

TITLE:

The role of pro-resolution lipid mediators in infectious disease

ABSTRACT:

Inflammation is an essential host defence against infection, but can be damaging when excessive. Resolution of inflammation is an active process, and the pro-resolution effects of lipoxins, resolvins and protectins have received significant interest. Here, we review emerging data on the role of these lipid mediators in infectious disease. Lipoxins influence host control of *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Trypanosoma cruzi* and *Plasmodium berghei* cerebral malaria in mice. Their effects are protective in toxoplasmosis, *T. cruzi* infection and cerebral malaria but detrimental in tuberculosis; related to the balance between pathogen-control and excessive immune response. Topical lipoxin abrogates the tissue damage seen in a rabbit model of *Porphyromonas gingivalis* periodontitis. The increased virulence of H5N1 influenza A virus in mice correlates with reduced expression of SOCS2, required to mediate the effects of lipoxin. Mice unable to synthesize lipoxin suffer increased lung pathology during respiratory syncytial virus infection. Protectin suppresses influenza A virus replication in vitro and increases survival in a mouse model of severe influenza infection. Resolvins were investigated in a number of animal models of systemic bacterial infection, and were found to enhance phagocytic clearance of bacteria, reduce inflammation severity, promote neutrophil apoptosis, modulate neutrophil chemotaxis and importantly, reduce mortality. Interestingly, resolvin also enhances the antibacterial effect of ciprofloxacin and vancomycin. Topical resolvin application reduces the severity of herpes simplex virus ocular infection in mice. If the effects of these mediators translate from pre-clinical studies into successful clinical trials, they represent promising new strategies in managing infectious disease.

Introduction:

Inflammation is an essential host defence mechanism, required for protection against pathogenic micro-organisms and viruses. However, an excessive and non-resolving inflammatory response is damaging to the host. For example, in sepsis there is loss of homeostatic control over the inflammatory response mounted against an infectious agent, resulting in a pro-inflammatory, pro-coagulant state that can result in organ dysfunction and death. Acute respiratory distress syndrome is another example, and describes the life-threatening hypoxic complication of excessive pulmonary inflammation that can be triggered by a range of insults, including pneumonia and sepsis.<sup>1</sup> Chronic, non-resolving inflammation also underpins a number of other pulmonary diseases, including asthma, cystic fibrosis and bronchiectasis. Inflammatory bowel disease and rheumatoid arthritis are extra-pulmonary examples of this. Resolution of inflammation and restoration of normal tissue function are therefore critical events following the clearance of an infectious agent, to prevent the development of these 'complications' of excessive inflammation. This is now known to be an active process, termed catabasis,<sup>2</sup> driven by the pro-resolution lipid mediators resolvin, protectin and lipoxin.<sup>3</sup> Lipoxins (trihydroxy-tetraene-containing eicosanoids) are derived from the omega-6 polyunsaturated fatty acid arachidonic acid through sequential reactions involving lipoxygenase enzymes (including 15-lipoxygenase type 1 and 5-lipoxygenase in human mucosal tissues).<sup>4–5</sup> Resolvins are derived from omega-3 polyunsaturated fatty acids and exist as two series (D and E). D-series resolvins are products of docosahexaenoic acid metabolism involving 15-lipoxygenase. E-series resolvins are synthesized from eicosapentaenoic acid involving aspirin-acetylated cyclo-oxygenase.<sup>2,6</sup> Protectins are also omega-3 polyunsaturated fatty acid derivatives, generated from docosahexaenoic acid through a 15-lipoxygenase-mediated pathway.<sup>7</sup> The pro-resolution effects of these mediators are exemplified by their role in pulmonary inflammation, the setting in which they are best studied. Resolvins, protectins and lipoxins all have a pro-resolution role in a mouse model of allergic airway inflammation.<sup>8–11</sup>

Given that an excessive inflammatory response is implicated in the pathogenesis of a number of infectious diseases and their complications, including sepsis syndrome and acute respiratory distress syndrome, we conducted a review of the literature to determine what is currently known about the role of the pro-resolution lipid mediators lipoxin, resolvin and protectin in infectious disease.

