Autophagy, cell death, and cytokines in K. pneumoniae infection: therapeutic perspectives

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ABSTRACT

*Klebsiella pneumoniae* is a notorious nosocomial pathogen causing a wide range of infections. The increasing trend of antimicrobial resistance obtained by the species immensely highly challenges the clinical treatment, representing a large threat to the global health care network. In particular, the recent convergence of multidrug resistance and hypervirulence in *K. pneumoniae* further worsens clinical outcomes, resulting in high mortality. Developments of new therapeutics become urgent, and immunotherapy based on antagonizing the anti-immune strategies of pathogens is a promising strategy, which requires the understanding of immune evasion mechanism in the context of the host–pathogen interactions. However, the underlying mechanisms employed by *K. pneumoniae* to counteract host immune responses, especially autophagy and cell death, have not been systematically reviewed and discussed yet. This review aims to summarize the tremendous progress that has been made to illuminate the landscape of cell signalling triggered by *K. pneumoniae* infection, especially in aspects of manipulating autophagy, cell death, and cytokine production.

Introduction

Over the past decades, *K. pneumoniae* has become one of the major causes of hospital- and community-acquired infections worldwide. In particular, the prevalence of multidrug-resistant (MDR) *K. pneumoniae* highly challenges antibiotic therapy, and infections caused by MDR *K. pneumoniae* are frequently associated with a high mortality [1]. Of greater concern, a convergence of carbapenem resistance and hypervirulence has recently emerged in epidemic clones worldwide, e.g. sequence type (ST) 11 and 258 [2,3]. Such convergence immensely exacerbates clinical outcomes of infections caused by the “superbug” resulting in a higher mortality, thus representing an emerging threat to the public health network. Developments of novel therapeutic approaches therefore become urgent to tackle the problem.

Accumulating evidence shows that immunotherapy is one of the promising strategies for the treatment of “superbug” [4]. Immunotherapy approaches based on passive immunization against *Klebsiella* spp. have been extensively studied. In particular, capsule polysaccharide (CPS)/lipopolysaccharide (LPS)-specific antibody and hyperimmune intravenous immunoglobulin (IVIG) from patients immunized with *Klebsiella* CPS/LPS have been tested in human clinical trials [5,6]. However, such strategies are largely limited by the highly diverse serotypes of *Klebsiella* spp.. Immunotherapy based on antagonizing the anti-immune strategies of pathogens could be an alternative solution, and a better understanding of the cross-regulation of the host and pathogen is a prerequisite for the development of host-directed therapeutics against pathogens. Current immunological concepts in bacteriology suggest that autophagy, cell death and cytokine production are critical host defense mechanisms against invading bacteria [4,7,8,9,10]. However, pathogens have correspondingly deployed devised immune evasion strategies, e.g. controlling cell death and limiting the emission of danger signals from dying cells to avoid elimination by the host. Despite *K. pneumoniae* is commonly considered as an extracellular pathogen, it can enter into macrophages through endocytosis dependent on
host cytoskeleton, cell plasma membrane lipid rafts, and the activation of phosphoinositide 3-kinase (PI3K), and further persist intracellularly within a vacuolar compartment inside host cells in vivo [11,12]. Furthermore, *K. pneumoniae* can manipulate phagosome maturation, inhibit apoptosis, impair efferocytosis, and promote necrosis/necroptosis of host cells to survive in the host.

Understanding the molecular pathways responsible for manipulating cell signalling provides critical insights into the bacterial pathogenesis. In this study, we aim to systematically review the current knowledge to illuminate the landscape of host–pathogen interactions associated with *K. pneumoniae*, especially in aspects of autophagy, cell death, and cytokine production.

**Virulence factors (VFs) of *K. pneumoniae* involved in the host–pathogen interactions**

Currently, a number of VFs have been identified in *K. pneumoniae*, and a set of VFs associated with immune evasion have been well characterized, e.g. CPS, LPS, siderophores, and fimbriae. Besides, a few untypical VFs, including out membrane proteins (OMPs), porins, efflux pumps, iron transport systems, and genes involved in allantoin metabolism, also play an important role in the pathogenicity of *K. pneumoniae* [13]. These VFs can influence the immune responses of host cells by various patterns, e.g. modulating cytokine secretions, and regulating autophagy and cell death, to escape the host killing. In this section, we will focus on those involved in circumventing early host responses for immune evasion during infection (Table 1).

Asymptomatic colonization is the first step for the infection development by *K. pneumoniae* [13–15]. *K. pneumoniae* is a versatile colonizer which mainly colonizes in the upper airways and the intestine [16,17]. Colonization by *K. pneumoniae* to human carcinoma or intestinal cell lines at the early stage of infection is usually a prerequisite for the pathogenicity, which is mediated by adhesive structures, mainly including type 1 and 3 fimbriae, F-28 fimbriae, a nonfimbrial factor called CF29K, and a capsule-like material [13,18]. Type 1 and 3 fimbriae are the major adhesive structures of *K. pneumoniae*, and for CF29K and KPF-28, their structures and functions were unclear in animal model systems. It is found that genes encoding type 1 fimbriae of *K. pneumoniae* are expressed in the urinary tract but not in the gastrointestinal tract or lungs, indicating that type 1 fimbriae contributes to the urinary tract colonization of *K. pneumoniae* but without effect on the ability to colonize the intestine or to infect the lung [19,20]. Besides colonization, type 1 fimbriae triggers an opsonin-independent form of phagocytosis called lectinophagocytosis which leads to increased bacterial killing [21]. Cooperative effect of type 1 fimbriae and LPS triggers oxygen-dependent apoptosis in human neutrophils. Type 3 fimbriae acts as a major contributor to the biofilm formation by *K. pneumoniae*, and mediates *in vitro* adhesion to epithelial cells and kidney and lung tissues [22]. Additionally, type 3 fimbriae stimulate an oxidative response in neutrophils [23] and mediate immunity to the infection in the murine model of respiratory diseases [24].

CPS was also involved in *K. pneumoniae* colonization. In the upper airway, the adult microbiota primes upper airway defenses through interleukin (IL) 17A, however, capsulation allows *K. pneumoniae* to overcome this barrier to establish colonization [25]. In adult mice, the encapsulated *K. pneumoniae* colonizes the upper airways at higher levels than an isogenic, unencapsulated mutant [26], and the ability to withstand microbiota-mediated defences is restored by complementation of the unencapsulated mutant [25], indicating the importance of CPS in the successful colonization by *K. pneumoniae*. Additionally, CPS is the first component that contacts with the immune cells during infection [27]. CPS can inhibit phagocytosis induced by immune cells [18,28], hinder the bactericidal action of antimicrobial peptides [18,29,30], and prevent the early immune response [31–34]. In an elegant study using a mouse model, it is found that in the early stages of infection, the levels of tumour necrosis factor (TNF)-α and IL 6 in broncho-alveolar lavage fluid (BALF) of mice infected with an non-capsulate mutant were significantly higher than those infected with a capsulate strain [31]. The results suggest that capsule allows

