**TLRs: Introduction**

- Discovered in 1990s
- Recognise conserved structures in pathogens
  - Rely on germline encoded pattern recognition receptors (PRRs) that have evolved to recognise pathogen-associated molecular patterns (PAMPs)
- Named for the Toll receptor in Drosophila which mediates fungal immunity
- “Toll” is German slang for “fantastic”
- LPS recognising TLR4 was first identified in mice
- Type-1 transmembrane proteins; The intracellular domain is highly homologous to the IL-1 type 1 receptor
- 10 TLRs identified in humans
**TLRs: Introduction**

- Studies of mice deficient in each TLR has demonstrated that each have distinct function in terms of PAMP recognition and immune response
- Most classes of TLRs are found in innate immune cells where they trigger immediate responses to pathogens:
  - Neutrophils
  - Monocytes/Macrophages
  - Dendritic cells
  - Mast cells
- Recently TLR expression on T- and B-cells has been described as well as epithelial cells, keratinocytes and malignant cells
- Interestingly TLR3 is predominantly expressed on tissue of the brain, heart, lung and muscle, suggesting a role in anti-infection/inflammation in these tissues.

**PAMPs: Pathogen-associated molecular patterns**

- Derived from a wide array of viruses, parasite and fungi
  - Lipids
  - Lipoproteins
  - Nucleic Acids
- Recognition of PAMPs by TLR occurs in various cellular compartments including:
  - Plasma membrane
  - Endosomes
  - Lysosomes
  - Endolysosomes
- TLRs are essential for generating immunity against infection
- Inappropriate TLRs contribute to acute and chronic inflammation and systemic autoimmune disease
PAMP recognition by cell-surface TLRs

- Recognise bacterial and fungal components
- TLR1, TLR2, TLR4, TLR5, TLR6, TLR11
- TLR2 and TLR6 form heterodimers

PAMP recognition by intracellular TLRs

- Recognised viral components
- TLR3, TLR7, TLR8, TLR9
TLR Signaling

- Individual TLRs trigger specific biological responses explained by the presence of TIR domain-containing adaptor molecules e.g. MyD88, TIRAP, TRIF and TRAM which are recruited by distinct TLRs and activate different signaling pathways/transcription factors
- MyD88 is the “universal adaptor” and interacts with all TLRs except TLR3 (receptor for dsRNA)
- There are also negative regulators for TLR signaling which suppress inflammation and deleterious immune responses. These include:
  - Splice variants for adaptor proteins
  - Ubiquitin ligases
  - Deubiquitinases
  - Transcriptional Regulators
  - MicroRNAs

Disruptions in negative regulators of TLRs

- Disruption of the negative regulators of TLRs can lead to persistent inflammation in vivo
- In mice a negative regulator is TANK (binds NFkB and IRF3). TANK deficient mice spontaneously develop glomerulonephritis.
Disruptions in negative regulators of TLRs

- More recently evidence is mounting that there is activation of TLR signaling during tissue damage in several diseases in the absence of infections suggests that endogenous molecules also serve as TLR agonists.
- These are known as “DAMPs” or Damage associated molecular patterns. This process may be important in autoimmune disease.
- TLRs have been shown to bind:
  - Heat-shock proteins
  - Intercellular matrix proteins
  - Mammalian genomic DNA