Influenza A virus ::: Lipoxins:

Cilloniz et al.<sup>12</sup> used a mouse model to investigate influenza A virus virulence, comparing host transcriptional responses to infection with reconstructed 1918 H1N1 virus to avian H5N1 virus (Vietnam/1203). This highly pathogenic avian H5N1 virus is more virulent in humans than the 1918 H1N1 virus, with a case-mortality rate of 60% compared with 2.5%.<sup>13</sup> In mice, H5N1 was more virulent (infection resulted in shorter survival time) than the H1N1 strain and disseminated to extra-pulmonary sites (brain and spleen) following intra-nasal inoculation. Global transcriptional responses to infection were analysed and showed that the different strains of influenza A virus caused different early responses in gene expression, and that many of the differentially regulated genes were components of the inflammatory response. H5N1 infection was associated with up-regulation of inflammasome genes [including caspase 1 and interleukin-1 (IL-1)], tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ) and protein kinase R, as well as other inflammation-related genes. Using a bioinformatics approach to analyse gene expression, the authors found that extra-pulmonary dissemination was associated with down-regulation of genes involved in mediating the pro-resolution effects of lipoxin on leucocyte recruitment and counter-regulation of pro-inflammatory cytokine induction. These included the suppressor of cytokine signalling (SOCS) 2 gene, the product of which is activated by lipoxins and is an essential intracellular mediator of lipoxin's effects on inflammatory cell trafficking and cytokine induction.<sup>14</sup> Therefore, loss of lipoxin's pro-resolution effects may be associated with greater influenza A virus virulence, suggesting a protective role for lipoxin in this infection, possibly related to the suppression of pro-inflammatory cytokines that are up-regulated in this model. The highly pathogenic avian H5N1 virus used in this study was less successful at spreading between human hosts compared with other influenza A virus strains, for example the 2009 H1N1 pandemic strain. A successful pathogen minimizes damage to its host to prolong the availability of its replicative niche and the high case-mortality seen with infection by this H5N1 strain indicates that it is not well adapted to humans. Reduction of lipoxin-mediated pro-resolution effects may contribute to the virus's poor adaptation to humans.

#### Respiratory syncytial virus ::: Lipoxins:

In a recent study, respiratory syncytial virus (RSV) infection of mice deficient in 5-lipoxygenase, an enzyme required for lipoxin production, failed to elicit alternative macrophage differentiation, which is known to be required for the resolution of RSV-induced lung injury.<sup>15–16</sup> Alternatively activated macrophages are thought to induce anti-inflammatory cytokine expression and drive lung tissue repair.<sup>16</sup> Significantly, RSV infection of these 5-lipoxygenase-deficient mice resulted in greater lung pathology on histological analysis (increased peribronchiolitis, perivascularitis, interstitial pneumonitis and alveolitis) in comparison to infected wild-type mice.<sup>15</sup> Treatment with lipoxin A4 and resolvin E1 partially restored the alternatively activated macrophage phenotype in the 5-lipoxygenase-deficient mice. These observations support a pro-resolution role for lipoxins in viral respiratory tract infection.

#### Other respiratory viruses ::: Lipoxins:

Alveolar macrophages are the major resident inflammatory cell of the respiratory tract and are able to produce lipoxins. Alveolar macrophages isolated from rats that had previously been infected with a range of respiratory viruses (murine parainfluenza virus type 1, rat coronavirus, pneumonia virus of mice and mouse adenovirus) produced higher levels of lipoxin than macrophages from rats that had been kept in sterile conditions since birth.<sup>17</sup> These findings suggest that virus-induced pulmonary inflammation is a stimulus for the production of lipoxins.

#### Human immunodeficiency virus ::: Lipoxins:

In a cell culture model of HIV central nervous system infection, following co-culture of HIV-infected monocytes with astroglia, TNF- $\alpha$  and IL-1 $\beta$  were produced and this production correlated with synthesis of large amounts of leukotriene B4, leukotriene D4 and lipoxin A4.<sup>18</sup> This is the only study to demonstrate, albeit in vitro, that lipoxins are produced in direct response to viral infection. However, the role of lipoxin in this model of infection has not been investigated.