**Table 1. Key VFs of *K. pneumoniae* involved in the immune evasion.**

| VFs     | Mechanism                                                                                     | References      |
|---------|------------------------------------------------------------------------------------------------|-----------------|
| CPS     | Mediate resistance to opsonophagocytosis and antimicrobial peptides; impede the expression of beta-defensins by airway epithelial cells; hamper bacterial binding and internalization to induce a defective immunological host response; subvert the activation of inflammatory responses in a NOD1-dependent manner; decrease the SUMOylation | [18,28–34,36]   |
| LPS     | Reduce C1q binding by O antigen; reduce complement C3 deposition and degradation; Increasing the propensity for bacteremia; decrease the SUMOylation | [18,27,36,41,42] |
| Siderophore | Bind to human granulocytes via the oligomannose and hybrid N-linked units of CD11/CD18; trigger oxygen-dependent apoptosis in human neutrophils | [47,48]         |
| Fimbriae | Prevent the activation of airway epithelial cells; resistance to antimicrobial peptides; induce dendritic cells maturation and deliver antigen into the MHC class I presentation pathway; activate human macrophages; | [21,23]         |
| OMPs    | Capture iron from the host                                                                    | [50–54]         |
| Porins  | Resistance to antimicrobial peptides; phagocytosis prevention;                                | [54,56,57]      |
| AcrAB   | Resistance to antimicrobial peptides                                                           | [60]            |
K. pneumoniae to grow in vivo by suppressing the host immunological responses. Besides, CPS can prevent K. pneumoniae from the adherence to the membrane of host cells, and reduce bacterial internalization [35]. Additionally, CPS plays an important role in the intracellular survival of K. pneumoniae [12,36,37]. Compared with the wild-type strain, an isogenic wzy knockout strain was significantly more susceptible to the killing by human heparinized blood, serum, and neutrophils [37]. Of note, some capsular serotypes are highly associated with hypervirulence, e.g. K1 and K2. Such hypervirulence variants are much more resistant than classic K. pneumoniae to in vitro killing by serum or to phagocytosis by neutrophils and macrophages, and induce high colonization levels. The enhanced virulence could be resulted from the specific structures of CPS. For instance, the K1/K2 capsules lack Manα2-Man structure that can be recognized by macrophages and SP-A protein [21], while they contain sialic acid involved in the resistance to phagocytosis [38]. Additionally, K1/K2 capsules can induce less respiratory burst in polymorphonuclear leukocytes (PMNLs) [39,40].

LPS is an important VF for K. pneumoniae to resist eliminations by the host. It prevents complement C1q from binding with bacteria, thus inhibits the activation of complement pathway [18,27]. LPS also protects K. pneumoniae against C3 by binding to C3b, resulting in the prevention of interactions between C3b and cell membrane, and abrogating pore formation [41,42]. Of note, a recent study revealed that capsule and LPS together can promote K. pneumoniae infection by decreasing the levels of small ubiquitin-like modifier (SUMO)-conjugated proteins which lead to the limited activation of inflammatory responses and increased intracellular survival in macrophages during infection [36].

A number of studies have shown that K. pneumoniae is able to persist in the host cells in vivo [43–46]. During this process, K. pneumoniae utilizes host nutrients to replicate, and iron is a limited resource for K. pneumoniae to thrive in the host. To cope with the situation, K. pneumoniae secretes siderophores to “steal” iron from host iron-chelating proteins or to scavenge it from the environment [47,48]. Siderophores expressed in K. pneumoniae mainly include enterobactin, yersiniabactin, salmochelin, and aerobactin [13]. The productions of more than one type of siderophore by K. pneumoniae may be as a means of optimizing successful colonization of different tissues and/or avoiding neutralization by the host [47,49]. And the diverse siderophores highly facilitate K. pneumoniae adaptations to the complex environment in vivo.

Notably, some membrane proteins (e.g. OMPs, porins and efflux pumps) as untypical VFs play a crucial role in modulating host cell immune responds. For example, OmpA is one of the major outer membrane proteins of Gram-negative bacteria, and it has been shown to be implicated in preventing the activation of toll-like receptors (TLRs) to inhibit inflammatory responses and responsible for resistance to antimicrobial peptides [50–54]. Besides, OmpA was found to induce IL-1β and IL-18 production in host cells, resulting in caspase-1 activation, which simultaneously stimulated pyroptosis, thus leading to the death of the host cells [55]. In K. pneumoniae, the porin OmpK36, a homology of OmpC, prevents phagocytosis of neutrophils and alveolar macrophages [56,57], but it is not required for the serum resistance [54]. AcrAB is an efflux pump mainly contributing to the resistance against antibiotics [58,59]. Padilla et al. found that the AcrAB mutant is more susceptible than the wild type to cationic antimicrobial peptides (CAMPs) presenting in human bronchoalveolar lavage fluid (BALF) and to human antimicrobial peptides [60]. Therefore, AcrAB is important for K. pneumoniae to cope with the killings of antimicrobial peptides produced by hosts. Additionally, the secreted proteins maybe involved in the pathogenicity of K. pneumoniae as well [61–66]. A study shows that ten of fourteen (71%) sera collected from patients with K. pneumoniae pyogenic liver abscess (PLA) were immuno-reactive with the recombinant secreted protein KP1_p307, and it is further confirmed that antibodies against KP1_p307 protein were elicited after infection. Hence this secreted protein could be used as an antigen for early serodiagnosis of K. pneumoniae PLA [66]. However, more functional study of KP1_p307 should be performed to understand its contribution to the pathogenicity of K. pneumoniae.

Albeit the biological function of most VFs discussed here have been well characterized, the role of VFs in the manipulation of cell death and autophagy is largely unknown. In the next sections, we will discuss in detail how K. pneumoniae counters with the host immune system in aspects of impairing autophagy, triggering apoptosis, promoting necroptosis, interfering efferocytosis, and inducing pyroptosis.

K. pneumoniae manipulates autophagy-related cell signalling to survive in host cells

Autophagy is a process that controls the quality and quantity of intracellular biomass in eukaryotic cells, whereby cytoplasmic components are enveloped and sequestered by membranous structures that subsequently fuse to lysosomes for degradation. In the aspect of pathogen cleaning, autophagy can directly eliminate the intracellular microbes and/or their products via a process termed xenophagy, in which the selective capture and degradation of intracellular
microbes are completed by lysosomes [67]. Upon autophagy activation, serine/threonine kinases ULK1 and Beclin-1 combine with autophagy-related protein (Atg) 14 and type III phosphatidylinositol 3-kinase Vps34 together with other Atgs to promote the formation of a cup-shaped isolation membrane to engulf the cargo [68]. Subsequently, the cup-shaped isolation membrane elongates into a double membrane vesicle, named autophagosome. The autophagosome further fuses with lysosomes to form an autolysosome in which the engulfed cargo will be degraded. Additionally, other cellular processes, e.g. neutrophil extracellular traps (NETs) formation, phagocytosis and inflammation activation, are also involved in the autophagy-mediated elimination of *K. pneumoniae*. The underlying mechanism is discussed here.

