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Targeting the "Cytokine Storm" for Therapeutic Benefit

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Inflammation is the body's first line of defense against infection or injury, responding to challenges by activating innate and adaptive responses. Microbes have evolved a diverse range of strategies to avoid triggering inflammatory responses. However, some pathogens, such as the influenza virus and the Gram-negative bacterium *Francisella tularensis*, do trigger life-threatening "cytokine storms" in the host which can result in significant pathology and ultimately death. For these diseases, it has been proposed that downregulating inflammatory immune responses may improve outcome. We review some of the current candidates for treatment of cytokine storms which may prove useful in the clinic in the future and compare them to more traditional therapeutic candidates that target the pathogen rather than the host response.

In the event of tissue damage, whether caused by injury or infection, inflammation is the body's first coordinated line of defense. It is responsible for activating both innate and adaptive immune responses so that the damage can be resolved and homeostasis restored. The characteristic signs of inflammation include heat, redness, swelling, and pain and are easily recognizable (1). There are four stages to a classical self-limiting inflammatory response: (i) recognition of the problem, (ii) recruitment of leukocytes and other immune system components, (iii) elimination of the threat, and (iv) resolution of the inflammatory state (i.e., a return to homeostasis).

RECOGNITION

In the case of infection, inflammation begins when the cells of the innate immune system recognize a pathogen-associated molecular pattern (PAMP) possessed by the invading organism. PAMPs are often an essential feature of the microbe and therefore are highly conserved, increasing recognition (2). The receptors on host phagocytic cells that recognize PAMPs are known as pattern recognition receptors (PRRs), of which there are several different categories. Soluble PRRs such as mannose binding lectin act as opsonins, preparing the microbe for phagocytosis (3). Intracellular PRRs, notably the Nod-like receptors (NLRs), are found in the cytosol for the detection of intracellular pathogens (4). Retinoic acid-inducible gene (RIG)-like receptors (RLRs) share a caspase recruitment domain (CARD) with NLRs and are mainly responsible for viral detection (5, 6). Transmembrane PRRs include the Toll-like receptors (TLRs) and C-type lectin receptors. Activation of a subset of NLRs, NLRP1, NLRP3, and NLRC4, induces the formation of a multiprotein complex called the inflammasome. Upon assembly, caspase proteins are cleaved from their proforms to an active state leading to the processing of interleukin-1β (IL-1β) and IL-18 (7).

Once the PRR is activated and ligand binding occurs, a signaling cascade is triggered, which results in expression of specific proinflammatory cytokines. Cytokines play a vital role throughout the four stages of inflammation. During the early phase of infection, these protein messenger molecules act as signals to the immune system, regulating the duration and gravity of the immune response to damage or infection. Depending upon the specific cytokine that has been secreted, their role can be to activate (proinflammatory) or dampen (anti-inflammatory) the host response. For example, stimulated TLRs induce proinflammatory cytokines, while the production of the anti-inflammatory cytokine IL-10 is important during the later stages of infection in controlling disease-induced tissue pathology (8). In the case of sterile inflammation caused by tissue damage, trauma, and ischemia, PRRs recognize certain host-specific molecules that are only released during cell injury or necrotic death, termed damage-associated molecular patterns (DAMPs). These molecules include heat shock proteins and high-mobility group box 1 (HMGB1) and are recognized in much the same way as PAMPs (9).

RECRUITMENT

Once recognition has occurred and inflammation has been initiated, certain host cells begin to secrete chemokines. Chemokines are relatively small proteins with a molecular weight of less than 10 kDa which activate and mediate the migration of leukocytes to the site of infection or inflammation (10). Many different types of cells are able to secrete these chemotactic cytokines, including phagocytic cells such as macrophages and neutrophils, though endothelial cells are responsible for over half of all produced. Chemokines activate integrins and bind to intercellular adhesion molecules (ICAMs) (11). Subsequently, cells roll along the endothelium, up a chemokine gradient to the site of inflammation where they transmigrate through cell junctions into the damaged or infected tissue (10, 12).