#### Mycobacterium tuberculosis

##### ::: Lipoxins:

Following aerosol infection with *Mycobacterium tuberculosis*, mice have been shown to produce high levels of lipoxin A4 during chronic infection.<sup>19</sup> Pulmonary endothelial cells and macrophages were responsible for this. Transgenic mice deficient in 5-lipoxygenase, an enzyme required for

lipoxin production, were able to control *M. tuberculosis* infection better than wild-type mice, as demonstrated by lower pulmonary and splenic titres of viable *M. tuberculosis* (at 21 and 42 days). On histological examination of lung tissue, extensive inflammation and areas of necrosis were seen in wild-type mice whereas the transgenic mice had a lesser degree of inflammation and little evidence of necrosis. Importantly, the transgenic mice unable to produce lipoxin enjoyed enhanced survival in this model of *M. tuberculosis* infection and had increased pulmonary levels of IL-12, IFN- $\gamma$  and inducible nitric oxide synthase, which is known to have a protective role in host control of *M. tuberculosis* infection in mice.<sup>20</sup> Oral administration of a lipoxin A4 analogue reversed the enhanced control of infection in the transgenic mice, and splenocytes from these mice showed reduced IFN- $\gamma$  production.

#### *Toxoplasma gondii*

##### ::: Lipoxins:

In a mouse model of *Toxoplasma gondii* infection (established through intraperitoneal inoculation of *T. gondii* cysts), serum levels of lipoxin A4 rise during infection and remain high once chronic infection has been established.<sup>21</sup> Transgenic mice unable to produce lipoxin A4 suffered increased mortality following infection, compared with wild-type control animals.<sup>14–21</sup> Interestingly, fewer *T. gondii* brain cysts were seen in the lipoxin-deficient mice and consistent with this, lower parasitic burden and increased serum levels of IL-12 and IFN- $\gamma$  were seen when compared with wild-type mice. Therefore, it seems likely that the increased mortality of the lipoxin-deficient mice is attributable to cytokine-mediated tissue damage, despite better parasite control. Corroborating this, increased histological severity of meningitis and encephalitis was observed in the transgenic mice. Administration of a lipoxin analogue rescued the lipoxin-deficient mice from this fatal phenotype and lowered IL-12 and IFN- $\gamma$  levels (to wild-type levels in the case of IFN- $\gamma$ ).

Interleukin-12, produced by antigen-presenting cells such as dendritic cells, has an important role in host control of intracellular pathogens such as *T. gondii* and viruses, but in excess can cause immunopathology. In mice, it has been shown that lipoxin A4 analogues suppress IL-12 production by dendritic cells stimulated with *T. gondii* extract.<sup>22</sup> Although this may be a host-driven response to curb excessive inflammation, induction of lipoxin production could be a strategy adopted by pathogens to modulate host immunity and perhaps facilitate chronic infection by reducing tissue damage.

The contrasting roles of lipoxins in these two models of infection may relate to the dynamics of the specific pathogen–host interactions. *Toxoplasma gondii* replicates more quickly than *M. tuberculosis* and the risk of excessive inflammation and ensuing immunopathology may therefore be greater.<sup>19</sup> By preventing this, lipoxins are beneficial to the host, and so enhance survival. *Mycobacterium tuberculosis* induces a weaker T helper 1 cell response in mice than *T. gondii* and replicates more slowly, therefore the enhanced inflammatory response seen in the lipoxin-deficient mice may be advantageous, by ‘topping-up’ existing host control. The reduced tissue damage seen in lipoxin-deficient mice may be a result of better control of *M. tuberculosis* replication. It is also important to note that the local (lung versus peritoneum/brain) biosynthesis and actions of lipoxin may differ between these two infection models and could provide an alternative explanation for the different outcomes observed.

#### *Trypanosoma cruzi* (Chagas disease) ::: Lipoxins:

In a mouse model of *Trypanosoma cruzi* infection, it was found that the administration of aspirin (acetylsalicylic acid) at doses of 25 or 50 mg/kg increased survival and decreased peak parasitaemia, whereas higher or lower doses had no effect.<sup>23</sup> The aspirin-triggered lipoxin, 15-epi-lipoxin A4, was found to be increased by administration of aspirin in both in vitro and in vivo models of infection, with infection alone also increasing 15-epi-lipoxin A4 levels. Exogenous administration of 15-epi-lipoxin A4 was found to decrease parasitaemia peaks and decrease cardiac inflammation and increase survival. The authors therefore hypothesized that the beneficial effect of aspirin on infected mice was a result of the production of 15-epi-lipoxin A4. This may represent a new therapeutic strategy in the acute phase of Chagas disease.