Human TLRs and their ligands

<table>
<thead>
<tr>
<th>TLRs</th>
<th>Major cell type</th>
<th>Genetic recognition ligands</th>
<th>Endogenous ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Murine cells, T cells, R cells, NK cells, splenocytes</td>
<td>H. influenzae, Rickettsia</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR2</td>
<td>Murine cells, T cells, R cells, splenocytes</td>
<td>Acinetobacter baumannii</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR3</td>
<td>Murine cells, R cells, splenocytes</td>
<td>Poly I:C, poly U, poly A, Poly(I:C)</td>
<td>RNA</td>
</tr>
<tr>
<td>TLR4</td>
<td>Murine cells, R cells, splenocytes</td>
<td>B. subtilis, E. coli, T. denticola</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR5</td>
<td>Murine cells, R cells, splenocytes</td>
<td>Bacteroides, Rickettsia, Borrelia</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR6</td>
<td>Murine cells, R cells, splenocytes</td>
<td>Mycoplasma, Chlamydia, Helicobacter</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR7</td>
<td>Murine cells, NK cells, R cells, splenocytes</td>
<td>B. subtilis, E. coli, T. denticola</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR8</td>
<td>Murine cells, R cells, splenocytes</td>
<td>Bacteroides, Rickettsia, Borrelia</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR9</td>
<td>Murine cells, R cells, splenocytes</td>
<td>B. subtilis, E. coli, T. denticola</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
<tr>
<td>TLR10</td>
<td>Murine cells, R cells, splenocytes</td>
<td>B. subtilis, E. coli, T. denticola</td>
<td>Lipopolysaccharide, heat shock proteins</td>
</tr>
</tbody>
</table>

Chang Inflamm Res 2010
Genetic variation in TLRs and disease susceptibility

- Monogenic disorders associated with complete deficiency in certain TLR pathways demonstrate susceptibility to specific pathogens
  - MyD88 deficiency leads to greater susceptibility to pyogenic bacteria
    - Life-threatening infections first appear in infancy and have a cumulative mortality of 30-40%. Interestingly these infections become less frequent during early adolescence after which no fatalities have been reported.
  - ? Effect of adaptive immunity
  - TLR3 deficiency leads to greater susceptibility to herpes viruses
    - Presents 3 months - 6 years
    - Present with a syndrome of recurrent herpes encephalitis

- Common polymorphisms in gene encoding several TLRs have been associated with infectious and autoimmune diseases.
- Effect at both individual and population levels
  - Mutations in 2 amino acids in TLR4 reduce its ability to bind LPS and increase the likelihood of gram-negative sepsis
  - SNPs in adaptor Mal (part of the TLR2 and TLR4 pathway) has been reported to be protective against tuberculosis particularly in South Indian and South African Populations
    - However a recent meta-analysis of over 6 000 pts concluded there was no survival advantage of the SNP in Mal
- Potential sources of Bias in population level studies:
  - Small sample sizes
  - Frequency of SNP allele
  - Population stratification/ non-bias sampling
• Interestingly where a genetic mutation has conferred a survival advantage against infection in a population there is a high probability that it reduces childhood mortality at a cost of promoting autoimmunity later in life.

Netea et al Nat Immunol 2012
Relationships between TLRs and cancer

- TLR-mediated (chronic) inflammation at the site of tissue injury may create a microenvironment that predisposes to tumorigensis e.g. Crohns disease and bowel cancer, hepatitis and HCC
- Pts with SNP Asp299Gly and H/Pylori infection have an increased risk of gastric cancer possibly the result of exaggerated inflammation.
- SNP is TLR4 and TLR1-6-10 cluster in Sweden have an increased risk of early onset prostate cancer

TLRs expressed on cancer cells and their signaling

- A number of reports suggest that functional TLRs are widely expressed on cancer cells and cell lines, although how tumor cells control or utilize TLR activation remains largely unknown.
- One hypothesis is that normal tissue utilizes NFkB activation regulates acute inflammation via short term expression of pro-inflammatory mediators. Some argue that malignant cells used the NFkB pathway to express pro-inflammatory and cell-survival genes to inhibit cell-death pathways and promote growth of malignant cells.
TLR expression in human cancer cells

