Nod2 and Crohn’s disease: many connected highways

In this issue of The Lancet, David van Heel and co-workers analyse the innate immunity driven by the mononuclear cells of patients with Crohn’s disease. Using nanomolar concentrations of muramyl dipeptide in an ex-vivo system, they report that pathways act synergistically for the nucleotide oligomerisation domain 2 (Nod2, also known as caspase-recruitment domain 15 or Card15) and toll-like receptor (TLR). Co-activation of these two pathways dramatically increased the production of proinflammatory chemokines. This observation argues for an integrated response of innate immunity.

Innate immunity is driven by a few pathogen-associated molecular patterns that are present in most of the non-eucaryote cells. The host molecules involved in recognition of pathogen-associated molecular patterns are known as pattern-recognition receptors. Two main families of pattern-recognition receptors have been discovered in human beings. TLRs are transmembranous molecules able to recognise lipopeptides (TLR1 and 6), lipoteichoic acid (TLR2 and 6), DNA from viruses (TLR3, 7, and 8), lipopolysaccharide (TLR4), flagellin (TLR5), bacterial DNA (TLR9), and still unknown other molecules.1 Nods are seen as the intracellular counterpart of the TLRs. Nod1 (also known as Card4) and Nod2 are activated by some fractions of peptidoglycan, a major component of the bacterial wall.

The poorly purified pathogen-associated molecular patterns used in initial experiments might have led to erroneous conclusions. For example, Nod2 was initially considered as a lipopolysaccharide receptor, but it is now known that it recognises muramyl dipeptide, a peptidoglycan fraction. More recently, Travassos et al showed that TLR2 is a receptor for lipoteichoic acid but not for peptidoglycan.2 As a result, Nod1 and Nod2 now appear with the peptidoglycan-recognition proteins as the only known proteins involved in the host response induced by peptidoglycan. Peptidoglycan-recognition proteins are considered as lytic enzymes (secreted or stored in vesicles) while the Nods are seen as intracellular sensors of peptidoglycan even if we do not yet know whether peptidoglycan products interact physically with the Nods.

The observation that pattern-recognition receptors act in synergy is not a surprise: living systems are highly integrated. Also, previous studies converged toward this conclusion. The serine/threonine kinase Rip2 (receptor-interacting protein 2, also known as RICK and CARDIAK) is activated by Nod1 and Nod2. However, in Rip2 knock-out mice, the response to lipopolysaccharide, peptidoglycan, and double-stranded RNA is impaired.3,4 These observations might be partly related to contaminated preparations of pathogen-associated molecular patterns as discussed above. However, the demonstration that Rip2 can be recruited to TLR2-signalling complexes after peptidoglycan stimulation and that the effect of the reduced cytokine production

in Rip2−/− macrophages was due to defective signalling from cell-surface receptors argue for a role of Rip2 in the TLR pathway.  

More recently, NOD2 knock-out mice were shown to be resistant to endotoxin challenge, suggesting here too a connection between TLR4 and Nod2 pathways.  

Watanabe et al studied splenocytes from these Nod2−/− mice and found excess production of interleukin 12 and interferon when stimulated with commercial non-purified peptidoglycan.  

Uehara et al recently observed a synergistic effect in terms of production of interleukin 8, in the monocyte leukaemia cell-line THP1 co-stimulated with specific peptidoglycan fragments and specific TLR2, TLR4, and TLR9 ligands.  

van Heel and co-workers’ report confirms the connection between the TLR and Nod pathways. Nod and TLR appear to act in synergy, in agreement with the experiments performed in Rip2−/− mice and THP1 cell line but not in agreement with Watanabe et al’s results on Nod2−/− splenocytes. Discrepancies also appear between studies for the types of TLRs involved in the cooperation with Nod2 and for the chemokines regulated by the interaction.  

The mechanism of the cross-talk between the pathways for pattern-recognition receptors is not known, but we can speculate that Rip2 has a crucial role (figure). There are physical and functional links between Rip2 and both TLR and Nod pathways. Interestingly, Rip2 seems to be also involved in adaptive immunity, suggesting a much more developed network of molecular interactions and the integration of both innate and adaptive immune responses. The recent reports that Nod2 interacts with other proteins containing neuronal apoptosis-inhibitory-protein domains and that Nod1 is negatively regulated by Card6 also argue for a complex nexus involving the Nods and Rip2.  

In Crohn’s disease, at least 30 non-conservative variations spread along the protein have been reported, including three common mutations (R702W, G908R, and 1007fs). However, the role of these mutations is unknown. For most of them, inactivation of nuclear-factor B after stimulation with muramyldipeptide is defective. In such cases, most data suggest a change of sensitivity rather than an all-or-none effect, as shown by van Heel and co-workers. Unfortunately, a clear relation between response to muramyldipeptide and lesions in Crohn’s disease is still lacking, limiting the impact of any functional characterisation of NOD2 mutations.  

We cannot yet fully explain the disease mechanisms in cases with NOD2 mutations. Consistent with van Heel’s data, defective production of proinflammatory chemokines is usually found for mutated Nod2. However, since the first studies, this observation was difficult to conciliate with the inflammation in Crohn’s disease. As a consequence, alternative functions of Nod2 have been looked for. Watanabe et al showed that NOD2 limits the proinflammatory effects driven by TLR2 stimulation. Chen et al also considered Nod2 as an anti-inflammatory molecule under certain circumstances. Finally, Netea et al recently suggested that Crohn’s disease results from defective production of anti-inflammatory cytokines (interleukin 10 and tumour growth factor B).
The walk from the disease to the gene is known to be long and difficult for complex genetic disorders. Crohn’s disease illustrates the fact that the return walk from the gene to the disease might also be hard.

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We declare that we have no conflict of interest.


Protecting everybody’s genetic data

An ambitious project of collection of DNA samples from indigenous peoples all over the world will undoubtedly provide valuable insights into humanity’s migratory past.

However, such an exercise generates many and complex questions about first, technical standards relating to taking, transporting, and storing samples for DNA analysis, and second, the protection of genetic data of people and populations.

Many of the samples taken for this project will, inevitably, come from developing countries, or countries involved in or recovering from some form of armed conflict, whether war or widespread political violence. In such countries, the International Committee of the Red Cross (ICRC) is attempting to promote technical standards and protection of genetic data mainly in relation to verifying or refuting kinship relationships, criminal investigation, and the identification of human remains.

Beyond the need for minimising both technical and human errors by adherence to certain standards, there is the question of what other objectives the analysis of a person’s DNA that serve. In most countries, the national laws that protect personal data have been extended to the protection of genetic data, because people’s genetic data represents powerful information and so could be misused or abused. Governments recognise this. The 2003 UNESCO International Declaration on Human Genetic Data recognises the special status of human genetic data because they may: “be predictive of genetic predispositions concerning individuals and the power of predictability can be stronger than assessed at the time of deriving the data . . . have a significant impact on the family, including offspring, extending over generations, and in some instances on the [person’s] whole group [and] contain information the significance of which is not necessarily known at the time of the collection of the biological samples”. The Declaration stipulates that “any collection, processing, use and storage of human genetic data, human proteomic data and biological samples shall be consistent with international law of human rights” and that “clear, balanced, adequate and appropriate information shall be provided to the person whose prior, free, informed and express consent is sought”. In other international institutions, governments have recognised the need for strict technical standards to protect genetic data. However, the resulting documents are not binding in a legal sense.

Some countries have no national laws to minimise errors in DNA tests or to protect people’s genetic data; the countries that are of greatest concern tend to be where the ICRC is working and from which the samples