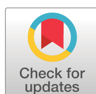
The deadly dance of alveolar macrophages and influenza virus

Camille David [1,2], Charles Verney [1,2,3], Mustapha Si-Tahar [1,2] and Antoine Guillon [1,2,3]

[1]INSERM, Centre d'Etude des Pathologies Respiratoires (CEPR), UMR 1100, Tours, France. [2]Université de Tours, Tours, France. [3]CHRU de Tours, Service de Médecine Intensive Réanimation, Tours, France.

Corresponding author: Antoine Guillon (antoine.guillon@univ-tours.fr)



Shareable abstract (@ERSpublications)
This review aims to summarise insights into the role of human alveolar macrophages during influenza infection to gain a better understanding of influenza virus pathogenesis. https://bit.ly/3AQMZUq

Cite this article as: David C, Verney C, Si-Tahar M, *et al*. The deadly dance of alveolar macrophages and influenza virus. *Eur Respir Rev* 2024; 33: 240132 [DOI: 10.1183/16000617.0132-2024].

Copyright ©The authors 2024

This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org

Received: 11 June 2024
Accepted: 22 Aug 2024

Abstract

Influenza A virus (IAV) is one of the leading causes of respiratory infections. The lack of efficient anti-influenza therapeutics requires a better understanding of how IAV interacts with host cells. Alveolar macrophages are tissue-specific macrophages that play a critical role in lung innate immunity and homeostasis, yet their role during influenza infection remains unclear. First, our review highlights an active IAV replication within alveolar macrophages, despite an abortive viral cycle. Such infection leads to persistent alveolar macrophage inflammation and diminished phagocytic function, alongside direct mitochondrial damage and indirect metabolic shifts in the alveolar micro-environment. We also discuss the "macrophage disappearance reaction", which is a drastic reduction of the alveolar macrophage population observed after influenza infection in mice but debated in humans, with unclear underlying mechanisms. Furthermore, we explore the dual nature of alveolar macrophage responses to IAV infection, questioning whether they are deleterious or protective for the host. While IAV may exploit immuno-evasion strategies and induce alveolar macrophage alteration or depletion, this could potentially reduce excessive inflammation and allow for the replacement of more effective cells. Despite these insights, the pathophysiological role of alveolar macrophages during IAV infection in humans remains understudied, urging further exploration to unravel their precise contributions to disease progression and resolution.

Introduction

Lung infections cause more loss of disability-adjusted life-years worldwide than highly recognised threats to the public's health such as cancer, heart attacks or strokes [1]. Influenza virus is one of the leading causes of respiratory infections. In 1918, over 50 million people succumbed to severe influenza viral pneumonia. Fast forward a century, despite significant medical advancements, we still face yearly epidemics that lead to 3–5 million severe illness cases worldwide, resulting in up to 650 000 deaths [2]. Therefore, it is crucial to develop new antiviral drugs for better treatment of influenza A virus (IAV) pneumonia. To achieve this, we need a deeper understanding of how IAV interacts with host cells, especially in the context of IAV-triggered lung inflammation, which is believed to be the cause of severe "flu" symptoms according to several studies [3, 4].

Influenza viruses typically start their infection in the respiratory tract. In uncomplicated IAV infection, virus replication is confined to the upper respiratory tract and alveolar macrophages are unlikely to be actively engaged. However, when the respiratory infection extends deeper into the distal lungs, alveolar macrophages are the most abundant resident leukocytes in the lower respiratory airways and play a pivotal role in the innate immune response [5]. IAV-infected cells are phagocytosed by alveolar macrophages through the recognition of viral RNA by various receptors, which leads to the secretion of type I interferons (IFNs), pro-inflammatory cytokines, eicosanoids and chemokines. Chemokines produced at the infection site bring in more immune cells, such as natural killer (NK) cells, monocytes and neutrophils, to the airways. Working alongside alveolar macrophages, these recruited leukocytes (including NK cells,

monocytes and neutrophils) play a crucial role in triggering apoptosis in the infected cells and/or in removing virus-infected cells.

Alveolar macrophages are specialised tissue-specific macrophages critical for innate immunity and maintaining lung homeostasis. As frontline defenders of the alveoli and airways, they must strike a delicate balance: providing rapid surveillance and defence against external pathogens while minimising inflammation to preserve the integrity of the thin alveolar–capillary membrane and ensure efficient gas exchange.

Comprising 85–95% of all cells sampled *via* bronchoalveolar lavage (BAL) in both mice and humans at steady state [5–8], alveolar macrophages inevitably interact with influenza viruses. However, their precise involvement in human influenza infection remains largely unclear. Some unanswered alveolar macrophage-related questions should be underscored and discussed, such as: Do influenza viruses infect human alveolar macrophage and, if yes, is the viral replication leading to infective viral particles? Why are alveolar macrophages the only lung leukocytes to "disappear" during the first days of influenza infection in mice? Is the alveolar macrophage immune response beneficial or deleterious for the host during influenza infection? Moreover, advancements in alveolar macrophage biology over the past decade have revealed long-term changes in "experienced" alveolar macrophages, previously exposed to infections or insults [9]. This leads to significant alveolar macrophage heterogeneity and variations in immune baseline between "experienced" and "naïve" organisms [10]. These discoveries challenge the traditional experimental models and their translational relevance, underscoring the need to integrate them into studies of host–pathogen interactions between influenza viruses and alveolar macrophages.