**K. pneumoniae can activate autophagy during infection, but the underlying mechanism remains poorly understood**

Autophagy serving as an innate immune response to defend against pathogens invading inside the host cells has been extensively studied on a set of intracellular microorganisms [69]. Despite *K. pneumoniae* is an extracellular pathogen, it can induce autophagy of alveolar type II epithelial (A549) cells as shown in a recent study [70], and autophagy constitutes an important mechanism to control intracellular growth of *K. pneumoniae* in macrophages. Enhancement of *K. pneumoniae* intracellular growth was observed when the autophagy process of macrophages was inhibited by bafilomycin A1, suggesting that autophagy is important for the physiological control of intracellular *K. pneumoniae* [71]. In a model of *K. pneumoniae*-induced sepsis, autophagy was enhanced by the activation of Beclin-1 through forced expression of active mutant Becn1F121A or treatment with TB-peptide, and adverse outcomes of pneumonia-induced sepsis were significantly alleviated [72]. These data underpin the role of autophagy in host defense against *K. pneumoniae* infection. It has been known that mTOR is an important inhibitor of autophagy [73], and PI3K-AKT signalling is a major pathway through mTOR to regulate cell survival and proliferation [74]. A recent study identifies that autophagy induced by *K. pneumoniae* infection is dependent on PI3K-AKT-mTOR pathway, and inhibition of PI3K-AKT-mTOR signalling pathway increased the susceptibility of the host to *K. pneumoniae* infection [75].

Although *K. pneumoniae* is able to initiate autophagy, the underlying mechanism has been poorly defined. Generally, two pathways are individually involved in the activation of autophagy, i.e. the cell surface recognition and cytosolic sensing of PAMPs and DAMPs [76]. In the pathway of cell surface recognition, pathogens bind to surface receptors (e.g. TLRs), and trigger LC3 conjugation directly onto the phagosomal membrane upon phagocytosis, followed by the initiation of autophagy [76]. For the cytosolic sensing pathway, the representative is *M. tuberculosis*-induced autophagy. *M. tuberculosis* through its ESX-1 secretion system damages the phagosomal membrane, and cytosolic sensor c-GAS recognizes its DNA, resulting in the ubiquitination of the bacterium to initiate autophagy [77]. It is unclear whether *K. pneumoniae* could damage the phagosomal membrane. Limited evidence shows that TLR4 can recognize LPS of *K. pneumoniae*, and is involved in autophagy activation during *K. pneumoniae* infection [78]. OmpA is also associated with TLR2 signalling activation [4]. We thus assume that *K. pneumoniae* may initiate autophagy through cell surface recognition of PAMPs and DAMPs. More studies are needed to further elucidate the pathway of *K. pneumoniae*-induced autophagy.

**K. pneumoniae elimination by autophagy is associated with NETs formation**

Neutrophils have been regarded as first line of defense to capture and destroy extracellular pathogens, and their activity is highly dependent on the formation of NETs. Recent studies suggest that TRPM2- and Mincle-mediated NETs formation is associated with autophagy and involved in the defence against *K. pneumoniae* infection (Figure 1(A)). During *K. pneumoniae* infection, autophagy is required for transient receptor potential melastatin 2 (TRPM2)-AMPK-p38- and Mincle-mediated NETs formation in a reactive oxygen species (ROS)-dependent and -independent manner, respectively [79–81]. Trpm2−/− neutrophils exhibited defective NET formation, impaired autophagy activation, and significantly reduced phosphorylation of AMPKα1 and p38 MAPK, and inhibition of AMPKα1 and p38 MAPK signalling additionally impaired H2O2-induced autophagy and NETs formation. Consistently, Trpm2−/− mice displayed more susceptible to pneumoseptic infection with *K. pneumoniae* [80]. These results indicate that the involvement of autophagy in TRPM2-mediated NETs formation via AMPK-p38 signalling pathway is an important antimicrobial mechanism for the protection against *K. pneumoniae*. Likewise, significantly reduced phagocytosis and NETs formation were observed in Mincle−/− neutrophils, resulting in impaired autophagy activation, and attenuated neutrophil-mediated bacterial uptake and clearance. The deficiency of NETs formation can be rescued by the activation of autophagy in Mincle−/− neutrophils, indicating that NETs formation is regulated by Mincle via the modulation of autophagy [79,82]. Further studies are needed to understand the molecular signalling pathway connecting autophagy and NETs formation.
**K. pneumoniae manipulates phagosome maturation to survive in the host cells**

As an important immune response, the role and machinery of bacteria phagocytosis-induced autophagy have been identified in multiple studies (Figure 1(B)). Autophagy initiated by bacteria infection can regulate phagocytosis to facilitate the host to eliminate pathogens, and this process is co-modulated by Lyn, TLR2, and Rab [76,83]. Once autophagy is blocked, phagocytosis and subsequent bacterial clearance by alveolar macrophages are reduced [76,83]. For some intracellular pathogens, e.g. *M. bovis* and *M. tuberculosis*, autophagy can regulate phagocytosis indirectly by altering the surface expression of phagocytic receptors [84]. Although it is unclear whether *K. pneumoniae* possesses a similar mechanism, a previous study reported that OmpA of *K. pneumoniae* can engage TLR2 to activate NF-κB signalling [50]. Further work should be conducted to understand whether *K. pneumoniae* could through OmpA regulate TLR2 activation and modulate Lyn dependent autophagy.

In general, the host can through autophagosome or phagosome to eliminate intracellular pathogens after phagocytosis being induced. A recent study has shown that *K. pneumoniae* can avoid killing by regulating phagosome maturation to persist within phagosome compartment inside host cells in vivo [12]. Although *K. pneumoniae*-containing phagosomes are acidic, they are negative for cathepsin D, a marker for the lysosomal soluble content, indicating that the fusion of the phagosome with lysosomes was prevented [12]. The perturbed maturation of the *K. pneumoniae*-containing phagosomes is further
found to be associated with the recruitment of Rab14 through PI3K-AKT signalling pathway [12]. These results demonstrate that *K. pneumoniae* perturbs the fusion of the *K. pneumoniae*-containing phagosomes with the hydrolases-rich lysosomal compartment by targeting a PI3K-AKT-Rab14 pathway, and the efficient clearance of bacteria is subsequently disturbed in the intracellular niche of macrophages (Figure 1(C)).

*K. pneumoniae* manipulates autophagy-related Atg7 to decrease inflammation activation

The initiation of autophagy can further activate inflammation to cope with bacterial infection. As a critical E1-like ubiquitin, Atg7 is responsible for the elongation of autophagosomes membrane during autophagy [85]. The expression level of Atg7 significantly increased in murine alveolar macrophages upon *K. pneumoniae* infection, and Atg7-knockout mice exhibited decreased survival rates, attenuated bacterial clearance, and intensified lung injury [86,87]. Simultaneously, excessive proinflammatory cytokines and superoxide were further induced in the lung of Atg7-knockout mice [87]. Loss of Atg7 in alveolar macrophages resulted in the upregulation of IL-1β and pyroptosis, and Atg7-/- mice manifested more intense proinflammatory responses and exhibited activated NF-κB and p38 MAPK following *K. pneumoniae* infection, suggesting that NF-κB activity is suppressed by Atg7 [78,86,87]. The suppression mechanism has been further studied, in which Atg7 binding to p-IκBα negatively regulates the ubiquitylation of p-IκBα and the activation of NF-κB signalling pathway [78] (Figure 1(D)).

In summary, autophagy plays a significant role in host defense against *K. pneumoniae* infection (Figure 1) (Table 2). However, in the context of bacterial pathogenesis, key virulence factors (VFs) and receptors involved in *K. pneumoniae*-induced autophagy and the inhibition of phagosome maturation are largely unknown.

