doing! We may find new therapeutic targets, including the mechanisms at work.’

Didier Cataldo’s team are now trying understanding what the ADAMTS-12 protease interacts with on the molecular level. ‘We have changed the level of research: we moved from the in vivo stage back to the purely molecular level. This means that this will take time, because these studies are highly complex. It’s unlikely you’ll hear any more about this molecule for a long time’, he smiles. Research is often a waiting game ...

'To conclude' continues Professeur Cataldo, 'we can say that asthma is a polygenic disease for which a whole series of genes have been identified, and the links between cause and effect are more or less complex. Our contribution is to have proven that one of these genes is effectively linked to asthma because when we suppress it, asthma is aggravated. There is still a very long way to go before we will understand all genes associated with asthma and how they function, but our little contribution is ADAMTS-12.'

(1) Kurtz et al, J allergy Clin Immunol Vol 118, 2, 396-402

(2) Control of Allergen-Induced Inflammation and Hyperresponsiveness by the Metalloproteinase ADAMTS-12. Geneviève Paulissen, Mehdi El Hour, Natacha Rocks, Maud M. Guéders, Fabrice Bureau, Jean-Michel Foidart, Carlos Lopez-Otin, Agnès Noel, and Didier Cataldo. J Immunol published online 7 September 2012 ol.1103739
http://www.jimmunol.org/content/early/2012/09/07/jimmun