<table>
<thead>
<tr>
<th>TLRs</th>
<th>Types of cancer cell(s)</th>
</tr>
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<tbody>
<tr>
<td>TLR1</td>
<td>Human biliary and neck squamous cell carcinoma (HNSSC) cell line [15], squamous cell carcinoma [19]</td>
</tr>
<tr>
<td>TLR2</td>
<td>HNSCC cell lines [15], laryngeal carcinoma cells [15]</td>
</tr>
<tr>
<td>TLR3</td>
<td>HNSCC cell lines [15], laryngeal carcinoma cells [15], lung cancer cell lines [94], breast cancer cell lines [94], melanoma cell lines [94]</td>
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<tr>
<td>TLR4</td>
<td>HNSCC cell lines [15], laryngeal carcinoma cells [15], lung cancer cell lines [94], breast cancer cell lines [94], ovarian cancer (oVCA) cell lines and oVCA cell lines [94], gastric carcinoma cells [98], colorectal cancer (CRC) cell lines [95], melanoma cell lines [95], prostate cancer cell lines [100]</td>
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<tr>
<td>TLR5</td>
<td>HNSCC cell lines [15], prostate carcinoma cells [98], colorectal cancer (CRC) cell line (SW480) [102]</td>
</tr>
<tr>
<td>TLR6</td>
<td>HNSCC cell lines [15]</td>
</tr>
<tr>
<td>TLR7</td>
<td>HNSCC cell lines [15], chronic lymphocytic leukemia (CLL) [104], breast cancer cell lines [105], [98]</td>
</tr>
<tr>
<td>TLR8</td>
<td>HNSCC cell lines [15]</td>
</tr>
<tr>
<td>TLR9</td>
<td>HNSCC cell lines [15], breast carcinoma cell lines [104], gastric carcinoma cells [98], chronic lymphocytic leukemia (CLL) [104], cervical cancer cells [107], prostate cancer cells [106], [98], lung cancer cell lines [98], non-small-cell lung cells and cell lines [109]</td>
</tr>
<tr>
<td>TLR10</td>
<td>HNSCC cell lines [15]</td>
</tr>
</tbody>
</table>

*Chang Inflamm Res 2010*

TLR-based therapeutics

- TLRs satisfy many criteria for consideration as a therapeutic target:
  - Overexpression in disease
  - KO mice being resistant to disease models
  - Ligands exacerbating disease in models
  - Genetic differences in TLRs correlating with disease risk
- Given TLR play an important role in promoting inflammation and innate immunity, as well as priming the adaptive response TLR agonists are being explored for use against infectious diseases and cancer.
- Conversely as TLR agonists promote chronic inflammation and autoimmunity TLR inhibitors are being explored in parallel for use in autoimmunity and inflammatory diseases.
- Cell surface TLRs can be targeted by small molecules and antibodies
- Intracellular TLRs require targeting with modified oligonucleotides
### TLR-based therapeutics for infectious diseases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Indication</th>
<th>Target</th>
<th>Drug class</th>
<th>Clinical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6001</td>
<td>Dynavax Technologies</td>
<td>Hepatitis B infection</td>
<td>TLR agonist</td>
<td>CpG oligodeoxynucleotide</td>
<td>Phase 3</td>
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<tr>
<td>CON17936</td>
<td>Dynavax Technologies</td>
<td>Hepatitis B infection</td>
<td>TLR agonist</td>
<td>CpG oligodeoxynucleotide</td>
<td>Phase 3</td>
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<tr>
<td>PN01</td>
<td>Neurimmune</td>
<td>Hepatitis C infection</td>
<td>TLR agonist</td>
<td>Syntetic CpG oligodeoxynucleotide</td>
<td>Phase 3</td>
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<tr>
<td>BN-9001</td>
<td>Valneva</td>
<td>Hepatitis B</td>
<td>TLR agonist</td>
<td>Syntetic CpG oligodeoxynucleotide</td>
<td>Phase 3</td>
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<tr>
<td>Hennessy et al Nat Rev 2010</td>
<td></td>
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### TLR-based therapeutics for autoimmune diseases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Indication</th>
<th>Target</th>
<th>Drug class</th>
<th>Clinical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>12024123</td>
<td>SanoTech</td>
<td>Psoriasis</td>
<td>TLR agonist</td>
<td>Smaller molecule</td>
<td>Clinical phase 3</td>
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<tr>
<td>BI 6550</td>
<td>GSK</td>
<td>Psoriasis</td>
<td>TLR agonist</td>
<td>Smaller molecule</td>
<td>Clinical phase 3</td>
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<tr>
<td>Hennessy et al Nat Rev 2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
TLR agonists for cancer