### Table 2. Strategies and mechanism involved in counteracting autophagy and cell death trigged by *K. pneumoniae*.

| Cell death          | Strategy                        | Mechanism                      | VFs involved            | References |
|---------------------|---------------------------------|--------------------------------|-------------------------|------------|
| Autophagy           | Promote autophagy                | PI3K-AKT-mTOR pathway          | Unknown                 | [75]       |
|                     | NETs formation                   | TRPM2-AMPK-p38 and Mincle-mediated NETs formation | Unknown | [79–81] |
|                     | Phagocytosis                     | Co-modulation of Lyn, TLR2, and Rab | Unknown | [83] |
|                     | Limit inflammatory responses     | Atg7 binding to p-IκBα negatively regulates the activation of NF-κB signalling pathway | Unknown | [78,87] |
| Apoptosis           | Inhibit phagosome maturation     | Unknown                         |                         |            |
|                     | Induce macrophage apoptosis      | Co-modulation of Bax, Bcl-xl, Cytochrome c, Apaf-1, and Caspase 9 | Unknown | [12] |
|                     | Induce HepG2 cells Apoptosis     | Unknown                         |                         | [12]       |
|                     | Induce platelets apoptosis       | TRPM4/Myd88 and JNK/MAPK pathways | Hypermucoc viscosity and LPS | [89]       |
|                     | Delay neutrophils apoptosis      | Co-modulation of Bax, Bcl-2, Mcl-1, Caspase-3, and IL-8 | K1-type capsule | [92] |
|                     | Impair effrocytosis              | Co-modulation of PS, Flipcase, and Caspase 8-RIPK1-RIPI3-MLKL signalling pathway | Unknown | [94] |
|                     | Necrosis of HepG2 cells          | Co-modulation of Endo G, AIF, DFF40, PARP, and Calpain 2 | Unknown | [88] |
| Necrosis/ Necroptosis| Necroptosis                      | Caspase 8-RIPK1-RIPI3-MLKL signalling pathway | Unknown | [95] |
|                     | Induce macrophage pyroptosis/pyronecrosis | Co-modulation of NLRP3, ASC, HMGB1, Caspase 1, Caspase11, IL-1β, and IL-18 | Unknown | [44] |
|                     | Effrocytotic uptake of the pyroptotic cells | Co-modulation of Inflammasome, Caspase 1, and IL-10 | LPS | [103] |

### K. pneumoniae induces apoptosis and further modulates apoptosis and effrocytosis of apoptotic cells to survive and emit in the host

Apoptosis is vital in counteracting microbe infection by restricting intracellular bacteria replication and delivering antigens to immune cells. Apoptosis triggered by intracellular pathogens has been intensively studied, while that by extracellular pathogens, like *K. pneumoniae*, is poorly characterized. Limited studies showed that *K. pneumoniae* enables to promote apoptosis of macrophages, platelet, epithelial cells, and liver cancer cells [12,88–90]. Cano and co-workers found that *K. pneumoniae* triggered programmed cell death in macrophages, and apoptosis features appeared at 6 h post infection [12]. To our knowledge, this is the orphan study of *K. pneumoniae*-induced macrophage apoptosis during the preparation of this work. Since only phenotypical characterizations were reported in that study, the underlying mechanism remains unclear. Compared with macrophage apoptosis, the molecular mechanism of apoptosis induced by *K. pneumoniae* in liver hepatocellular cells HepG2 has been studied relatively clearly. Apoptosis of HepG2 cells is induced at the early stage of *K. pneumoniae* infection, and the expression of Bax is increased and that of Bcl-xl is decreased, resulting in mitochondrial release of Cytochrome c [88]. The released Cytochrome c interacts with Apaf-1 to activate caspase 9, leading to apoptosis in the early hours of infection [88]. These results indicate that *K. pneumoniae*-triggered apoptosis of HepG2...
cells is dependent on the intrinsic pathway, also known as BCL-2 family-mediated apoptosis pathway.

The factors of *K. pneumoniae* involved in triggering apoptosis are largely unknown. A recent study identified a relationship between hypermucoviscosity and apoptosis. Although both hypermucoviscous *K. pneumoniae* (hmKP) and non-hmKP clinical strains can induce apoptosis of platelets and cause decreased level of platelet *in vivo*, the highest apoptosis proportions (45%) were observed in the infection caused by the hmKP strain [89]. It suggests that hypermucoviscosity could perhaps enhance apoptosis during infection. However, a discrepancy was found in another study that no significant differences were detected for apoptosis of bovine mammary epithelial cells triggered by hmKP and non-hmKP isolates at 9 h post infection, and the proportion of apoptosis cells was very low (ca. 6%) [90]. Of note, *K. pneumoniae* strains used in this study were isolated from bovine clinical mastitis, and the hypermucoviscous phenotype was negatively correlated to the pathogenicity. We thus suppose that the discrepancy might be due to the diverse pathogenicity of strains used in the two studies.

*K. pneumoniae* has evolved capacities to inhibit host cell apoptosis signalling to survive intracellulary. Delayed apoptosis and impaired efferocytosis are supposed to be two major strategies for this bacteria to counteract apoptosis, and the details are discussed below.

**K. pneumoniae delays apoptosis to survive in the host**

A study has shown that the interaction of *K. pneumoniae* with neutrophils inhibits the surface exposure of PS induced by anti-Fas antibody, an agonist known to cause neutrophil apoptosis, suggesting that *K. pneumoniae* can delay neutrophil apoptosis to escape the killing of neutrophils [91]. Capsule, an important virulence factor of *K. pneumoniae*, was identified as a factor involved in delaying neutrophil apoptosis. Compared with acapsular mutants, the strain with K1-type capsule significantly delayed the apoptosis of infected neutrophils [92]. Additionally, the K1-type capsule can modulate the anti-apoptotic effects by downregulating the ratio of Bax to Bcl-2 and by upregulating the expression of Mcl-1 (Figure 2). This leads to the delay of caspase-3 activation in the neutrophils, accompanied by the induction of anti-apoptotic cytokine IL-8 [92].

**K. pneumoniae impairs efferocytosis to emit in the host**

Apoptosis is not the last step to eliminate intracellular pathogens, and efferocytosis is responsible for the control of bacterial transmissions after apoptosis. If apoptotic cells are not swiftly eliminated by efferocytosis, secondary necrosis will be initiated mediated by gasdermin E- or DFNA5 (deafness, autosomal dominant 5), causing bacterium spread, autoimmunity and persistent inflammation [93]. Therefore, the capacity of inhibiting efferocytosis is crucial for pathogens to develop severe infection. A recent study indicated that *K. pneumoniae* can impair the efferocytic uptake of neutrophils *in vitro* and *in vivo* with the assistance of macrophages [94]. Impaired efferocytosis is linked to reduced externalization of PS via the modulation of flippase activity, and obstructs the recognition of apoptotic cells by macrophages to promote *K. pneumoniae* dissemination (Figure 2). Efferocytosis thereby is inhibited resulting in the promotion of *K. pneumoniae* dissemination. Together, these results evidence that *K. pneumoniae* is able to impair efferocytosis by inhibiting apoptosis of neutrophils to establish infection (Figure 2) (Table 2).