RESOLUTION

Following recruitment of immune cells to the site of inflammation, resolution of the damage begins. The cytokines induced by PAMPs and produced by leukocytes are proinflammatory cytokines and include tumor necrosis factor alpha (TNF-α), IL-6, and members of the IL-1 family, all of which have different proinflammatory roles. TNF-α and IL-1β induce vasodilation and permeability, allowing immune cells to reach the site of damage, while IL-β and IL-6 induce complement and opsonization (2). As well as mediating the inflammatory response, proinflammatory cyto-

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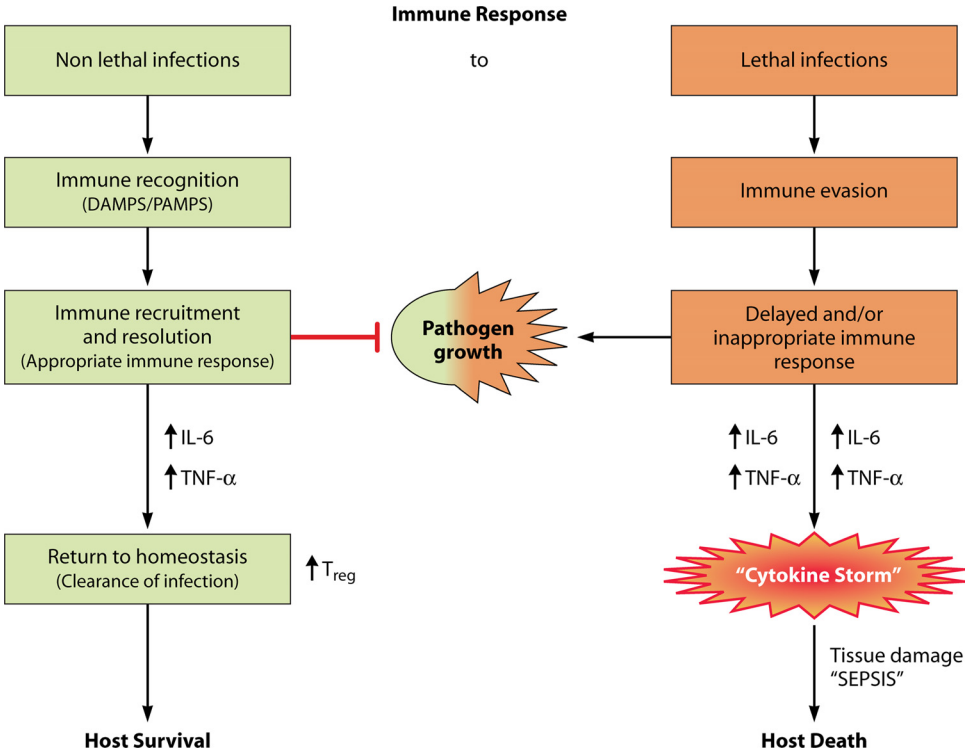

FIG 1 During infection, the host recognizes the pathogen, which leads to cellular recruitment and a proinflammatory cytokine response including IL-6 and TNF-α. This inflammatory response leads to pathogen clearance, thus allowing the return to immune homeostasis and host survival. In some infections, immune recognition is delayed and/or evaded, causing a delayed and/or inappropriate response. This can allow the pathogen to proliferate, triggering hypercytokinemia that leads to tissue damage and potentially death of the host.

kines can affect the brain, inducing behavioral and physiological symptoms such as fever, nausea, and anorexia (13).

RETURN TO HOMEOSTASIS

Throughout its activation, the inflammatory response must be regulated to prevent a damaging systemic inflammation, also known as a "cytokine storm." A number of cytokines with anti-inflammatory properties are responsible for this, such as IL-10 and transforming growth factor β (TGF-β) (14). Each cytokine acts on a different part of the inflammatory response. For example, products of the T_h2 immune response suppress the T_h1 immune response and vice versa (15). Without the ability to resolve the inflammation, the collateral damage to surrounding cells has the potential to be catastrophic, resulting in sepsis and even death. However, if it is controlled correctly, inflammation can be resolved effectively, with little or no long-term damage to the host (16).

PATHOGENS

Pathogens attempt to skew the response of the finely balanced immune system in order to evade immune responses and have evolved a diverse range of strategies to favor their own growth, survival, and replication. At one extreme, some pathogens have strategies to appear invisible to the immune system and thus fail to induce an effective immune response, while at the other extreme, other pathogenic organisms are capable of hyperstimulating the immune system, commonly known as a cytokine storm. This can prevent the clearance of infection and induce tissue damage (i.e., necrosis, a potentially fatal condition). Many reviews have focused on immune evasion as a means to establish infection (17), but the importance of cytokine storms in disease is only just becoming apparent.