- **BCG as intravesical therapy for bladder carcinoma**
  - BCG contains many TLR agonists
  - Antitumor effects largely driven by IFN-gamma, IL-2 and promotion of antigen-specific T-cells
- **TLR7 ligand imiquimod was licensed in 1997 by the US FDA for the treatment of HPV-associated genital warts and BCC**
  - Acts largely through promoting IFNs which has antiviral and anti-tumor action
  - Better effects when administered locally rather than systemically
- **Other ligands of TLR7 e.g. imidazoquinoline have been used to treat B-CLL in clinical trials**
- **TLR9 ligand ODN entered phase I trial for B-NHLCPG**
  - ODN has also been used to treat RCCC, NSCLC and melanoma

TLR-based therapeutics in development for cancer treatment

Table 1: Development status of compounds that target TLRs for cancer indications

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Target *</th>
<th>Drug class</th>
<th>Clinical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monophosphoryl</td>
<td>Hemispheris/Bristol-Myers</td>
<td>TLR1</td>
<td>dsRNA-molecule</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CDX-1102 (Ref. 16)</td>
<td>Invirex</td>
<td>TLR3</td>
<td>dsRNA-mimics</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CR8520 (Ref. 42)</td>
<td>Cleveland BioSciences</td>
<td>TLR5</td>
<td>Faspeptide</td>
<td>Preclinical</td>
</tr>
<tr>
<td>IMO-2125 (Ref. 25-28)</td>
<td>ImmPharmaceuticals</td>
<td>TLR5</td>
<td>Cyclophosphamide</td>
<td>Phase I</td>
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<tr>
<td>MGN-1798 (Ref. 29)</td>
<td>Nalogen</td>
<td>TLR9</td>
<td>Non-coding stem-loop RNA</td>
<td>Phase I</td>
</tr>
<tr>
<td>ANA773 (Ref. 44)</td>
<td>Anadyo Pharmaceuticals</td>
<td>TLR7</td>
<td>sRNA-molecule</td>
<td>Phase I</td>
</tr>
<tr>
<td>OIM-174 (Ref. 36-37)</td>
<td>OIMPharma</td>
<td>TLR7, TLR9</td>
<td>Lipid-A derivative</td>
<td>Phase I</td>
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<tr>
<td>SSS103 (Ref. 30)</td>
<td>Dynax Technologies</td>
<td>TLR9</td>
<td>Short ODN-cyclic dinucleotide</td>
<td>Phase I</td>
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<tr>
<td>AGN105 (Ref. 31, 32)</td>
<td>Pizer</td>
<td>TLR9</td>
<td>CpG oligonucleotides</td>
<td>Phase I</td>
</tr>
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<td>SSA:181.9-071 (Ref. 35)</td>
<td>3M Pharmaceuticals</td>
<td>TLR7</td>
<td>Small molecule sRNA</td>
<td>Phase I</td>
</tr>
<tr>
<td>Imiquimod (Ref. 8-12, 20)</td>
<td>3M Pharmaceuticals</td>
<td>TLR7</td>
<td>Small molecule sRNA</td>
<td>Phase I</td>
</tr>
<tr>
<td>Card-58 (Ref. 14)</td>
<td>Celldyn Pharmaceuticals</td>
<td>polyTLR</td>
<td>Antibody conjugate</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

*All agonists, double stranded RNA, single stranded RNA, TLR, TLR agonist.

Hennessy et al Nat Rev 2010
Conclusions

• TLR are germline encoded receptors that recognise pathogen-associated molecular patterns

• Initially defined by expression on cells of the innate immune system but more recently found to be expressed on cells of adaptive immune system and wider tissues

• Ligand binding results in a signal cascade which results in upregulation of pro-inflammatory cytokines

• The inflammation generated by TLR has been shown to aid immunity against a wide variety of bacterial, viral and fungal pathogens
Conclusions

• Additionally it is likely that TLRs cross react with endogenous/host proteins and play a role in chronic inflammation/autoimmunity
• TLR-based therapeutics are being explored in treatments for infectious diseases and malignancy either alone or as adjuvants
• Additionally treatments aimed at inhibiting TLR-mediated inflammation are being explored in the area of autoimmunity.