**K. pneumoniae promotes necrosis/necroptosis to escape the killing of hosts**

Necrosis is a form of lytic cell death employed by some bacteria to disseminate *in vivo* and to evade host immune responses. Compared with necrosis, necroptosis is a programmed cell death, which also promotes pathogens’ transition. Necrosis occupies an important role in the pathogenicity of *K. pneumoniae*. Apoptotic HepG2 cells induced by *K. pneumoniae* infection further progress to secondary necrosis and primary necrosis, and PARP is inactivated by caspase 7 at this stage [88]. As the infection persists over time, Endo G, AIF, and DFF40 are translocated from mitochondria to the nucleus, causing DNA fragmentation. The DNA damages lead to PARP activation and ATP depletion, and calpain 2 is subsequently upregulated resulting in the digestion of cellular components, followed by the induction of necrosis at the final, which promotes *K. pneumoniae* dissemination [88] (Table 2). Alternatively, *K. pneumoniae* activates cell necroptosis through the RIPK1/RIPK3/MLKL cascade to escape the host killings [95], and can further interfere apoptosis activation to inhibit efferocytosis. Inhibition of necroptosis by RIPK1 and RIPK3 restores the efferocytic uptake of *K. pneumoniae* infected neutrophils by macrophages *in vitro*, suggesting that *K. pneumoniae* induces necroptosis of neutrophils [94]. Therefore, inhibition of necroptosis is able to rescue the efferocytic uptake for *K. pneumoniae*-infected neutrophils (Figure 2). Compared with WT, Ripk3<sup>−/−</sup> mice promoted bacterial clearance from the BALF and the lung at 96 h post infection [95]. These data suggest that necroptosis facilitates *K. pneumoniae* survival in the host by reducing the efferocytic uptake of infected neutrophils via macrophages (Figure 2). In the context
of therapy, inhibitions of necrosis/necroptosis could be an assistant treatment for *K. pneumoniae* infection.

*K. pneumoniae* is counteracted by pyroptosis/pyronecrosis during infection

Pyroptosis is a programmed cell death that depends on the inflammatory caspases, including caspase-1, murine caspase-11, and human caspase-4/5 [96]. The most important character of pyroptosis is the maturation and secretion of pro-inflammatory cytokines (IL-1β, IL-18, and IL-33) promoted by active caspase 1 [97]. Recently, pyronecrosis is proposed as a new form of pathogen-induced cell death similar to pyroptosis [98]. The common feature of pyroptosis and pyronecrosis is the release of pro-inflammatory cytokines, and both can be stimulated by similar factors and produce similar outcomes. However, compared with pyroptosis, pyronecrosis needs cathepsin B instead of caspases’ activities [99]. Abundant evidence supports the significance of pyroptosis/pyronecrosis in the anti-infection by hosts that pyroptosis/pyronecrosis of infected cells removes the pathogens’ protective intracellular niche and promotes neutrophil-mediated killing.

**Figure 2.** Cell death triggered by *K. pneumoniae* in neutrophils. *K. pneumoniae* modulates the anti-apoptotic effects by downregulating the ratio of Bax to Bcl-2 and upregulating the expression of Mcl-1, resulting in the delay of Caspase 3/7 activation and the inhibition of apoptosis. *K. pneumoniae* also can increase the activity of flippase to reduce PS exposure, which is the signal of "eat me" for efferocytosis, followed by decreasing the efferocytic uptake of neutrophils by macrophage. In parallel, *K. pneumoniae* inhibits caspase 8, which promotes the formation of necroptosome complex RIPK1/RIPK3/MLKL to induce necroptosis. Necroptosis emits *K. pneumoniae* to the external environment, and decreases efferocytosis. (Created with BioRender.com).
**K. pneumoniae induced macrophage pyronecrosis is NLRP3 and ASC dependent**

A few studies have suggested that *K. pneumoniae* can induce pyroptosis/pyronecrosis, and the underlying mechanism has been explored. In a *K. pneumoniae*-infected human macrophage cell line, NLRP3 or ASC knockdown abrogated IL-1β release and cell death, and further attenuated the release of high-mobility group box 1 protein (HMGB1), suggesting that *K. pneumoniae* induced macrophages pyronecrosis is dependent on NLRP3 and ASC characterized by IL-1β release, cell death, and HMGB1 release [44]. As a result, deficiency of Nlrp3 and Asc significantly increased the mortality of mice infected by *K. pneumoniae* [44], validating the protective function of NLRP3 and ASC in the host against *K. pneumoniae* (Table 2).

The factors of *K. pneumoniae* responsible for triggering pyroptosis/pyronecrosis are largely unknown. Recently, K1-CPS was identified as one of such factors in an orphan study. Caspase-1 activation and IL-1β secretion in the presence of ATP were induced by K1-CPS using mouse J774A.1 macrophages, and the reduced expression of NLRP3 and ASC by shRNA plasmids attenuated caspase-1 activation and IL-1β secretion [100], which is consistent with the previous study aforementioned [44]. K1-CPS-induced NLRP3 expression was significantly lower in TLR4 knockdown cells than in control, indicating that TLR4 is involved in the NLRP3 inflammasome activation induced by K1-CPS [100]. K1-CPS induced NLRP3 inflammasome activation is also associated with ROS generation, mitogen-activated protein kinase phosphorylation, and NF-κB activation [100]. These findings indicate that K1-CPS is a factor that induces IL-1β secretion through the NLRP3 inflammasome (Figure 3).

**K. pneumoniae infection is inhibited by neutrophil recruitment through NLRC4 and caspase11 activation**

A set of pyroptosis-related proteins is additionally involved in the protection against *K. pneumoniae* infection, such as NLRC4 and caspase11. Cleavage of caspase-1, IL-1β, and IL-18 were reduced in NLRC4-

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**Figure 3.** Pyroptosis triggered by *K. pneumoniae* infection. TLR4 receptor can recognize *K. pneumoniae* LPS and K1-CPS, and activate NLRP3 inflammasome and caspase-11. Inflammasome formation allows caspase-1 activation, resulting in mature IL-1β and IL-18 release by cleavage of pro-IL-1β and pro-IL-18, respectively. Subsequently, pore formation and release of intracellular soluble components, such as LDH and HMGB, is culminated by the induction of pyroptosis/pyronecrosis. IL-1β together with other mediators recruit new cells to the infection site, promoting efferocytosis of pyroptotic-infected cells and bacterial clearance. But this progress can be inhibited by IL-10. Simultaneously, *K. pneumoniae* can activate NLRC4 to promote caspase-1 activation, and further increase the release of IL-1β and IL-18. NLRC4 is also associated with IL-17 releasing, which was independent with caspase1. The released IL-1β through the interaction with its receptor IL-1R promotes the secretion of ICAM-1 and VCAM-1, and further promotes neutrophil recruitment.
deficient mice, suggesting that the activation of caspase-1 and cleavage of IL-1β and IL-18 are NLRC4 dependent [101] (Figure 3). NLRC4−/− mice further exhibited higher lung and spleen bacterium burden, suggestive of a defect of bacterial clearance and dissemination in the NLRC4−/− mice. This well explains the decreased survival of NLRC4−/− mice caused by K. pneumoniae infection [101]. Notably, NLRC4, did not induce pyroptosis in pulmonary macrophages and neutrophils though it promoted IL-1β and IL-18 production, and the main function of NLRC4 signalling coping with K. pneumoniae infection is neutrophil recruitment and innate immunity [101]. Additionally, Caspase11 was activated by K. pneumoniae in BMDMs and mice, and caspase-11 deficiency blocked the secretion of IL-1α and IL-1β in BMDMs, indicating that caspase-11 mediates K. pneumoniae induced BMDM pyroptosis [102] (Figure 3). Caspase-11 deficiency also impaired neutrophil recruitment and bacterial clearance in the early stage of pulmonary infection caused by K. pneumoniae [102], suggesting that caspase-11 is involved in defending against K. pneumoniae infection by recruiting neutrophils in the early stage of infection [102].