Diverse pathogenic viruses (e.g., influenza A) and bacteria (e.g., *Francisella tularensis*) have been found to induce cytokine storms or hypercytokinemia (Fig. 1) (18–20). These pathogens disrupt the delicate balance of a suitable inflammatory response, tipping it from being beneficial to destructive by causing large amounts of positive feedback in immune cells and upregulation of proinflammatory markers, in particular cytokines TNF-α, IL-1β, IL-8, and IL-6. This soon results in symptoms such as hypotension, fever, and edema and can eventually cause organ dysfunction and death (21).

One of the most studied examples of an organism that can cause cytokine storms is influenza A virus, in particular the pandemic subtypes. For example, the H1N1 strain that caused the 1918 pandemic has been shown to induce higher levels of proinflammatory immune cells and cytokines in the lungs than seasonal influenza viruses. This contributes to its high virulence and may account for the unusually high mortality rate seen in otherwise healthy young adults during the outbreak (22). Severe influenza infections caused by highly virulent subtypes such as H1N1 and H5N1 are characterized by overinduction of proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, and monocyte chemotactic protein-1 (MCP-1) (23, 24), which eventually results in multiple organ dysfunction and failure and increased vascular hyperpermeability (23).

Infection by the inhalation route of the zoonotic bacterium *F.*

tularensis can also result in a systemic inflammatory response. Less than 10 *F. tularensis* type A strain bacteria are required to initiate disease (25). *F. tularensis* is an intracellular pathogen and, upon infection, rapidly invades macrophages, where it can multiply in the cytoplasm to high levels (reviewed in reference 26). Interestingly, it has been shown in animal models that when they are infected via the inhalational route, there is a delay of several days between the initial infection and induction of cytokines and chemokines, allowing bacterial replication and dissemination uncontrolled by the immune system (27, 28). Once activated, however, proinflammatory cytokines such as IL-6 are quickly upregulated by up to 1,000 times their resting level. As with influenza, the unchecked hypercytokinemia and subsequent secondary cascades such as coagulation eventually result in widespread necrosis, organ and system failure, and death (25).

THERAPEUTIC STRATEGIES FOR TREATING INFECTIOUS DISEASE

The last century saw enormous leaps forward in the advancement of medicine, resulting in the development of more and more strategies to protect against infectious diseases, many of which have been very successful. Some of these, such as antibiotics, target the pathogen, but increasingly, approaches to elicit a beneficial immune response are being developed as our understanding of the human immune response and host-pathogen interactions develops.

TARGETING THE PATHOGEN

Antibiotics are the best known and most widely used weapon to combat bacterial infections. When antibiotics were discovered in the first half of the 20th century (29), they were heralded as wonder drugs, the beginning of the end for infectious diseases. However, the strong selective pressure exerted by antibiotics, combined with inappropriate use, resulted in the rapid emergence of resistance. Some species of bacteria, such as *Mycobacterium tuberculosis*, have become multidrug resistant (MDR) or even extensively drug resistant (XDR). XDR *M. tuberculosis* has now been reported in over 45 countries (30). Indeed, there are now worrying reports of totally drug-resistant *M. tuberculosis* in India (31). As resistance renders many antibiotics ineffective, there is a pressing need for new compounds for use in the clinic. However, very few new classes of antibiotic have been discovered in the last three decades (32, 33), most new antibiotics appearing on the market being derivatives of beta-lactams and quinolones.

The situation with antivirals is even more desperate: there are far fewer licensed antiviral treatments available than there are antibiotics, and those that are available suffer from being highly specific and thus only target a narrow proportion of viruses. One of the underlying issues is that viruses exploit host cell machinery; thus, identifying effective compounds that inhibit the viral life cycle without affecting the host is challenging. For example, the nucleoside analogue ribavirin targets viral nucleic acid replication. The compound is activated by viral, but not human, enzymes, thus preventing replication (34, 35). Primarily used to treat hepatitis C virus (HCV) as part of combination therapy, it has also been shown to be effective against other viruses, such as measles virus, influenza virus, and arenaviruses, in particular, the virus causing Lassa hemorrhagic fever (36). However, it has a high prevalence of side effects and is thought to be teratogenic in humans (35). Similarly to antibiotics, resistance is also an issue with antiviral drugs, especially for those viruses which have high rates of mutation. Herpes simplex virus, for example, has developed resistance to the antiviral acyclovir. Resistance in patients on long-term treatment regimens for recurrent herpes outbreaks began to emerge within a decade of the drug's original release in the 1980s (37).