#### Cerebral malaria ::: Lipoxins:

Wild-type and 5-lipoxygenase-deficient mice were infected with *Plasmodium berghei*-ANKA in a model of cerebral malaria.<sup>24</sup> Wild-type mice enjoyed significantly increased survival in comparison to 5-lipoxygenase-deficient mice. Levels of parasitaemia were similar between

groups, but lipoxin-deficient mice had an increased cerebral parasite burden after 5 days despite reduced cerebral inflammation and less CD8<sup>+</sup> IFN- $\gamma$ <sup>+</sup> T cells in brain tissue. The lipoxin-deficient mice were found to have higher serum levels of IL-12 and IFN- $\gamma$ , both of which are associated with the pathogenesis of murine cerebral malaria. Significantly, treatment with 15-epi-lipoxin A4 significantly prolonged survival in both wild-type and lipoxin-deficient mice, as well as reducing expression of IL-12 and IFN- $\gamma$  in the brain. The mortality rate did not differ significantly if lipoxin was administered on the day of infection or 3 days later. Lipoxin therefore appears to increase host survival in a mouse model of cerebral malaria by limiting inflammation, not by enhancing control of parasite levels.

#### Periodontitis ::: Lipoxins:

Serhan and co-workers used a rabbit model of periodontitis to investigate the effects of lipoxin in this infection.<sup>25</sup> The application of a dental ligature plus *Porphyromonas gingivalis* resulted in soft tissue loss, histological (leucocyte infiltrates) and radiological (bone loss) features of periodontitis in wild-type rabbits. The authors then used a transgenic rabbit that over-expresses the enzyme 15-lipoxygenase, resulting in threefold to fourfold increased lipoxin production. In these transgenic animals, following the same procedure to establish periodontitis, none of the clinical, histological or radiographic features were found. In the wild-type animals, bone loss could be prevented by the administration of intravenous metronidazole, confirming that *P. gingivalis* infection was responsible for the features seen. Wild-type rabbits subject to periodontitis induction were then administered a topical lipoxin analogue and this protected the animals from gross soft tissue destruction, leucocyte accumulation and bone loss. Periodontitis is an infectious disease, though the tissue damage characteristic of the condition is thought to be driven by an aberrant host response to infection.<sup>26</sup> In this set of experiments, Serhan et al. demonstrate that lipoxin has a role in curbing this excessive response and that this has a protective effect in vivo.

#### Salmonella typhimurium

##### ::: Lipoxins:

By performing transcriptional profiling of model epithelial cells exposed to lipoxin, Canny et al.<sup>27</sup> found that lipoxin up-regulated the expression of bactericidal/permeability-increasing protein (BPI). BPI is an innate immune defence molecule that is active against Gram negative bacteria, through bactericidal effects on bacterial cell membranes, neutralization of lipopolysaccharide and as an opsonin. This was then confirmed in four other epithelial cell lines (OKF6, T84, Caco2 and pulmonary epithelial cells A549). Building on the known bactericidal effect of epithelium-derived BPI on Gram-negative bacteria, the authors sought to examine the effect of lipoxin on epithelial killing of *Salmonella typhimurium*. Epithelial cell culture was pre-exposed to lipoxin analogue and then incubated with *S. typhimurium*. This resulted in concentration-dependent increased bacterial killing compared with non-pre-treated cultures. This enhanced bactericidal activity could be significantly inhibited by the addition of anti-BPI, indicating that induction of BPI was largely responsible for this lipoxin-mediated enhanced killing of *S. typhimurium*. Interestingly, in a mouse model of (non-infectious) dextran sodium-sulphate-induced colitis, oral administration of lipoxin analogue reduced weight loss, passage of blood per rectum and mortality, affirming its role in gastrointestinal mucosal inflammatory conditions.<sup>28</sup>