**K. pneumoniae clearance by pyroptosis needs efferocytic uptake of the pyroptotic cells**

Similar to apoptotic cells, pyroptotic cells also launch “eat me” signals to promote efferocytosis by phagocytes. However, if the infected pyroptotic cells cannot be cleared in time by hosts, it can promote the spread of pathogens. Indeed, Codo et al. showed that the survival of K. pneumoniae in macrophages increased when the infected pyroptotic cells had not been engulfed through efferocytosis [103]. Although pyroptotic cells were engulfed efficiently after being co-cultured with freshly collected macrophages, this process could be inhibited by IL-10, followed by the further inhibition of inflammasome activation, pyroptosis, and efferocytosis of infected cells [103] (Table 2). This study demonstrates that the assistance from efferocytic uptake of the pyroptotic cells is needed for the clearance of K. pneumoniae by pyroptosis (Figure 3). Additionally, K. pneumoniae might employ pyroptosis to harness the host immune response to promote its infection [103]. We therefore suppose that promoting pyroptosis induction and/or enhancing the efferocytosis of pyroptotic cells by phagocytes may be feasible to improve the treatment efficacy for K. pneumoniae infection.

**K. pneumoniae induced cytokine network during infection**

Cytokines, secreted by immune and non-immune cells, are cell-to-cell signalling small proteins (~6–70 kDa). Cytokines make up an exceptionally large and diverse group of pro- and anti-inflammatory factors, acting as regulators of host responses to infection, immune responses, inflammation, and trauma [104]. The release of pro-inflammatory cytokines leads to the activation of immune cells and to the further release of other cytokines. Inflammation may bring beneficial effects to hosts in terms of pathogen clearance and phagocytosis of apoptotic cells, however, uncontrolled inflammation could cause adverse outcomes resulting in a variety of chronic inflammatory diseases [104]. To maintain the balance, anti-inflammatory factors can neutralize excessive inflammation induced by pro-inflammatory cytokines. Simultaneous release of pro- and anti-inflammatory cytokines is suggested to be mandatory in any immune responses, and inflammatory cytokines are crucial for hosts to defend against infections. Currently, a set of cytokines has been reported associated with K. pneumoniae infection (Table 3).

**K. pneumoniae promotes the production of chemokines responsible for neutrophil migration and subverts the activation of CXCL8 secretion to survive in the host**

Chemokines are an extended family of small-size chemotactant cytokines that facilitate leukocyte migration and positioning via binding to receptors located at the target cell surface. Multiple chemokines have been identified involved in counteracting K. pneumoniae infection, like CXCL15, MIP-1α/CCL3, CXCL1 (keratinocyte cell-derived chemokine, KC), CXCL8 (IL-8), and CXCL5. CXCL15, a novel CXC chemokine, mediates neutrophil migration from the lung parenchyma into the airspace [105], and MIP-1α/CCL3, a member of the CC chemokine family, is required for the phagocytic activity of alveolar macrophages during K. pneumoniae infection [106]. Mice lacking CXCL15 (lungkine) or MIP-1α/CCL3 was susceptible to K. pneumoniae infection [105,106]. Transient intrapulmonary expression of MIP-1α/CCL3 or CXCL1 showed a striking improvement in survivals associated with recruitment of neutrophils [107–109]. Hence, CXCL15, MIP-1α/CCL3 and CXCL1 are necessary for K. pneumoniae elimination by hosts.

CXCL8 is mainly secreted by leukocytes and endothelial cells dependent on the activation of NF-κB signalling pathway under special conditions, such as exposure to IL-1 or TNF-α, bacterial or viral products and cellular stress [110]. Similar to the function of CXCL15 and MIP-1α/CCL3, CXCL8 recruits neutrophils and other immune cells to the sites of infection to cope with K. pneumoniae [111]. Correspondingly, the pathogen has developed multiple strategies to subvert the activation of CXCL8 secretion (Figure 4). K. pneumoniae attenuates pro-inflammatory...
mediators-induced CXCL8 secretion by upregulating the expression of CYLD and MKP-1 in alveolar epithelial cells [34]. Alternatively, through Rho GTPase (Rac1)–NOD1–CYLD and MKP-1 pathways, K. pneumoniae regulates NF-κB and mitogen-activated protein kinases (MAPKs) signalling activation to attenuate IL-1β-induced CXCL8 secretion [34]. A mechanistic study further showed that the activation of NF-κB is attenuated by K.pneumoniae via activating an EGF receptor (EGFR)-phosphatidylinositol 3-OH kinase (PI3K)–AKT–ERK–GSK3β signalling pathway, and CYLD is identified as the downstream effector of the signalling pathway [112]. The activation of EGFR is stimulated by CPS dependent on a TLR4–MyD88–c-SRC pathway [112]. However, the factors responsible for Rac1 activation remain unknown.

**K. pneumoniae induces the pro-inflammatory cytokines production which mediates K. pneumoniae elimination**

The synergy of multiple pro-inflammatory cytokines constructs the key network of host resistance to K. pneumoniae, mainly including IL-1β, IL-6, TNF-α, IFN-γ, IL-12, IL-17, and IL-23. As the most well-known pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α are critical for hosts to cope with infection caused by various pathogens. Indeed, the expression of IL-1β, IL-6, and TNF-α is upregulated during K. pneumoniae infection [44,100,113–115]. TNF-α receptors (TNFR1) deficiency and neutralization of TNF-α consistently caused an increased mortality resulting from impairing lung antibacterial host defense and/or dysregulating production of cytokines detected in the mouse model [113,114]. CPS is an essential factor to stimulate the production of TNF-α and IL-6 in macrophages, dependent on TLR4/ROS/PKC-δ/NF-κB, TLR4/PI3-kinase/AKT/NF-κB, and TLR4/MAPK signalling pathways [116].

IFN-γ is one of the most well-studied pro-inflammatory cytokines involved in defence against K. pneumoniae infection. Different from those common pro-inflammatory cytokines aforementioned, IFN-γ signalling directly activates numerous antimicrobial programmes in host defense, such as the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), and generation of antimicrobial peptides and cytokines [117]. Intratracheal inoculation of IFN-γ knockout mice infected by K. pneumoniae caused a significantly increased mortality, associated with a 100-fold increase of pulmonary bacteria within 2 days post infection and the upregulation of lung-associated IL-10 mRNA [118,119]. At the late stage of infection, the level of serum IL-6 in IFN-γ-/- mice was significantly higher than that of controls, suggestive of the defective immunological host response [119]. These results support the critical role of IFN-γ in the host defense against K. pneumoniae.

