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Toll-Like Receptors in Innate Immunity: Signalling Pathways and Therapeutic Targets

Solene Morvan¹●, Mahesh Rao²●, Hannah Kowalczyk³●

¹Associate Professor, Department of Immunology, Vosges Institute of Applied Microbiology, Epinal, France.

²Senior Scientist, Department of Transfusion Medicine, Tungabhadra Institute of Biotechnology, Hospet, India.

³Associate Professor, Department of Transfusion Medicine, Cumberland Plateau Institute of Life Sciences, Cookeville, USA.

Email: ¹s.morvan@viam-e.fr, ²m.rao@tib.ac.in, ³h.kowalczyk@cpils.edu

ORCID: ¹0000-0001-1415-1465, ²0000-0001-4711-7394, ³0000-0001-4456-0032

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ABSTRACT

Toll-like receptors (TLRs) are among the best-characterised sensors of the innate immune system, and the signalling pathways downstream of them have been mapped in considerable detail over roughly two decades. This narrative review summarises what the published literature says about how TLRs recognise microbial and host-derived molecules, how the adaptor proteins MyD88 and TRIF route the signal to inflammatory and interferon responses, and what has happened when those pathways have been used as therapeutic targets. It reports no new data and rests on a non-systematic reading of the literature up to 2020. The pathway map is clearer than the clinical picture: agonists have found a place as vaccine adjuvants and topical immune modifiers, whereas antagonists tested in sepsis did not deliver the benefit that the biology had suggested. Redundancy among receptors, differences between species, context-dependent roles for the same receptor in defence and in tissue injury, and uncertainty about endogenous ligands help to explain that gap. The review closes with the questions that future work needs to address before receptor-level intervention can be expected to perform reliably in patients.

Keywords: Toll-like receptors; Innate immunity; MyD88; TRIF; NF-kappaB; Interferon regulatory factors; Immunotherapy; Narrative review

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Article Class: Review Article

1. Introduction

The innate immune system has to recognise danger quickly, before an adaptive response has had time to form. It cannot do that by learning each pathogen in turn. Charles Janeway proposed that it relies instead on a limited set of

germline-encoded receptors that detect molecular features shared by many microbes (1). Toll-like receptors (TLRs) are the clearest realisation of that idea. The story began with a developmental protein in the fruit fly, Toll, which turned out to control the antifungal defence of adult flies



(2), and within a year a human homologue was shown to activate genes of the immune response (3). A mutation in the gene for TLR4 then explained why certain mouse strains failed to respond to bacterial lipopolysaccharide (4).

Since then the field has produced a large and detailed literature. The receptors, the adaptors that follow them and many of the kinases and transcription factors beyond have all been described, and several excellent reviews cover the ground (5, 6, 7). What is less settled is how far that molecular detail translates into reliable therapy. This article is a narrative review of the published work on TLR ligand recognition, intracellular signalling and therapeutic targeting. It reports no new data and no measurements of the authors, and it does not describe any local patient group. The aim is a fair account of what is known and what is still argued about. It is written for readers who know the outline of innate immunity and want a candid account of where the pathway map is firm and where it is not.

2. Literature Review

2.1 Discovery and the Receptor Family

Humans carry ten TLRs, each a transmembrane protein with an extracellular leucine-rich domain that binds the ligand and an intracellular domain shared with the interleukin-1 receptor family (5, 8). That shared intracellular domain, usually called the TIR domain, is what links recognition to signalling. The family divides loosely by location. Some receptors sit at the plasma membrane and face the outside world, while others are confined to endosomes, where they meet nucleic acids released by internalised microbes (6).

The distinction is more than tidy bookkeeping. Nucleic acids are not foreign in themselves, since every host cell is full

of them, and an endosomal location is one way the system avoids reacting to its own DNA and RNA under normal conditions. Medzhitov frames the larger problem as one of discrimination: the receptors must separate microbial features from host material and then match the strength and kind of the response to the threat (9). Differences like these look minor on a diagram and can matter a great deal inside a cell.

