Toll-like receptor 2 as therapeutic target in lung disease

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Toll-like receptor 2 (TLR2) is activated by pathogens and nonpathogen insults leading to innate immune responses and inflammation. TLR2 is a promiscuous receptor being activated by a variety of pathogen-associated molecular patterns (PAMPs), but especially by those expressed by Gram-positive bacteria. TLR2 functions in heterodimers with either TLR1 or TLR6, and there is some evidence for tissue-specific preferences for TLR1 versus TLR6 in the activation of TLR2-mediated responses. Activation of TLR2 has now been implicated in a number of respiratory-based conditions including asthma and chronic obstructive pulmonary disease. The role of TLR2 in lung disease and its emerging prominence as a therapeutic target is discussed below.

The target

Pathogen-associated molecular pattern agonists (ligands)

Toll-like receptor 2 (TLR2) senses pathogen-associated molecular patterns (PAMPs) in bacteria and fungi. In general terms, TLR2 functions predominately as a receptor for Gram-positive bacteria while TLR4 functions as a receptor for Gram-negative organisms. However, TLR2 is considered the promiscuous member of the TLR family with a wide range of ligands capable of activating it. This feature of TLR2 makes it, not only interesting to study in terms of immune function, but also an ideal target for drug therapy. Specific PAMPs for TLR2 include lipoteichoic acid, diacylglycerides and triacylated molecules found in the outer membrane of Gram-positive bacteria. While TLR2 favors Gram-positive bacterial sensing, it can also be activated by PAMPs from some types of Gram-negative organisms (1). A recent review by Ulrich Zähringer lists a full range of TLR2 PAMP agonists (1).

Oxidants

In addition to pathogen-derived agonists, there is now growing evidence to suggest that host-derived molecules can also activate TLR2 (2). Of particular importance and relevance to lung inflammation is the finding that oxidants, including those in cigarette smoke, can activate macrophages and airway cells to release cytokines such as CXCL8 (3) via a mechanism involving TLR activation (4, 5). It has been known for some time that oxidants activate cells and induce inflammation although the signaling pathways involved in oxidant-induced responses are not completely understood. A specific function for TLR2 as a ‘receptor’ for oxidant stress was first suggested by Frantz and coworkers in 2001 using cardiac myocytes or fibroblasts stimulated with H2O2 (6). In this study the activation nuclear factor-κB (NF-κB) by H2O2 was only seen in TLR2-expressing cells and was prevented when TLR2 was blocked with an antibody (6). Our group and others have also shown, using animal models, that oxidant-induced inflammation associated with inhaled ozone (7) or cigarette smoke extract is absent in TLR2 or TLR4 knockout animals. These findings suggest that either TLR2 or TLR4 can act as oxidant sensors.

TLR2/6

In the earlier study by Frantz et al., using isolated cells identifying TLR2 as an oxidant sensor, it was specifically reported that TLR4 was not involved (6). Preliminary data from our group using human embryonic kidney cells transfected with specific TLRs support the observation that TLR2 and not TLR4 senses oxidant insult and, interestingly, that TLR6, more than TLR1, is the preferred heterodimer partner for oxidant-induced CXCL-8 release (Paul-Clark et al., unpublished observations). When we analyzed data at early time points (< 2 hours) in our in vivo models of oxidant-induced inflammation, we found, consistent with cell-based data, that TLR2, but not TLR4, receptors were critical in the sensing of the insult (5).

These findings clearly substantiate the hypothesis that TLR2, but not TLR4, is a sensor of early oxidant-induced inflammation. The involvement of TLR4 in oxidant-induced inflammation in vivo, shown at later time points, may well be due to a secondary inflammatory insult mediated by bacterial leakage from the gut or the lungs.