The mechanism of IFN-γ stimulation by K. pneumoniae has also been characterized recently (Figure 4). IFN-γ is mainly secreted by natural killer (NK) cells during infection, highly dependent on the DC signalling pathway or macrophage type I IFN signalling pathway [120,121]. In the DC signalling pathway, NK cells are actively recruited after physical contact with membrane fractions of K. pneumoniae-matured DC, either in a CCR5-dependent manner or by the induction of CCR7 expression in CD56 (dim) CD16 (+) NK cells [120]. Both pathways stimulate enhanced migratory responsiveness of NK cells to the lymph node, and increase the production of IFN-γ [120]. It is accordingly hypothesized that in vivo K. pneumoniae infection is responsible for the
Induction of innate immune responses characterized by DC-dependent NK-cell migration accompanied by IFN-γ production, and NK-DC interactions are facilitated both in the periphery and in the lymphoid tissue [120]. However, in vivo validations are needed for this conclusion. Alternatively, the IFN-γ production can be additionally promoted by K. pneumoniae matured DC cells through the interaction of CCL5-CCR5 receptor and CCL19-CCR7 receptor on NK cells. K. pneumoniae-infected DC cells secrete IL-23 which further induces IL-17 production by T cells to promote bacterial clearance. IL-17 promotes CXCL5 production through IL-17R on the epithelial cells to induce the recruitment of neutrophils. The recruitment of neutrophils can also be promoted by IL-8 and IFN-λ secreted by K. pneumoniae infected epithelial cells. Neutrophils are involved in the elimination of K. pneumoniae through NETs formation. IL-22 interacts with IFN-λ to help maintain the integrity of epithelial barrier to inhibit neutrophil transmigration and microbial invasion. The MDSCs produced IL-10 also has a crucial role in K. pneumoniae clearance. The synergy of these cytokines constructs the complex network of host defense against K. pneumoniae. The detailed signalling pathways of IL-8 and IL-10 production upon K. pneumoniae infection are shown on the right panel. (Created with BioRender.com).

Figure 4. Complex network of cytokines coping with K. pneumoniae infection. K. pneumoniae can directly interact with macrophages, neutrophils, DC cells and epithelial cells in alveoli (left panel). The macrophage is the major immune cell in alveoli, and can produce type I IFNs upon infection with K. pneumoniae, resulting in NK cell migration and IFN-γ production. IFN-γ further activates the macrophage anti-microbial armament; both macrophages and DC cells can produce IL-12, which is required for IFN-γ production. IFN-γ production can be additionally promoted by K. pneumoniae matured DC cells through the interaction of CCL5-CCR5 receptor and CCL19-CCR7 receptor on NK cells. K. pneumoniae-infected DC cells secrete IL-23 which further induces IL-17 production by T cells to promote bacterial clearance. IL-17 promotes CXCL5 production through IL-17R on the epithelial cells to induce the recruitment of neutrophils. The recruitment of neutrophils can also be promoted by IL-8 and IFN-λ secreted by K. pneumoniae infected epithelial cells. Neutrophils are involved in the elimination of K. pneumoniae through NETs formation. IL-22 interacts with IFN-λ to help maintain the integrity of epithelial barrier to inhibit neutrophil transmigration and microbial invasion. The MDSCs produced IL-10 also has a crucial role in K. pneumoniae clearance. The synergy of these cytokines constructs the complex network of host defense against K. pneumoniae. The detailed signalling pathways of IL-8 and IL-10 production upon K. pneumoniae infection are shown on the right panel. (Created with BioRender.com).

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IL-12 is regarded as another important inducer for the production of IFN-γ during K. pneumoniae infection. Significantly attenuated lung IFN-γ levels were found in IL-12 deficient K. pneumoniae-infected mice [122]. Consistently, overexpression of IL-12 in
K. pneumoniae-infected mice promoted host survival, and the treatment using IFN-γ antibody partially attenuated this survival benefit [123], suggesting that the synergy of IL-12 and IFN-γ can improve host defense against K. pneumoniae. The production of IFN-γ may be regulated by IL-12 signalling through STAT4, however, this conclusion needs to be further validated [124,125].

The other well-studied pro-inflammatory cytokine involved in K. pneumoniae elimination is IL-17. IL-17 is produced in a compartmentalized fashion in the lung after being challenged by K. pneumoniae. IL-17R-deficient mice displayed a significant delay in neutrophil recruitment into the alveolar space resulting in a greater dissemination of K. pneumoniae, and were exquisitely sensitive to intranasal K. pneumoniae with 100% mortality after 48 h [126]. This defect in response to the K. pneumoniae challenge was associated with a significant reduction in lung chemokine gene expression, e.g. granulocyte colony stimulating factor (G-CSF) and macrophage inflammatory protein (MIP)-2 [126]. Consistently, overexpression of IL-17 in the pulmonary compartment can improve K. pneumoniae clearance and host survival [127]. These data demonstrate that IL-17R signalling is critical for the lung chemokine gene expression and early lung neutrophil recruitment against K. pneumoniae [126]. IL-17 induced neutrophil recruitment is associated with the expression level of the chemokine CXCL5. Reduced expression of CXCL5 was found in the IL-17R deficient epithelium, and recombinant CXCL5 administration restored neutrophil recruitment and K. pneumoniae clearance [128]. The results suggest that IL-17 through epithelial IL-17R signalling regulates Cxcl5 expression followed by the recruitment of neutrophils to clean K. pneumoniae in lungs.

The regulation mechanism of IL-17 production during K. pneumoniae infection has been further revealed. IL-17 was induced in CD4+ and CD8+ T cells response to conditioned medium from K. pneumoniae-pulsed DC, and the signalling requires biologically active IL-23 [129]. Blocking IL-23 signalling can significantly reduce the amount of IL-17 in CD4+ and CD8+ T cells [129], and a decreased survival of IL-23 deficient mice with pulmonary K. pneumoniae challenge was observed [122,130]. TLR4 signalling in the lung is additionally required for the induction of the IL-23 p19 subunit transcript and IL-17 protein elaboration [129]. Defective bacterial clearance in IL-23 p19−/− mice was abolished by the administration of exogenous IL-17, and the treatment further restored the reduced production of lung G-CSF and LPS-induced C-X-C chemokine (LIX) [122]. These findings indicate that IL-23 is dependent on IL-17 to protect against K. pneumoniae. IL-17 can also be produced by γδ T cells (a subset of γδ T cells) in vivo in the presence of IL-23 and IL-1β. However, IL-17 production is negatively regulated by STAT6 signalling, since IL-4 through IL-4R of γδ T cells increases STAT6 phosphorylation, which further inhibits the production of IL-17A in γδ T cells in vitro [131]. Notably, K. pneumoniae has no effect on IL-4 production in vivo [131], thus the signalling of IL-17 production in γδ T cells during K. pneumoniae infection needs to be further studied.

K. pneumoniae manipulates the production of anti-inflammatory cytokines

Together with pro-inflammatory cytokines, a few anti-inflammatory cytokines are involved in protection against K. pneumoniae to balance the immunity reaction of the host. One of the most well-studied anti-inflammatory cytokines is IL-10, a core member of IL-10 family. In early studies, IL-10 was considered as the assistance of pathogens to counteract the host immune system. Neutralization of IL-10 leads to a significant decrease of K. pneumoniae loads in both lung homogenates and plasma harvested at 48 h post infection, resulting in a significant increase in survival. The expression of pro-inflammatory cytokines, such as TNF, MIP-2, and MIP-1α, was simultaneously increased [132]. In agreement, a lower 5-day survival rate of IL-10 overexpression mice was observed when being infected by K. pneumoniae. Alveolar macrophages from the IL-10 overexpression mice displayed increased alternative activation (M2 macrophages), whereas those from control exhibited classical activation (M1 macrophages) with a much higher intracellular bacterial killing potential [133]. These results suggest that K. pneumoniae exploits IL-10 to attenuate host immune response.