Due to the lack of promising antibiotics and antiviral compounds in development, alternative approaches have been considered. For example, two historically evaluated approaches, phage therapy and passive protection, have experienced an increase in interest. While they were largely disregarded after the discovery of antibiotics, they are now being considered again, as levels of antibiotic resistance continue to rise (38). While bacteriophages are easier to produce than antibiotics and have been shown to have very few, if any, side effects, they must be used as a cocktail of several different phages in order to prevent resistance from rapidly emerging. They are also highly specific, so an exact diagnosis, possibly even to the strain or serotype level, must be made before the correct bacteriophage can be administered (38).

Similarly, the idea of using antibodies to directly and immediately boost the immune system during infection has a long history of use but is rarely used today. Sera from immune individuals or animals have been used to treat disease such as *Corynebacterium diphtheriae* as early as the end of the 19th century (39). However, problems with side effects such as serum sickness and narrow specificity caused this approach to fall out of favor for treatment of most diseases. The exception was for prophylaxis of rabies: part of the postexposure rabies treatment consists of rabies immune globulin, which provides short-term, immediate protection with minimal side effects. More recently, developments in monoclonal antibody technology and antibody humanization have made passive therapy a more attractive option by decreasing the risk of adverse side effects.

TARGETING THE HOST

Both chronic infections and acute infections result in the induction of cytokine storms. It is therefore becoming apparent that a combination therapy approach involving an antimicrobial compound along with an immunotherapy may produce a more favorable outcome, and this is an area of intense investigation.

For an immunomodulatory therapeutic to be considered for treatment of infection, it must not deleteriously affect "helpful" elements of the immune response. It also needs to be specific for highly conserved networks that are essential to the host in either maintaining immune homeostasis and/or combating infection. In this review, we focus on promising new therapeutic approaches in this area (Fig. 2) and discuss the advantages and disadvantages which will influence whether they gain acceptance for the clinic.

PROINFLAMMATORY CYTOKINES

The therapeutic use of cytokines as nonspecific immunomodulators that boost the host defenses has traditionally been used to treat long-term or chronic diseases, such as hepatitis, and several are already licensed for human use, including IL-2 and interferons (IFNs) (40). One of the most widely used therapeutic cytokines is IFN-α, a type 1 interferon which inhibits viral replication. It is used in combination with ribavirin for the treatment of chronic HCV, resulting in greater viral RNA clearance together than when administered alone (41, 42).