TLR2 expression

TLR4 is expressed on many cell types in relatively high levels. By contrast, on macrophages, for example, TLR2 is expressed in relatively low amounts compared to TLR4. However, TLR2 is rapidly induced when cells are stimulated with cytokines or PAMPs (9-11). By contrast to macrophages, vascular (12) and airway smooth muscle (13) express TLR2 in comparable levels to TLR4. These observations suggest that the effects of TLR2 agonists in some tissues may well be functionally relevant before responses in others. This seems to be the case for nitric oxide synthase II induction in blood vessels by Gram-positive Staphylococcus aureus which occurs earlier in vascular smooth muscle cells than in macrophages (10, 12).

TLR2/TLR1 and TLR2/TLR6 heterodimers

TLR2 is unique among TLR receptors as it functions as a heterodimer with either TLR1 or TLR6. For some ligands that activate the TLR2/6 heterodimer, the scavenger receptor CD36 is required for maximum activation (14). In this setting, it is thought that CD36 acts in a parallel manner to CD14 in the lipopolysaccharide–TLR4 signaling pathway maneuvering the ligand to the receptor. Recent evidence suggests while...
TLR2 and TLR6 complexes are clearly ligand specific the signaling pathways and pattern of genes induced are identical (15).

There is increasing interest in the specific roles of TLR1 and TLR6 in the signaling of TLR2. In our hands we found that the sensing of Gram-positive bacteria by blood vessels and the resultant vascular dysfunction was mediated by TLR2 and TLR6 and not TLR1 (12). In fact, we found that activation of the TLR2/1 heterodimer in blood vessels had no effect on vascular function (12).

By contrast, activation of macrophages (10) or human bronchial epithelial cells (16) to release cytokines in response to selective PAMPs occurs equally well via the TLR2/1 and TLR2/6 pathways.

Figure 1. Toll-like receptor 2 (TLR2) as a therapeutic target in lung disease. TLR2 is activated by pathogen and non-pathogen agonists including Gram-positive Staphylococcus aureus and oxidants. It forms heterodimers with TLR1 or TLR6. Some agonists of the TLR2/6 heterodimer are facilitated by the scavenger receptor CD36. Once activated, TLR2 recruits the adapter proteins MAL (MyD88 adapter-like) and MyD88 (myeloid differentiation marker) which initiate signaling resulting in nuclear factor-κB (NF-κB) activation and induction of inflammatory genes. Early data suggest that in childhood, activation of the TLR2 pathways protects against the development of atopy and asthma. By contrast, in adults with established disease, activation of TLR2 may well lead to exacerbations. TLR2 activation by oxidants in smoke may well be the initiating factor in the development of respiratory disease associated with smoking, including chronic obstructive pulmonary disease (COPD). As a target, we may predict that ligand of TLR2 could have use in susceptible infants to force a Th1 response and prevent asthma. In adults, by contrast, TLR2 antagonists may be useful in the treatment of asthma and COPD. PAMPs, pathogen-associated molecular patterns; IRAK1, interleukin-1 receptor-associated kinase 1.

Cell signaling

Once activated, TLR2 recruits the adapter proteins MyD88 (myeloid differentiation marker) and MAL (MyD88 adapter-like) via interactions with the Toll interleukin (IL)-1 receptor (TIR) domains (17). Phosphorylation of IL-1 receptor-associated kinase 1 (IRAK1) is an early event in the preceding signaling cascade followed by activation of NF-κB and induction of a range of proinflammatory genes (17).

Gene profiles

Gene profiles induced by activation of TLR2 are often compared with those induced by TLR4. TLR4 differs from TLR2 as it recruits TRIF (Toll/IL-1 receptor domain-containing adaptor-inducing interferon-β) and TRAM (TRIF-related adapter molecule) in addition to MyD88 and MAL.
adapter proteins. Early work revealed that MyD88-dependent and MyD88-independent TLR signaling resulted in the induction of separate responses typified by the expression of specific genes. MyD88-dependent genes include tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and are regulated by early activation of NF-\(\kappa\)B. MyD88-independent, TRIF-mediated genes are typically interferon mediated and regulated by IRF-3 and a later phase activation of NF-\(\kappa\)B (18).