In contrast, a recent study showed that IL-10 produced by myeloid-derived suppressor cells (MDSCs) protected the host against K. pneumoniae infection, albeit it functioned divergently in the defense against carbapenem-resistant clinical isolates (CR-Kp) and the hypervirulent strain (KP1R1) [134,135]. During CR-Kp infection, MDSCs-produced IL-10 (MDSCs-IL-10) was highly responsible for the pathogen elimination. Overexpression of IL-10 in MDSCs not only improved CR-Kp clearance but also slightly reduced the lung injury. Using IL-10−/− mice, the protective effect of MDSCs-IL-10 was found through the prevention of lung damage and improvement of host survival to enhance CR-Kp clearance in the airways [134]. The immune response is an early recruitment of neutrophils and MDSCs that are able to produce IL-10 as well as other suppressive effectors to promote the pathogen elimination, resulting in tempering the inflammatory response and minimizing lung injury at early time points [134]. Nevertheless, MDSCs-IL-
10 was thought to be essential for the resolution of lung inflammation but not for bacterial clearance upon KPPR1 infection [135]. Although the bacteria was eliminated in IL-10−/− mice, an increased morbidity with progressive weight loss and persistent lung inflammation was found in the later phase [135]. In assistant with IL-10, the MDSCs efficiently effec
tively programed apoptotic neutrophils, which was critical to promote lung recovery [135]. In comparison to CR-Kp, the immune response against KPPR1 is a much slower infiltration of MDSCs but companied with a rapid bacterial clearance and a rapid infiltration of neutrophils [134], suggesting that the time of MDSCs recruitment and MDSCs-IL-10 production are divergently triggered by CR-Kp and KPPR1, which determines the outcome. However, the underlying mechanism remains unclear. Considered that KPPR1 is a hyper-virulent strain, it is reasonable to assume that different virulence genes carried by CR-Kp and KPPR1 may be responsible for the functional divergence of MDSCs-IL-10 observed.

Two pathways have been identified as responsible for the production of IL-10 during K. pneumoniae infection, namely the cardiolipin pathway and SARM1 pathway (Figure 4). In the cardiolipin pathway, PPARγ SUMOylation is induced by cardiolipin at the onset of infection, which causes the recruitment of a repressive NCOR/HDAC3 complex to the IL-10 promoter, resulting in the attenuated transcription level of the IL-10 [136]. Alternatively, SARM1 is required for IL-10 induction by fine-tuning the p38-type I IFN axis in the SARM1 pathway. The level of IL-10 transcription and p38 activation was reduced in the absence of SARM1 during K. pneumoniae infection. In turn, the activation of p38 and IL-10 production was increased in Sarm1−/− macrophages treated with IFNAR1 receptor blocking antibody. These results suggest that K. pneumoniae induced p38 activation is modulated by SARM1 through negatively regulating the expression levels of type I IFN, which further induces the production of IL-10 [137].

Another two members of IL-10 family, IFN-λ and IL-22, are also involved in synergistically regulating the host immune response against K. pneumoniae. Both IFN-λ and IL-22 are associated with mucosal defenses to regulate the barrier functions, but with differing kinetics. In the early infection, the elevated IFN-λ amplifies epithelial permeability, which enables bacterial transmigration from the airway lumen into the lung and bloodstream, and also promotes the recruitment of neutrophils across a polarized monolayer of airway epithelial cells. The production of IL-22 is subsequently increased, which helps in maintaining the integrity of epithelial barrier to block dextran permeability and to inhibit neutrophil transmigration and microbial invasion, resulting in enhanced bacterial clearance and bacteraemia prevention. The divergent and opposing expression of these two related cytokines suggests a regulated interaction in the host response to K. pneumoniae infection [138].

Besides IFN-λ, IL-22 can additionally work in concert with other inflammatory cytokines like IL-17. IL-17 and IL-22 cytokines are often co-expressed and further co-regulate CXC chemokine and G-CSF production in the lung to eliminate K. pneumoniae [138–140]. Similar to IL-17, IL-22 expression was markedly attenuated in Il23a−/− mice, suggesting that IL-23 is required for the induction of IL-22 [140]. In the liver, IL-22 additionally shows potent bacteriostatic activity against K. pneumoniae by producing antimicrobial peptides (i.e. lipocalin 2 and serum amyloid A2) through IL-22Rα1 and STAT3 singling [141], suggestive of pleiotropic effects of IL-22 on K. pneumoniae elimination. Therefore, development of agents targeting IL-22-associated signalling pathways may provide selective alternatives for the management of K. pneumoniae infection.

**Concluding remarks and future directions**

With the exhausted traditional antibiotic pipeline, the emergence of MDR K. pneumoniae promotes the growing clinical needs for novel therapeutic strategies. At present, the therapeutic targeting is mainly gathered in metabolic pathway and certain virulence factors (e.g. LPS and CPS) of K. pneumoniae [142]. Phages, phage polysaccharide depolymerases and antimicrobial peptides represent additional promising tools for antimicrobial therapy [143,144]. However, most of the strategies are highly specific, even strain-dependent, thus largely comprising their cost efficiency and/or outcomes in clinical settings. Accordingly, immunotherapy based on antagonizing the anti-immune strategies of pathogens could be an alternative solution to satisfy these requires. According to the various functions of cytokines during K. pneumoniae infection, compartmentalized cytokine delivery as adjuvant therapy may be a promising strategy. For instance, treatment with growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) can protect alveolar epithelial cells from apoptosis in response to K. pneumoniae. Intratracheal administration of GM-CSF significantly decreased albumin leak, lung cell apoptosis, and bacteraemia in K. pneumoniae infected mice [145]. Additionally, recombinant IL-22 treatment efficiently ameliorated the ST258 pulmonary infection [146]. Thus, GM-CSF and IL-22 treatment may represent a promising immunomodulatory method for the prevention or treatment of pneumonia. Development of agonists and inhibitors for cell signalling could also be an adjunct treatment. Toll-like receptor 4 (TLR4), which can recognize LPS of gram-negative
bacteria, is a potential target for inhibiting undesired inflammatory responses. Some Traditional Chinese Medicine, e.g. Sodium houttuynifate, LianQinJieDu decoction, and Ugonin M et al., which can modulate TLR4 signalling pathway, have been suggested to be the potential drugs for the treatment of gram-negative bacteria pneumonia [147]. Use of complement inhibitors is another approach to downregulate the host’s immune response to protect organs from damages caused by bacterial infection. For instance, blocking of complement C5a can protect lung and extrapulmonary organ failure in pneumonia-induced sepsis [148].

To further promote the development of immuno-therapy based on antagonizing the anti-immune strategies of pathogens, a better understanding of the interaction between K. pneumoniae and the host is indispensable. However, the underlying mechanisms have not yet been systemically studied, and numerous knowledge gaps are needed to be filled in. In particular, it is critical to identify the VFs involved in the host–pathogen interaction, especially those essential for the K. pneumoniae-induced autophagy, cell death and cytokines production. Furthermore, it is imperative to uncover the type(s) of cell death that is more efficient for hosts to cope with K. pneumoniae infection. It is largely unclear whether the triggers for each type of cell death are identical or strain/clone-specific. Understanding these questions would provide us with novel insights into the pathogenicity of K. pneumoniae and the development of selective alternatives for the management of K. pneumoniae infection.

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