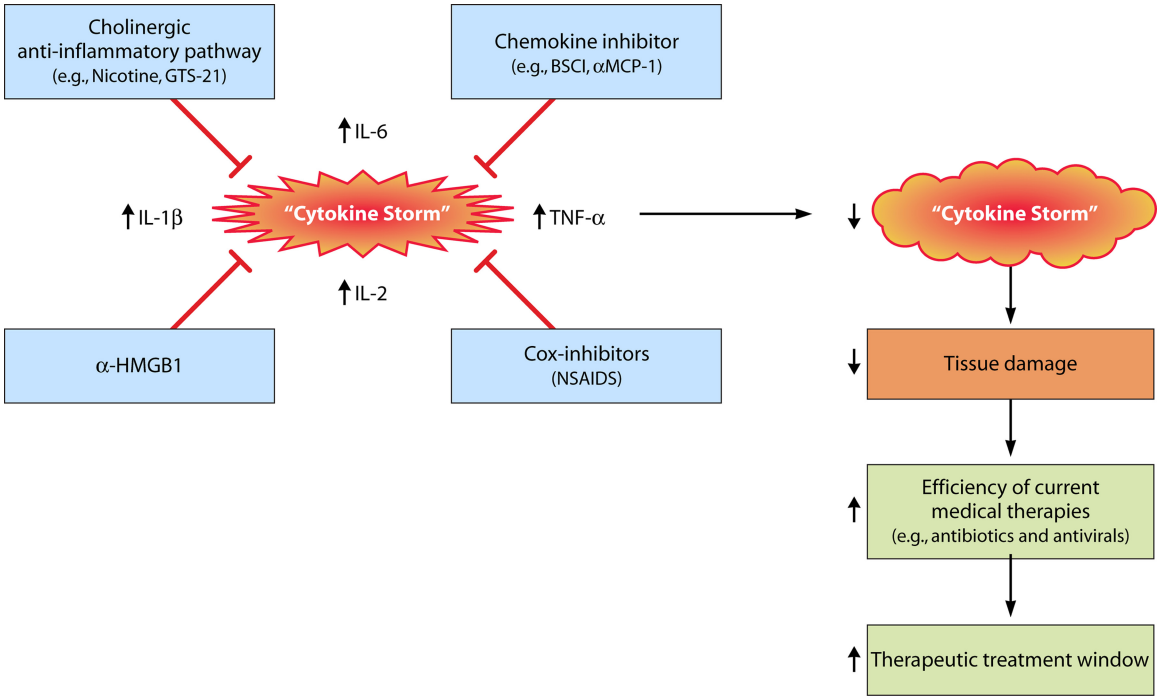

FIG 2 When a cytokine storm has arisen, conventional therapeutics may not be sufficient. Strategies to combat this cytokine storm have included compounds that target fundamental immune pathways, such as the chemokine network and the cholinergic anti-inflammatory pathway, and more specific strategies have included the use of HMGB1 antibodies and COX-2 inhibitors. All these lead to a downregulation of the cytokine storm, reducing the risk of tissue damage and allowing time for conventional therapies to target the pathogen directly.

Having a strong proinflammatory response at the time of infection often results in survival of the host following infection with what would normally be a lethal dose of a microbial pathogen. If treatment is initiated either just before or the same time as infection, then the induction of proinflammatory cytokines by stimulatory molecules such as CpG oligonucleotides has beneficial effects on host survival. Prophylactic use of CpG has been demonstrated to be effective in murine models of *F. tularensis* subsp. *holarctica* live vaccine strain (LVS) (43), *Burkholderia mallei* (44), and *Burkholderia pseudomallei* (45) infection. However, it should be noted that such treatments are not universally successful. For example, recent studies have demonstrated that a preexposure CpG treatment strategy failed to protect mice that were subsequently infected with the highly virulent *F. tularensis* strain SchuS4 (46). Furthermore, there are no reported data on the efficacy of CpG treatment given postexposure against highly virulent pathogens. Indeed, unpublished observations from our laboratory suggest that CpG treatment following infection/onset of symptoms may indeed be detrimental to the host. This therefore implies that causing a rapid increase in proinflammatory cytokine production once infection has occurred may not be suitable.

An alternative approach to induction of proinflammatory host responses could be the use of proinflammatory cytokines themselves. However, the use of common proinflammatory cytokines like IL-1β, IL-6, and TNF-α induces the pathophysiological effects associated with severe infection (47, 48). Indeed, intravenous administration of IL-1β has been shown to cause generalized fatigue, headache, nausea, vomiting, myalgias, and arthralgias (49).

The timing of administration of proinflammatory cytokine treatments requires careful management, since there is a danger that their use may exacerbate the symptoms of disease in infected individuals. It is therefore likely that the window for treatment has probably closed once symptoms present. This uncertainty, coupled with a paucity of effective triggers for the use of proinflammatory treatments, suggests that such an approach is unlikely to find widespread application.

TARGETING THE OVERACTIVE IMMUNE RESPONSE

Once an infection has progressed to a late stage and an individual begins to suffer symptoms of disease (e.g., fever, pyrexia), the immune response generated at this point can be detrimental to the host if cascades are not appropriately controlled. Therefore, balancing the inflammatory network may represent a more effective means of treatment for postsymptomatic infections than stimulating a broad response with proinflammatory cytokines. One cause of death in infectious disease is the collateral damage caused by the immune response as it attempts to clear the pathogen rather than the effect of virulence factors produced by the organism. By controlling the proinflammatory response (e.g., leukocyte recruitment to the site of infection), an immunomodulatory treatment has the potential to reduce this tissue damage by preventing immune "overcrowding." While such an approach may not clear the infection, it can support survival until a successful adaptive immune response is mounted, allowing antimicrobial therapy to be effective.

It is clear that for infections with pathogens such as influenza A virus and *F. tularensis*, where a dysregulated immune response can cause significant damage, one therapeutic strategy would be to bring the inflammatory response back under control.

STIMULATING THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

The cholinergic anti-inflammatory pathway uses the neurotransmitter acetylcholine (Ach) to interact specifically with α7 subunit of nicotinic acetylcholine (α7nAch) receptors on innate immune cells such as macrophages. These receptors are able to respond to Ach from a number of sources, including other immune cells and the vagus nerve, and their activation results in the suppression of proinflammatory cytokines. NF-κB, the main transcription factor for proinflammatory cytokines, is activated by PAMPs such as lipopolysaccharide (LPS) and triggers a pathway which results in the translocation of NF-κB and the transcription of proinflammatory genes. Stimulation of the vagus nerve can inhibit this pathway, downregulating the immune response and even reversing the symptoms of sepsis (50, 51).