Role of TLR2 in health and disease

Currently, it is not clear precisely how TLR2 interacts with TLR1 and TLR6 nor what relevance specific tissue distribution has to immunity or disease. However, this is an active area of TLR research which will inevitably identify new targets.

Finally, TLR2 activation has also been shown to result in the inhibition of inflammatory genes (19, 20). The inhibitory function of TLR2 may explain in part the protective effect it appears to have in the development of asthma (see below).

Evidence for a role for TLR2 in lung disease

The importance of TLR2 as a target for treatment of lung disease has been heightened by the understanding that bacterial infections can initiate and propagate disease and by the rapid increase in Gram-positive infections. In addition, above other members of the TLR family, TLR2 seems particularly sensitive to nonpathogen ligands including host-derived molecules and oxidants. These points strongly suggest that TLR2 will be an important therapeutic target in the treatment of lung diseases including chronic obstructive pulmonary disease (COPD) and asthma.

COPD

In COPD, a link between TLR2 has recently been described although the area is new and results inconsistent. TLR2 is either unregulated in monocytes (21) or downregulated in macrophages (22) in samples from COPD patients. However, the strongest indication comes from preclinical models described above, showing that oxidants including cigarette smoke induce cell activation and inflammation in a TLR2-dependent manner.

Atopy and the hygiene hypothesis

By contrast, there is increasing evidence that a functional TLR2 system is protective against the development of asthma. This fits well with the “hygiene hypothesis” that activation of the innate immune system and induction of an appropriate Th1 response protects infants from a Th2 skew and atopy. Early studies showed increased expression of Toll-like receptor 2 protein on blood cells of children on farms. This was taken to explain their reduced risk of atopy (23). Later polymorphisms in TLR2 gene were also associated with reduced risk of asthma in children of farmers—it was suggested that the TLR2/–16934 polymorphism could result in increased TLR2 protein expression (24).

Protection from asthma

TLR2 forms heterodimers with TLR1 or TLR6, and a very recent study suggests that polymorphisms in TLR1 or TLR6, which result in increased expression of TLR protein, are associated with reduced susceptibility to asthma (25). Clearly then, TLR2 signaling may coordinate the development of a Th1 phenotype in some individuals and where this function is reduced there could be an increased risk of atopy and asthma.

Causation of asthma

However, there is also the possibility that in adults in whom asthma has developed, activation of TLR2 may lead to exacerbations in inflammation and symptoms. Recent evidence suggests that bacterial infection, which will result in activation of TLR2, contributes to disease severity (26). Activation of TLR2 on pulmonary mast cells results in the release of various mediators associated with asthma including leukotrienes (27). Moreover, activation of TLR2 in animal models appears to predispose to an atopic Th2 phenotype consistent with a role for this receptor in exacerbation of asthma (28).

The tools

Genetically modified mice lacking TLR2, TLR1 or TLR6 have been developed. There are also highly specific ligands available that activate the TLR2/1 or TLR2/6 heterodimer complexes. However, there are currently no antagonists of TLR2, TLR1 or TLR6. Doubtless these are being developed and will become available over the coming years at which point we will be able to test the hypothesis that “TLR2 is a therapeutic target for the treatment of human lung disease.”

Summary and conclusions

TLR2 is implicated in lung inflammation. It is a receptor for oxidant stress and for Gram-positive bacteria. There is strong evidence that in childhood, activation of TLR2 may preserve a Th1 phenotype and protect against the development of asthma. TLR2-specific antigens are already available and it is therefore possible that these may be used as adjuvants in the prevention of asthma. Once asthma is established, or in the pathogenesis of COPD, TLR2 activation by oxidants or bacteria may exacerbate inflammation. In these settings, antagonists of TLR2 may prove therapeutically useful.

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