#### Sepsis ::: Resolvins:

Caecal ligation and puncture in mice establishes microbial sepsis that is considered similar to the pathophysiology of sepsis in humans.<sup>29</sup> In this model, in comparison to a control treatment, intravenous administration of resolvin D2 reduced the viable bacterial load in blood and peritoneal exudates (in the absence of direct antibacterial effects) and reduced neutrophil migration into the peritoneal cavity. In addition, histological evidence was seen of enhanced phagocytosis of bacteria in inguinal lymph nodes and in vitro, incubation with resolvin D2 enhanced zymosan phagocytosis by macrophages obtained from the peritoneum of resolvin-naïve mice. When incubated with resolvin D2, human neutrophils demonstrated enhanced phagocytosis of *Escherichia coli* and also increased intracellular production of reactive oxygen species. Furthermore, plasma levels of pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , IL-23 and TNF- $\alpha$ ) were reduced in resolvin D2-treated mice. Importantly, the effect of these pro-resolution actions of resolvin was to increase survival from sepsis in this mouse model.

Chiang et al.<sup>30</sup> investigated the effects of resolvin using a mouse model of *E. coli* peritonitis, involving intraperitoneal inoculation of *E. coli*. They found that the intraperitoneal administration of

resolvin D5 enhanced phagocytosis of bacteria in comparison to mice inoculated with *E. coli* without resolvin. Resolvin D1 had a similar but smaller effect. Administration of resolvin (D1 or D5) was also associated with a significantly lower titre of viable bacteria in the blood and peritoneal exudate, as well as a lesser degree of hypothermia. Importantly, resolvin D1 increased survival in this model. As for the model of microbial sepsis discussed previously, plasma levels of pro-inflammatory cytokines (including TNF- $\alpha$  and IL-1 $\beta$ ) were reduced by resolvin administration. Interestingly, it was found that resolvin D1 enhanced the antimicrobial effect of ciprofloxacin in resolving *E. coli* peritonitis. When given in combination, resolvin and ciprofloxacin had an additive effect on reducing the 'resolution interval' (time from maximum concentration of peritoneal leucocytes to 50%), greater than either agent given alone. In addition, the combined administration of ciprofloxacin and resolvin D1 resulted in lower titres of viable bacteria in blood and peritoneal exudate, and enhanced macrophage phagocytic activity (not seen with either agent alone). The effect of reducing bacterial titres was still seen even when ciprofloxacin was administered at sub-optimal concentrations with a cocktail of resolvin D1, D5 and protectin D1. In *Staphylococcus aureus* skin infection, using mouse dorsal skin pouches, resolvins D1 and D5 plus protectin D1, when administered with vancomycin (at a sub-optimal concentration) into the pouch, enhanced the clearance of bacteria, resulting in lower viable bacterial titres in pouch exudate and reduced neutrophil infiltration. This effect was also seen when either resolvin or vancomycin was administered alone, but again, an enhanced effect was seen when both were given together.

#### Burns-related sepsis ::: Resolvins:

Infection secondary to burns is an important clinical problem and burns-related sepsis is responsible for significant mortality in burns patients. Using a rat model of burn injury, Kurihara et al.<sup>31</sup> demonstrated that neutrophils isolated from burned rats were impaired in their ability to migrate towards a chemoattractant. This defect in neutrophil chemotaxis could result in an inability of neutrophils in burns patients to successfully reach and kill infectious agents. As a consequence, these 'lost' neutrophils activated by the burn injury may accumulate and initiate tissue damage inappropriately in healthy tissues. By administering resolvin D2 to the burned rats, Kurihara et al. found that neutrophil chemotaxis was restored to almost normal. When burned rats received intravenous lipopolysaccharide 9 days after their burn injury, pre-treatment with intravenous resolvin D2 improved survival significantly. Similarly, resolvin D2 pre-treatment increased survival following caecal ligation after burns injury. A shorter duration of resolvin treatment that was insufficient to normalize neutrophil chemotaxis did not significantly improve survival in these two models of burns-related sepsis. Therefore, this study suggests that administering resolvin D2 could improve outcomes in burns-related sepsis by modulating neutrophil chemotaxis.