2.2 Ligand Recognition and Structure

Crystal structures have made the ligand story concrete. The TLR1–TLR2 heterodimer is drawn together by a triacylated lipopeptide, with the lipid chains lodged in the receptor pockets (10). TLR4 behaves differently and cannot respond to lipopolysaccharide on its own. It needs the accessory protein MD-2 (11), and structures of the complex, one with an antagonist bound and one with the endotoxin itself, show how lipid A is held and how binding brings two receptor complexes together (12, 13). Table 1 lists several receptors with their usual location and ligand class.

Table 1. Selected human TLRs, where they sit and what they recognise.

Receptor	Location	Typical ligand class
TLR1/TLR2	Cell surface	Triacylated lipopeptides (10)
TLR3	Endosome	Double-stranded RNA (14)
TLR4/MD-2	Cell surface, then endosome	Lipopolysaccharide (11, 13)
TLR5	Cell surface	Bacterial flagellin (15)
TLR7/TLR8	Endosome	Single-stranded RNA (16, 17)
TLR9	Endosome	Unmethylated CpG DNA (18, 19)

Note: Qualitative summary of the cited literature; several receptors recognise more than the examples listed.



Protein ligands are recognised too. TLR5 detects flagellin, the building block of bacterial flagella, and cells that express it respond to the purified protein (15). Among the nucleic-acid sensors, TLR9 responds to unmethylated CpG motifs that are common in bacterial DNA (18) and that it meets after trafficking from the endoplasmic reticulum to the endolysosome (19). TLR3 detects double-stranded RNA, a common by-product of viral replication (14), and TLR7 and TLR8 detect single-stranded RNA (16, 17). The last pair sit close to one another in sequence and yet differ in what they do and where they act, and the work of Heil and colleagues showed that even the same ligand can be handled differently in mouse and human cells (17).

2.3 Adaptors and Signalling Pathways

Once a receptor is engaged, its TIR domain recruits TIR-containing adaptors. A useful review of this small family describes five of them, each with a particular job (20). MyD88 was found first, as an adaptor shared between interleukin-1 and Toll-family receptors (21), and mice lacking it lose responses to interleukin-1 and interleukin-18 (22). At the membrane, TIRAP (also called Mal) helps to bring MyD88 to TLR2 and TLR4 (23, 24). MyD88 then assembles with IRAK kinases into a helical oligomer that acts as a signalling platform (25), and onward signalling through TRAF6 and the kinase TAK1 activates the IKK complex and, in turn, the transcription factor NF- κ B (26). The usual output is the production of inflammatory cytokines.

A second route exists. TLR3 and TLR4 can signal through TRIF, which does not need MyD88, and cells lacking TRIF lose the MyD88-independent response (27). For TLR4, a further adaptor, TRAM, couples the receptor to TRIF only after the receptor has been taken into the cell by

endocytosis, which means the two branches are separated in space and in time (28). The TRIF branch leans on the kinases TBK1 and IKK-epsilon, phosphorylation of IRF3, and the production of type I interferon (29). Table 2 sets the two branches side by side, and Figure 1 draws them as a flow. Timing matters in innate immunity in a way that is easy to forget.

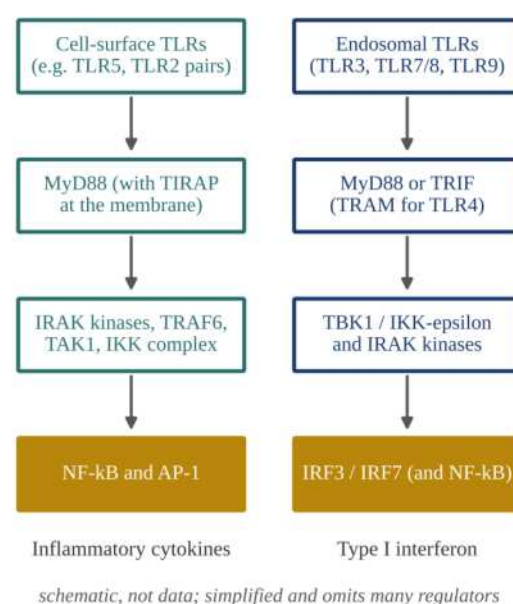


Figure 1. Routes from receptor to response in TLR signalling (schematic, not data).

The picture is neater than the biology. The branches cross-talk with one another and with other innate sensors, including cytosolic receptors for nucleic acids, and the strength and duration of the output depends on negative regulators that this review does not cover (30, 31). In plasmacytoid dendritic cells the MyD88 branch can lead to interferon production through IRF7, which blurs the neat association of MyD88 with inflammation and TRIF with interferon (29). That is one reason the same receptor can look essential in one report and dispensable in the next.

