

Repositioning the 'Cell 1996' discovery in the context of Toll/TLRs as key receptors regulating innate immunity

The discovery of the receptors activating the innate immune response (acknowledged by the award of the 2011 Nobel prize to B. Beutler and J. Hoffmann) involved many individuals working in different fields and, to me, it is a good illustration of the complexity of the discovery process in general. The following text seeks to reposition the 1996 Cell paper in the general timeline that led to the discovery of TLRs (Toll-like receptors) as key pattern recognition receptors in mammals. This is obviously a simplified account, focussing as it does on just the main papers. I have also included comments on the positive and downside aspects of each paper **with respect** to the final story which revealed that mammalian TLRs are key pattern recognition receptors.

I-Background to the discoveries

I will not mention the outstanding work that identified the effect of **Toll gene mutations** on Drosophila embryogenesis (C. Nusslein-Volhard, E. Wieschaus), **IL1 signaling** (this I know less well), and **NF- κ B signaling** (D. Baltimore and others), that are at the root of the discovery that TLRs are key molecules in immunity.

A) Identification of the first inducible antibacterial peptides (Steiner et al. Nature 1981):

Hans Boman's (1924-2008) contribution was seminal and extremely innovative at that time. His work done in insects was followed a year later by Robert Lehrer's identification of antibacterial peptides in mammals.

Downside aspects:

- The discovery of antimicrobial peptides did not lead to the same radical changes that followed the identification of Toll/TLRs as immune receptors.

B) The concept of Pattern-recognition Receptors (Charles J. Janeway, Cold Spring Harbor symposia on quantitative biology 1989):

Charles Janeway's (1943-2003) idea of pattern recognition receptors provided the conceptual framework for the field and attracted attention towards innate immunity. It also provided a model explaining how innate immunity affects the adaptive immune system. Those are two important basis of modern immunology.

Downside aspects:

- This was just a model, it was not supported by any experimental work. I think that the field of immunology has traditionally been filled with too many speculative theories (idiotypic network, danger theory...).

- Note all ideas were not completely new (e.g. work on LPS recognition in Limulus, characterization of immune stimulatory molecules in the adjuvant field), however Charles Janeway's charisma did attract the attention of immunologists to topics the vast majority of them had been ignoring.

Personal remarks:

For me, both Hans Boman and Charles J. Janeway deserve a specific mention in the textbook of immunology.

C) Identification of Toll function in development (Anderson et al., Cell 1985a and b, Hashimoto Cell 1988, Schneider et al., 1991)

Kathryn Anderson, first in C. Nusslein-Volhard's laboratory (Tubingen), then with her own group in the US, clarified the position of Toll in the dorso-ventral pathway and identified its molecular nature as a receptor.

Downside aspects:

- This outstanding contribution focuses on the role of Toll in development.

Contributions to the elucidation of the Toll pathway in fly development also came from the groups of S. Wasserman, R. Steward, M. Levine and C. Nusslein-Volhard.

II- Toll in Drosophila immunity

A) Studies pointing to a role for Toll in Drosophila immunity (1993-1995):

Several reports already pointed to a possible role for Toll in immunity:

- Gertulla-Anderson (Genetics, 1993) indicated the presence of melanotic tumors in Toll^D mutants (melanotic tumors are cellular immune-like reactions).
- Ip and Levine (Cell, 1993) showed a role for Toll in Dif nuclear translocation upon bacterial infection (the function of Dif was not known).
- Reichhart et al. (CRAS, 1993) pointed to a role for Dorsal in immunity (although Dorsal mutants had no immune phenotype).
- Lemaitre et al. (EMBO J., 1995) showed that Toll regulated Dorsal nuclear translocation upon bacterial infection, as well as lamellocyte differentiation.
- Petersen.... and Engstrom (EMBO J., 1995) analyzed the role of Dif in the expression of the antibacterial peptide gene, cecropin, using cell transfection.
- Rosetto-Hultmark (BBRC, 1995) showed that a transfection of Toll in cultured cells activated the cecropin gene.

From my point of view of geneticist, none of these studies clearly identified the role of Toll *in vivo* since these studies were based either on experiments in which Toll was over-expressed, or they used a gain-of-function allele of Toll. Nevertheless, a role for Toll in immunity was in the "air" before our 1996 Cell paper.