It has been shown that direct electrical stimulation of the vagus nerve can substantially reduce the levels of LPS-induced TNF-α in both the liver and serum of rats (52), as well as inhibiting secondary sepsis cascades such as systemic coagulation (53), increasing the rates of survival in both cases. However, electrical stimulation of the nervous system in humans would be too invasive and risky to be considered a feasible treatment for sepsis and hypercytokinemia, and so pharmaceutical methods of activating the α7nAch receptor are currently being investigated.

Nicotine is a nonselective agonist of the α7Ach receptor and is able to suppress the production of proinflammatory cytokines by mimicking the binding of acetylcholine. It has been demonstrated that nicotine can selectively reduce the inflammatory response in a number of infection scenarios, including *Legionella pneumophila* (54) and *Chlamydia pneumoniae* (55) infection; however, it is highly unlikely that nicotine will ever be used clinically due to its toxicity, addictive nature, and lack of specificity.

GTS-21, also known as DMXB-A, is another selective α7Ach receptor agonist already undergoing clinical trials for schizophrenia and Alzheimer's disease (56–58). It produces the same inflammatory modulation as nicotine but is nontoxic, does not result in an addiction, and has no known side effects (59). GTS-21 significantly reduces TNF-α and the late mediator of sepsis, HMGB1, downregulates IFN-γ pathways, and prevents the LPS-induced suppression of IL-10 and STAT 3 mechanisms (60), all of which contribute to a significant increase in survival in murine sepsis (59). In 2011, GTS-21 underwent *in vivo* human trials for the treatment of sepsis. The effects of orally administered GTS-21 at the highest known safe dose were examined in response to endotoxin-induced sepsis. The effects, while clearly dose-dependent, were highly variable between subjects and the mean plasma concentration of GTS-21 was low, resulting in a lack of statistically significant results. Within each individual, however, low levels of IL-6 and TNF-α were observed, proportional to the GTS-21 plasma concentration, indicating that high doses or different methods of administration may produce a more significant effect (61). There may also be potential for other α7Ach receptor agonists such as CNI-1495, which has already been shown to increase survival in murine sepsis models (62, 63).

PROSTAGLANDINS AND CYCLOOXYGENASE INHIBITORS

Prostaglandins are a large, varied family of fatty acids, a number of which, such as prostaglandin E_2, are early markers of inflammation. These prostaglandins are produced by activated macrophages during infection and increase symptoms of sepsis and systemic inflammation, such as vascular permeability and edema, as well as stimulating other immune cells (64). Prostaglandins are synthesized by cyclooxygenase (COX) enzymes, COX-1 and COX-2, which can be inhibited by pharmaceuticals in order to prevent the production of inflammatory prostaglandins. There are many COX inhibitors already widely and cheaply available, many of which are classed as nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen. However, many NSAIDs are not suitable for long-term use, as the lack of specificity causes side effects in the gastrointestinal tract (65).

Selective inhibition of COX-1 has been shown to increase mortality and disease symptoms in experimental sepsis and systemic inflammation (66). In comparison, selective COX-2 inhibitors have been shown to significantly reduce levels of inflammation, without the damaging gastrointestinal side effects. One such drug is celecoxib, a safe, inexpensive COX-2 inhibitor which has shown promising results in decreasing symptoms of severe systemic inflammation caused by influenza infection in mice. Infection with the virus results in the production of very high levels of COX-2, especially in alveolar epithelial cells. In particular, lethal strains such as H5N1 induce COX-2 at higher levels than nonlethal, seasonal strains (67). The downregulation of COX-2, through prostaglandin inhibition, results in a decrease in proinflammatory cytokine levels and leukocyte activation without causing immune suppression. This event effects viral clearance and disrupts the formation of protective immunity (68, 69). A combination therapy of celecoxib along with an antiviral such as zanamivir results in a significant increase in survival (68). Overall, the data currently available in the literature suggest that the use of COX-2 inhibitors as therapeutics may represent a promising approach for the treatment of viral and bacterial infectious diseases.

PLATELET-ACTIVATING FACTOR INHIBITORS

The phospholipid platelet-activating factor (PAF) plays an important and varied role in mediating the inflammatory response. It is produced by a number of different cells and acts on the PAF receptor, which is primarily found on the plasma membrane of cells such as leukocytes, platelets, and endothelial cells (70). The effects induced by PAF binding are dependent on the type of cell the PAF receptor is located on. Binding to the PAF receptor on platelets activates platelet aggregation and coagulation cascades, while binding to receptors on endothelial surfaces encourages neutrophil adhesion and permeability (71). PAF binding also increases the production of proinflammatory cytokines such as TNF-α, IL-8, and IL-1β, as well as contributing to the formation of pulmonary edema and organ dysfunction when overexpressed during severe sepsis and systemic inflammation (70, 71).