A practical consequence of this layered arrangement is that readouts need care. A cytokine measured at one time point in one cell type reflects one slice of a response



that unfolds in stages. A receptor that looks silent early may be contributing later through the endosomal branch, and a knockout that looks harmless in a laboratory mouse may matter a great deal in an animal exposed to a wider range of microbes (28, 32).

Table 2. The two main signalling branches downstream of TLRs.

Feature	MyD88 branch	TRIF branch
Receptors	Most TLRs; TLR4 included (22)	TLR3; TLR4 (27)
Helpers	TIRAP at the surface (23, 24)	TRAM for TLR4
Key factors	NF-kB; IRF7 in some cells (29)	IRF3 and NF-kB
Typical output	Inflammatory cytokines	Type I interferon
Timing	Early	Later, after uptake (28)

Note: TLR4 is unusual in using both branches, one after the other.

2.4 TLRs in Host Defence and Disease

Genetics offers some of the most persuasive evidence for what TLRs do in people. Children with inherited deficiency of IRAK-4 or MyD88 suffer severe pyogenic bacterial infections, but, remarkably, many other infections are handled normally and the susceptibility seems to lessen with age (33, 34). TLR3 deficiency has been reported in patients with herpes simplex encephalitis, a rare complication of a common virus (35). A TLR5 stop codon polymorphism that abolishes flagellin signalling has been associated with susceptibility to Legionnaires' disease (36). Reviews of the human and mouse genetic data stress that individual TLRs are often less essential in people than the mouse work had implied (32, 37).

The same receptors are not purely defensive. In mice, signalling by TLRs in response to commensal bacteria appears to

be needed for the normal maintenance of the intestinal epithelium, which turns the usual picture of microbial sensing as a purely hostile business on its head (38). In autoimmune disease, immune complexes containing nucleic acids can activate B cells through both the B-cell receptor and TLRs (39). In sepsis, the host response to infection itself becomes harmful, and the place of innate sensing within that response is still debated (40). Several endogenous molecules have been proposed as TLR ligands, although many of the early reports have been challenged because preparations were contaminated with microbial products (41). The point is not that the work is poor, only that its conclusions travel less far than its headline suggests.

3. Materials and Methods

3.1 Scope and Question

The review asks three linked questions. How do TLRs recognise their ligands, how is the signal routed inside the cell, and what has been learned from attempts to turn that knowledge into treatment? It covers work published up to 2020. Clinical statements are limited to what the cited papers report, and no advice on individual care is offered.

3.2 Literature Assembly

This is a narrative synthesis. No formal systematic search was carried out, no search strings were registered and no database was screened according to a protocol. The sources are original papers and reviews that the authors know to be influential or representative, supplemented by following citations from those papers. None of these steps is complicated, but each is easy to skip. That approach has an obvious weakness, which is that it favours well-known work and can miss negative or less visible reports, and the Discussion



returns to it.

3.3 Appraisal Framework

Each source was read for the type of evidence it offered: structural, biochemical, genetic, animal or clinical. Claims were treated with more confidence when they were supported by more than one kind of evidence, and with less when they rested on one model or one laboratory. No numerical values were extracted or pooled, and none are reported here. It is a habit worth keeping even when the answer seems obvious. Figure 2 places some of the landmark papers on a timeline.

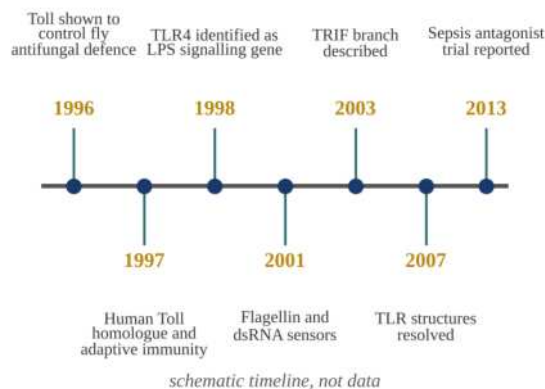


Figure 2. Selected milestones in TLR research (schematic timeline, spacing not to scale).