#### Pneumonia and acute lung injury ::: Resolvins:

The role of resolvin E1 in bacterial pneumonia and acute lung injury was investigated using a mouse model of aspiration pneumonia.<sup>32</sup> Mice were administered hydrochloric acid followed by *E. coli* into a lung. Resolvin E1, when given intravenously before this insult, reduced pulmonary neutrophil infiltration, enhanced bacterial clearance and decreased levels of pro-inflammatory cytokines (including IL-1 $\beta$  and IL-6) present in lung tissue homogenates. Importantly, mice administered resolvin E1 enjoyed increased survival (100% at 3 days, in comparison to 50% when only 0.9% saline was administered).

Neutrophil apoptosis is known to be a critical event in the resolution of pulmonary inflammation, and suppression of this process is thought to contribute to the pathology seen in acute respiratory distress syndrome and sepsis. In a case-control cohort study of 121 intensive therapy unit patients with or without sepsis, the level of neutrophil apoptosis in peripheral venous blood was found to inversely correlate with the severity of sepsis.<sup>33</sup> The role of resolvin E1 in this process has been investigated in mouse models of acute lung injury, including an *E. coli* pneumonia model and an *E. coli* peritonitis-associated acute lung injury model.<sup>34</sup> Six hours after intra-tracheal instillation of *E. coli* (pneumonia model), intraperitoneal administration of resolvin E1 enhanced the resolution of *E. coli*-evoked pneumonia in this mouse model. In contrast to the control group, mice administered resolvin had lower bronchoalveolar lavage neutrophil counts, higher monocyte/macrophage counts, increased neutrophil apoptosis, reduced bronchoalveolar lavage IL-6 levels and less severe pulmonary inflammation when assessed histologically. When a pan-caspase inhibitor was also added intraperitoneally, these pro-resolution effects of resolvin were abrogated, suggesting that neutrophil apoptosis plays a critical role here. In the *E. coli* peritonitis-associated

acute lung injury model, intraperitoneal administration of resolvin E1 also reduced bronchoalveolar lavage neutrophil counts and IL-6 levels, increased neutrophil apoptosis and reduced the histological severity of pneumonia as well as lung myeloperoxidase content (where myeloperoxidase is used as a marker of neutrophil activity). Importantly, resolvin administration reduced 6 hr mortality from 70% to 30% in this model.

In terms of a possible mechanism for increased neutrophil apoptosis, in vitro, resolvin E1 mitigates cellular survival signals generated in response to a number of stimuli relevant to the pathogenesis of acute lung injury in *E. coli* pneumonia and sepsis (neutrophil myeloperoxidase, serum amyloid A and bacterial CpG DNA). Similarly, 15-epi-lipoxin A4 also has a pro-apoptotic effect on human neutrophils and in a mouse model of *E. coli* sepsis-induced lung injury, intravenous lipoxin reduced bronchoalveolar lavage neutrophil counts and increased caspase-mediated neutrophil apoptosis.<sup>35</sup>

#### Herpes simplex virus ::: Resolvins:

As discussed, although inflammation is an essential response to infection, an excessive response is detrimental and stromal keratitis demonstrates this. It is an inflammatory lesion of the cornea that is a sequela of ocular herpes simplex virus infection, usually appearing 1 week after primary infection. Importantly, when stromal keratitis manifests, replicating virus cannot be detected; therefore the initiating virus has been cleared and the disease is a consequence of non-resolving excessive inflammation. In mice, ocular herpes simplex virus infection is a model of stromal keratitis in humans. Topical administration of resolvin E1 was able to control the immunopathological manifestations of ocular herpes simplex virus and reduce the severity of stromal keratitis lesions.<sup>36</sup> The influx of CD4<sup>+</sup> T cells and neutrophils was reduced, as were corneal levels of pro-inflammatory cytokines known to be involved in the pathogenesis of stromal keratitis. Levels of the anti-inflammatory cytokine IL-10 were increased.

#### Protectins:

Through a screen of the effect of a range of lipid mediators on the replication of H1N1 influenza A virus in human lung epithelial cells, protectin D1 was found to significantly reduce viral replication in vitro.<sup>37</sup> In a model of human severe influenza, virus was introduced through the intra-tracheal route in mice and protectin D1 levels were found to be reduced, suggesting that endogenous production was suppressed by the virus. When other influenza A virus strains were tested, the reduction in protectin levels was found to inversely correlate with the virulence of the virus strain used. In this same model of severe influenza, intravenous administration of protectin increased survival. Administration of resolvin and lipoxin had no effect. Significantly, the protective effect of protectin was still seen when administered 2 days after the start of infection.