PAF plays a key role in inflammation and its concentration is controlled by the enzyme PAF acetylhydrolase (PAF-AH) (72). However, during systemic inflammation, the levels of PAF-AH are suppressed, preventing the PAF-linked immune response from being controlled (73). As a natural, highly efficient process, it has been hypothesized that artificially increasing the concentration of PAF-AH could control the effects of PAF by inhibiting the PAF cascade before it can bind to the receptor. Recombinant PAF-AH has been expressed in *Escherichia coli* and shown promising early results in both rodents and human clinical trials, especially when combined with antibiotic therapy (73), resulting in decreased mortality and levels of inflammation, especially when administered early in infection, without demonstrating serious side effects

(74). However, a subsequent phase III clinical trial showed no significant reduction in mortality or sepsis and eventually was discontinued (75). A number of pharmaceutical PAF receptor antagonists have also been tested and successfully shown to reduce symptoms and mortality in diseases where systemic inflammation and PAF play key roles, such as the antagonist UK-74,505 in dengue virus infection (76).

CHEMOKINE MANIPULATION

Chemokines and their receptors are a large and diverse family of proteins. It has become clear that these molecules contribute to a large number of biological functions. Among these, however, the recruitment of leukocytes to specific tissues is the most extensively studied and likely the most important (77). Over the last decade, a key role for chemokines and their receptors has been demonstrated in many inflammatory diseases, including atherosclerosis, rheumatoid arthritis, and gastrointestinal diseases (78). This, in turn, has led to the idea that modulating the chemokine response may be a useful target for generating novel therapeutics (79).

It has been reported that pretreatment of mice with monocyte chemotactic protein-1 (MCP-1) completely protected against lethal systemic infection by *Pseudomonas aeruginosa* or *Salmonella enterica* serovar Typhimurium (80). Further therapeutic agents have been developed, including an IL-8 (CXCL8) inhibitor to block inflammatory states and angiogenesis (81), GROα E6A to treat malaria, and I-309 (a human monocyte chemoattractant) to treat tumors (82). This approach is made more attractive by the unusually attractive pharmacokinetics of injected chemokine proteins; unlike most biological therapeutics, where the protein is rapidly cleared from the bloodstream, a single injection of chemokine protein results in a sustained increase in activity as a result of the rapid equilibrium with the abundant, promiscuous chemokine receptor DARC (Duffy antigen/receptor for chemokines), which is present on red blood cells. Red blood cells, therefore, act as a storage depot for the injected chemokine which is then gradually released over the following hours into the plasma in order to facilitate leukocyte trafficking.

Research looking to identify treatments that block specific chemokines/chemokine receptors has proved challenging. This is predominantly due to the chemokine network having a significant level of redundancy within the system (i.e., there are more chemokines than chemokine receptors), with some chemokine receptors binding multiple chemokine ligands (83, 84). Therefore, the blockage of one chemokine-receptor interaction does not fully stop its function and, as a result, research involving inhibitors or antagonists for a specific target rarely repeat the promising results found using knockout mice, e.g., studies of experimental autoimmune encephalomyelitis (85, 86). Nevertheless, promising early results have demonstrated that the administration of PF-04178903, an antagonist of the chemokine receptor CCR2, prior to challenge with the influenza H1N1A/Puerto Rico/8/34 strain reduced the mortality and the morbidity in a mouse model of infection while also reducing pulmonary immune pathology (87). In addition, the development of broad-spectrum chemokine inhibitors (BSCIs) that can affect multiple chemokine signaling pathways simultaneously while leaving other cytokine signals unaffected offer the potential to target the chemokine network specifically in order to dampen the overactive immune response. A compound called NR58-3.14.3 was shown to be effective against bacterial endotoxin-induced inflammation in the skin (88), and extensive data suggest that this compound is a useful therapeutic for asthma, atherosclerosis, stroke, and bronchiolitis obliterans syndrome.