We should also mention two very important papers in 1991 (Gay and Keith, Nature 1991; Schneider-Anderson, Genes & Dev. 1991) that indicated the existence of a conserved intracellular domain in Toll and IL1-R (the TIR domain) pointing to a possible link between the two pathways. Finally, one very interesting paper in the plant field had already implicated proteins with TIR and LRR domains in the immune process (Whitham et al., Cell 1994).

B) Imd and Toll in Drosophila immunity (Lemaitre et al., Cell 1996, but also Lemaitre et al. PNAS 1995, 1997 (Imd pathway, specificity):

- Provided the first genetic demonstration for a role of Toll in immunity (using loss-of-function mutations).
- Illustrated the power of the genetic approach for deciphering innate immunity: Toll and Imd mutations caused severe immune deficiencies with striking phenotypes. The 1995 PNAS and 1996 Cell papers, which presented the Toll and Imd models, were seminal for our comprehension of antimicrobial peptide gene regulation in insects. Of note, the next mutant regulating such genes in *Drosophila* was not identified until 1999 in the Hultmark group (Hendegren et al. Dev. Cell 1999).
- Demonstrated the specificity of the innate immune system.

Downside aspects:

- The 1996 Cell paper did not describe the identification of a “pattern-recognition receptor” since it was already clear in 1996 that the Toll receptor in *Drosophila* is activated by Spaetzle, an endogenous ligand, during the immune response. In effect, Toll in *Drosophila* is a signaling receptor, unlike the TLRs in mammals which are pattern-recognition receptors.
- The notion that Toll was involved in *Drosophila* immunity was already in the air in 1993-1995 (see above).

III Toll in mammalian immunity (a view from outside):

It was clear that several groups were close to identifying the role of TLRs in mammalian immunity. It was fruit ripe for the picking! This led to an intense competition that was not exempt from bullying, at least as viewed from the outside.

A) Toll activates NF- κ B in mammalian cell culture (Medzhitov and Janeway, Nature 1997):

- Provided the first link between Toll and NF- κ B dependent regulation of immune genes in mammals and attracted attention to mammalian TLRs.
- Suggested a key role for TLRs linking innate and adaptive immunity

Downside points:

- It is based on cell transfection experiments using a fusion (between CD4 and the intracellular domain of TLR4) and does not provide a solid proof.

Personal remarks:

- It is probably not a coincidence that the first paper on a human TLR emanated from the Janeway lab, since they initiated the concept of pattern recognition receptors!! It shows that both Medzhitov and Janeway were very open scientists curious about what was happening in other fields. Following Janeway’s death, Ruslan Medzhitov has continued to be one of the most creative scientists in the TLR field. He has always had a strong influence by anticipating many of the questions in this domain.

B) 1998 Science paper from Beutler’s group (Poltorak et al. Science 1998):

- Provided the first genetic evidence that a TLR is involved in bacterial sensing. This paper has the merit of being the first genetic proof in the mammalian field.
- Cloning the *lps* gene by positional cloning was a courageous approach.

Downside points:

- This paper was quickly followed by two other papers describing the same findings at intervals of a few months (the knockout mutation of TLR4 by Hoshino et al. (group of S. Akira), Journal of Immunology 1999; the independent identification of the lps locus as TLR4 by Qureshi et al. (group of D. Malo), also published in 1999). This suggests the discovery was merely a question of months which, given typical delays in scientific publication, might be considered as equivalent.

C) General contribution of Shizuo Akira's group:

The Akira group produced several seminal findings on TLR function and downstream signalling molecules) within a short time frame (generating many knockouts of various TLR genes). The group was a few months behind in terms of publishing the "first paper", but his laboratory has probably contributed more than all the others to the rapid development of the TLR field.

Remarks relating to the 2011 Nobel prize.

The good point about this year's Nobel prize is that it acknowledged two good and solid papers (Poltorak-Beutler Science 1998 and our 1996 Cell) that have marked their time. They are important contributions in the dissection of innate immune mechanisms and also reveal the power of the genetics approach.

If we now analyze this story in the perspective of the discovery of TLRs as pattern-recognition receptors, the situation is more complex since many findings were already "in the air". It is probable that the TLR field would have moved at the same speed without one of the key papers mentioned above. As so often in science, there was a convergence of studies that led different researchers in this same direction. However, in this tight, competitive atmosphere scientists with good communication skills, and fewer scruples about over-simplifying the historical details, had a tendency to shine when it came to "marketing" the TLR discoveries.