4. Results

4.1 What the Pathway Map Shows

Three features stand out from the literature. First, recognition is combinatorial: receptors work as pairs or with accessory proteins, and the same microbe usually presents several ligands at once (5, 10, 12). Second, signalling is modular, so that a handful of adaptors and a shared set of kinases and transcription factors are reused by many receptors (6, 20). Third, location and timing are part of the code. The same TLR4 can deliver a quick inflammatory signal from the surface and a slower interferon signal from the endosome (28). A therapy aimed at one

point therefore has to consider which of these several outputs it intends to change.

4.2 Therapeutic Strategies

Two broad strategies follow from the biology. One activates TLRs to strengthen an immune response, and the other blocks them to dampen one. Reviews of the field give a clear framework for both (42, 43). The first has produced real products. A TLR4 agonist, a modified lipid A, is used with an aluminium salt in a vaccine adjuvant system, and the work on it shows a brief and local innate response that goes along with an enhanced adaptive one (44). TLR7 and TLR8 agonists have been studied as topical agents in skin disease and cancer, where the aim is to recruit the patient's own immune system against abnormal cells (45). CpG oligodeoxynucleotides acting on TLR9 have been explored as adjuvants and as stand-alone agents, with a mixed record (46). In each case the result depends on where and for how long the receptor is stimulated (42). Table 3 summarises these approaches. A pathway that is easy to draw is not necessarily easy to drug.

Table 3. Therapeutic strategies aimed at TLRs and how they have fared.

Strategy	Example	Outcome so far
Agonist as adjuvant	TLR4 agonist system in a vaccine (44)	Established in practice
Topical agonist	TLR7 activation in skin disease (45)	Established for some uses
CpG DNA agonist	TLR9 activation (46)	Promising, still debated
TLR4 antagonist	Eritoran in sepsis (47)	No clear benefit
TLR4 signalling inhibitor	TAK-242 in sepsis (48)	No clear benefit

Note: Status reflects the published literature up to 2020 and is not a statement about any individual product.



4.3 Experience with TLR4 Antagonists in Sepsis

The second strategy has been harder. In sepsis a harmful excess of inflammation, driven partly by bacterial products such as lipopolysaccharide, seemed a natural target for a TLR4 blocker (40). Eritoran, a lipid A analogue that occupies the binding pocket of MD-2 (12), was tested in a large randomised trial in severe sepsis and did not lower mortality (47). TAK-242, a small molecule that interferes with intracellular TLR4 signalling, was tested in an earlier randomised trial and did not suppress circulating cytokine levels or improve outcomes (48). The two compounds act at different points, yet both ended without a clear benefit. Read this way, a negative trial is information about the strategy and not only about the drug.

Several explanations have been offered, and none is proven. Sepsis is not one disease, and patients differ in organism, site and stage. Blocking a receptor late in a syndrome that is already under way may be too late, and several receptors respond to the same organisms, so blocking one leaves the others in place (32). The human genetic data add a caution of their own, because a defence pathway that is switched off can leave the patient open to infection (33, 37).

4.4 Why Targeting Is Difficult

Putting these observations together, four obstacles recur. The first is redundancy among receptors and among downstream branches, which makes a single blocked step easy to bypass (30). The second is that the same pathway protects and harms depending on setting, as the intestinal and autoimmune examples show (38, 39). The third is the difference between species, which limits what animal models can predict about human responses (17, 37). The fourth is uncertainty about endogenous ligands, which makes it

unclear what exactly a drug would be blocking in sterile injury (41). Figure 3 arranges these questions as a simple decision tree. A reader mostly wants to know whether a result seen in a dish is likely to matter in a patient.

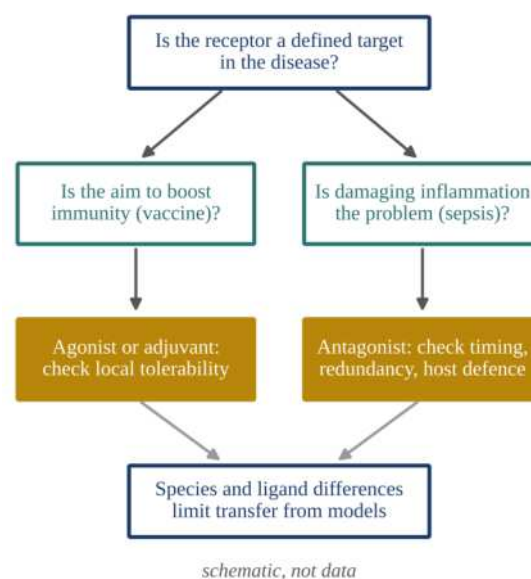


Figure 3. Questions that shape a decision to target a TLR (schematic decision tree, not data).