#### Conclusions:

Lipoxins, resolvins and protectins are endogenous lipid mediators with a pro-resolution effect in inflammation. Much work has focused on their now well-recognized role in allergic airway inflammation.<sup>38</sup> However, an emerging theme is their importance in the resolution of infection and associated inflammation, and this article has reviewed emerging data relevant to this. Table 1 summarizes what has been learnt from animal studies.

The role of lipoxins may vary depending on the specific infection. In a mouse model of *M. tuberculosis* infection, transgenic mice unable to produce lipoxin were able to control the bacteria better with reduced mortality in comparison to wild-type, and expressed IL-12, IFN- $\gamma$  and inducible nitric oxide synthase 2 at higher levels. In contrast, using the same transgenic mice in *T. gondii* infection, mortality was higher despite a lower parasitic burden and this was attributed to excessive cytokine-mediated tissue damage. Beneficial effects were also seen in a mouse model of *Trypanosoma cruzi* infection and cerebral malaria. Topical lipoxin abrogated the tissue and bone damage seen in a rabbit model of *P. gingivalis* periodontitis. Therefore, the pro-resolution effects of lipoxin may be advantageous in protecting the host from the damaging effect of excessive inflammation, but this benefit may be out-weighed in some situations by permitting unchecked pathogen replication (as seen in the model of *M. tuberculosis*). The finding that administration of lipoxin enhances survival in a mouse model of cerebral malaria suggests a potential new therapeutic strategy for human cerebral malaria.

The effects of resolvin were investigated in a number of mouse models of systemic bacterial infection, and were found to enhance phagocytic clearance of bacteria, reduce neutrophil influx and inflammation severity, promote neutrophil apoptosis and clearance, modulate neutrophil chemotaxis and importantly, reduce mortality. Interestingly, resolvin administration also enhanced

the antibacterial effect of ciprofloxacin (in *E. coli* peritonitis) and vancomycin (in *Staphylococcus aureus* skin infection) when the antimicrobial was administered at a sub-optimal concentration. Topical resolvins application also reduced the severity of herpes simplex virus ocular infection in mice. Finally, protectin was found to increase survival in a mouse model of severe influenza, even when administered 2 days after infection.

Given the well-recognized importance of these mediators in allergic airway inflammation, it is interesting to consider how their regulation may be disturbed in states of airway inflammation induced by infection, for examples acute respiratory distress syndrome. Notably, pulmonary production of protectin was found to be suppressed by highly pathogenic influenza A strains.<sup>37</sup> Evidence from Cilloniz et al.<sup>12</sup> suggests that lipoxin-mediated activity is also altered during influenza A virus infection. Disturbed lipoxin regulation could have a role in the predisposition towards asthma seen in infants following RSV bronchiolitis,<sup>39–40</sup> especially when considering the finding that mice unable to synthesize lipoxin fail to elicit alternative macrophage differentiation, required for the resolution of RSV disease.<sup>15–16</sup>

If the beneficial effects of these mediators translate from pre-clinical studies into clinical trials, they represent promising new strategies in the management of infectious disease. The pro-resolution, anti-inflammatory and antimicrobial-enhancing effects of resolvins, protectin and possibly lipoxins make these appealing candidates for further study in humans. From a therapeutic perspective it is important to note that these pro-resolution mediators enjoy a substantial advantage over steroids for use in the treatment of infectious inflammation, or other systemic inflammatory states, as they are not immunosuppressive agents. 'Aspirin-triggered' lipoxins and resolvins share the pro-resolution effects of lipoxin A4 and resolvin D1, respectively, and act by the same intracellular pathways.<sup>11,14</sup> This effect is unique to aspirin, and is not shared with non-steroidal anti-inflammatory drugs, which do not trigger the biosynthesis of these mediators. Due to its ability to trigger the production of these pro-resolution mediators there may be a new role for aspirin in managing the inflammatory sequelae of infectious disease.

#### Disclosures:

The authors declare that they have no competing interests.