MANIPULATION OF REGULATORY T CELLS

Regulatory T (Treg) cells are the host's natural anti-inflammatory cell and are important in maintaining homeostasis and controlling tissue damage that occurs during infection. Two main types of Treg cell have been described: those that are called induced and others that are called natural. Induced Treg cells (Tr1 cells) are dependent on IL-10 for their differentiation and regulation (89, 90). Natural Treg cells have been studied and characterized in much more detail. Typically, these $CD4^+$ T cells express IL-2R alpha (CD25), glucocorticoid-induced TNF receptor family-related gene (GITR), and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (91, 92). Highlighting their role as Treg cell mediators, depletion of each of these proteins individually can lead to autoimmunity. However, the expression of these markers alone is not sufficient to determine whether a Th cell is of the regulatory subset because activated nonregulatory T cells have the potential to express all these markers. The discovery of Forkhead box P3 (Foxp3) as a more specific marker of natural Treg cells has been essential to their experimental study. Foxp3 is a transcription factor that has now been shown to be the key regulator in the development of natural Treg cells (93, 94). The importance of this transcription factor is exemplified by the human disease immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, in which a mutation in the Foxp3 gene leads to severe autoimmunity (95–97).

It has been suggested that one Treg cell can influence numerous surrounding cells (98). With such potency, it is no surprise that these cells have been investigated as possible therapies to many diseases. Manipulation of Treg cell numbers by the addition of cytokines or targeting cell surface proteins has been shown to be effective in controlling many aspects of inflammation. Removal of Tregs with cocktails of antibodies has been shown to affect survival of pathogens within several mouse models of infection. The literature in the field of Treg manipulation for treatment of diseases is extensive (reviewed in references 99–101). Targeting these cells directly may be difficult in the human context but their effector molecules may act as useful targets to combat the overactive immune response observed in several diseases.

NOVEL THERAPIES PROMOTING IMMUNE RESOLUTION FOLLOWING INFECTION

Resolution of tissue damage is no longer seen as a passive process but more accurately as an active process that involves a range of biomolecules. Resolvins, lipoxins, and protectins are proresolution molecules involved in restoring tissue homeostasis. They elicit their effects via a range of mechanisms and are able to reduce neutrophil infiltration (102), increase the uptake of apoptotic neutrophils (103), and increase cellular exit via the lymphatic system. Collectively, these molecules work in concert in order to return the immune system to a resting state.

Resolvins are a set of newly identified lipid-based mediators that are derived from omega-3 polyunsaturated fatty acid (EPA) and docosahexaenoic acid (DHA). What separates these molecules from the traditional anti-inflammatory compounds is their ability to promote resolution without necessarily dampening down the inflammatory response (104). Resolvins can be further

divided into two groups: the E series and the D series, derived from EPA and DHA, respectively. Both types of resolvins have been shown to have an effect *in vivo* in several mouse models of diseases and stop neutrophil recruitment in peritonitis (105, 106). Resolvin E1 has been shown to increase host survival in models of colitis (107), and resolvin D2 has been shown to protect from ischemia-reperfusion kidney damage (108). This family of lipid mediators offer a novel and exciting avenue to treat inflammatory diseases. The ability to control tissue damage without disrupting the beneficial inflammatory response means these molecules represent a promising therapeutic strategy for treating infection that is worthy of further investigation.

SUMMARY

Inflammation is an essential part of an effective immune response, without which successful resolution of cellular damage and infection would not be possible. The inflammatory response is responsible for initial recognition of an invader or trauma, recruitment of the correct cells to allow resolution of the problem, and, eventually, a return to homeostasis. Pathogens are constantly adapting to be one step ahead of the immune system by evading or suppressing certain aspects. The immune system is unable to adapt at the same rate as microbes, and so pharmaceuticals have been developed to support the body's defenses, such as antibiotics and antivirals. However, many pathogens have developed resistance to such drugs. Therefore, attention has turned to immunomodulation as a therapeutic approach to enhance the efficacy of antimicrobials. Unfortunately, generically enhancing the broad immune response can sometimes worsen the outcome of disease. By modulating rather than upregulating the immune response via mechanisms such as the cholinergic anti-inflammatory pathway, COX-2 pathways, and PAF, the damaging positive feedback loops of sepsis and cytokine storms can be prevented. This may allow a longer window for diagnosis and treatment. Methods such as direct stimulation of the vagus nerve, treatment with nicotine, and PAF-AH have proven to be effective in murine studies but are unsuitable for clinical use in humans. However, more appropriate compounds are currently undergoing trials, including the nicotinic AchR agonist GTS-21. Though still in its infancy as an immune modulation drug, it shows promise *in vitro* and requires more investigation of its effects *in vivo*. Within the next decade, it is very possible that immune modulators such as GTS-21 and PAF-AH may be widely available and at the forefront of disease therapy.

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