One more thing shows up when the literature is read as a whole: the two strategies have not been tested on equal terms. Agonists have mostly been studied in settings where a short and controlled stimulus is the aim, while antagonists have been tried in a setting, severe sepsis, where almost everything about the patient is changing at once (40, 47). A fair comparison between the two strategies is therefore not possible from the published record, and the review does not attempt one.

5. Discussion

5.1 Interpretation

The central lesson of this literature is that detailed knowledge of a pathway and a successful intervention on it are different achievements. The agonist strategies work in part because they are modest. An



adjuvant needs only a short, local push to the immune system, and a topical agent acts on a small area of tissue where its effect can be tolerated (44, 45). The antagonist strategies ask for much more, namely a clean and timely suppression of a signal that the patient may also need. It would be wrong to read the negative sepsis trials as showing that TLR4 does not matter. They show that the specific compounds, given in the specific way and to the patients studied, did not help (47, 48).

A broader reading is also available. The structural and signalling work has been so successful that the pathway can be drawn with confidence, and that confidence has probably encouraged the expectation that a drug could be designed to order. Yet genetic studies in people have repeatedly shown that the importance of a receptor depends on context, with some deficiencies producing narrow phenotypes and others sweeping ones (34, 37).

Another point deserves mention. Because TLR signalling also participates in tissue maintenance and in the tolerance of resident microbes, a drug that blocks it may have effects well beyond the disease it is aimed at (38). Safety questions for long-term antagonism are therefore different from, and probably harder than, those for the short exposure involved in an adjuvant (43).

5.2 Limitations

Several limitations apply. The review is narrative and was not conducted as a systematic search, so selection bias is likely and the weight given to individual papers reflects the authors' judgement. It stops in 2020 and does not include later work. Much of the mechanistic evidence comes from mice or from cell lines, and the human data are mainly from rare inherited conditions that may not generalise. Reviews are more useful when

they say where the evidence stops than when they smooth it over. The clinical section rests on a small number of trials and cannot support firm conclusions about the strategy as a whole. Finally, a descriptive review of this kind cannot say how large any effect is, and it deliberately reports no numbers.

There is also a communication problem. Pathway diagrams, including Figure 1 in this review, give an impression of certainty that the underlying experiments do not always justify. Many arrows in such diagrams rest on overexpression systems, on a single cell type or on one mouse strain, and a reader who meets the diagram before the evidence may not realise how much of it is provisional (20, 31).

5.3 Directions for Future Work

Four lines of work seem worth pursuing. The first is better characterisation of the ligands that are actually present in sterile injury, with preparations free of microbial contamination (41). The second is the identification of patient subgroups, using genetic or immunological markers, in which blocking a receptor is reasonable and in which it is not (37). The third is attention to timing, for example by testing agents earlier in the course of illness than the sepsis trials did. The fourth is a closer look at the many regulators that tune signalling, which may offer gentler points of intervention than the receptor itself (30, 31). These points apply to most innate immune targets and not only to TLRs.

6. Conclusion

TLR biology is one of the clearest success stories in modern immunology. The receptors, the adaptors and the main transcription factors are known, and the map of how a microbial molecule becomes a cytokine or an interferon is largely in place. The translation into medicine is more uneven. Agonists have a secure place



as adjuvants and as topical agents, while the antagonists tested in sepsis have not delivered. Redundancy, context and species differences explain much of the gap, and the limits of narrative review mean this summary may itself be incomplete. Nothing here argues against studying these receptors; it argues for being precise about what each result shows. Future progress is likely to depend less on new receptors than on better matching of intervention to patient, setting and timing.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

S.M. conceived the review and wrote the sections on receptor biology and signalling. M.R. wrote the sections on host defence and disease. H.K. wrote the sections on therapeutic strategies and clinical experience. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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