Alveolar macrophages are likely to be underrated players of lung innate immunity during influenza infection. In this review, we aim to summarise insights into the role of human alveolar macrophages during influenza infection to gain a better understanding of influenza virus pathogenesis, while also pointing out key questions for future research.

Search strategy

This mini-review is structured as a narrative review. The published data in the area is non-homogeneous and not conducive to meta-analysis. In addition, various high-quality reviews have been published on either respiratory viral infection, on alveolar macrophages or on innate immune defence towards influenza infection. Here, we focus on overlapping aspects of alveolar macrophage physiology and IAV infection between animal models and humans. To restrict our findings to the state of the art, the search period covers 1980–2024. To find relevant English language publications in peer-reviewed journals, the following databases were interrogated: PubMed, Google, Scopus and MEDLINE (Ovid). The search algorithm was based on combinations of the following MeSH (Medical Subject Headings) terms or keywords: ("Respiratory virus" OR Influenza) AND ("Innate Immunity" OR "Trained Immunity" OR "Innate immune cells" OR Macrophages OR "alveolar macrophages") AND (lung OR "respiratory tract") AND (("Models, Animal" OR mice) OR human). We evaluated the search results to ensure they aligned with the research question and considered citation tracking to identify additional relevant literature.

Do influenza viruses infect alveolar macrophages?

As resident lung leukocytes, alveolar macrophages are among the first innate immune cell types to encounter the virus. However, *in vivo* evidence of influenza-infected alveolar macrophages remains limited. Intracellular viral nucleoprotein was detectable by day 2 post-infection in murine alveolar macrophages collected from BAL [11]. Single-cell mapping of host and viral transcriptomes in IAV-infected mice suggested that ~25% of the "mononuclear phagocyte system" were infected cells [12]. Cross-sections from *ex vivo* infected human lung explants also revealed IAV antigen staining in alveolar macrophages [13]. Despite these important observations, distinguishing between actual infected alveolar macrophages and macrophages that have engulfed infected cells remains challenging.

To assess if alveolar macrophages are permissive for influenza virus, *in vitro* models were used. Blood-derived macrophages were found to be susceptible to influenza infection; however, no efficient influenza virus propagation was detected independently of the viral strain used for infection [14]. The lack of productive virus replication has been inconstantly observed in these models [15]. The various conditions used to culture peripheral monocyte-derived macrophages may account for these observed differences. However, it is not clear if any of these macrophage subsets accurately reflect the biological phenotype of alveolar macrophages as they lack essential characteristics, such as expression of Siglec-F (sialic acid-binding Ig-like lectin-F), and the specific phenotype imprinted by the lung environment. In 1982, Rodgers and Mims [16, 17] demonstrated that *ex vivo* infections of murine and human alveolar

macrophages were permissive to influenza virus but no release of infectious virus was detected. Subsequent studies focusing on the susceptibility of human alveolar macrophages to IAV revealed varying degrees of uncertainty, with some studies suggesting that human alveolar macrophages could be infected by IAV [18–20] and that viral replication is active within the infected alveolar macrophages [19–21], while others reported an abortive virus cycle, resulting in no significant production of influenza neovirions [18–20, 22]. These disparities underscore the necessity to elucidate the interaction between alveolar macrophages and IAV in humans.

Changes induced by influenza virus infection on alveolar macrophage metabolism and functions

Upon contact with influenza virus, one of the earliest alveolar macrophage changes observed is a robust pro-inflammatory response, positioning alveolar macrophages as tissue sentinels orchestrating immune cell recruitment during the initial inflammation phase. In human and porcine alveolar macrophages infected by IAV *in vitro*, tumour necrosis factor-α stands out as the most secreted cytokine [19, 20], peaking between 4 and 8 h post-infection. Additionally, alveolar macrophages secrete a type I IFN response, a potent antiviral cytokine signal, upon *in vitro* influenza infection [20]. This triggers an amplification loop for viral sensing, evidenced by increased expression of the Toll-like receptors TLR3 and TLR7 at 24 h post-infection [19, 23].

Despite their pivotal role in initiating inflammation, alveolar macrophages produce fewer inflammatory mediators compared to blood-derived macrophages [24, 25]. After IAV infection, peroxisome-activated receptor (PPAR)-γ, a negative regulator of inflammatory cytokine production, is downregulated in alveolar macrophages through type I IFN-dependent signalling. PPAR-γ downregulation is associated with persistent inflammation and impaired pneumonia resolution [26]. Furthermore, alveolar macrophages exhibit diminished phagocytic function for several weeks post-influenza infection [27], a phenomenon known as immunoparalysis. This impairment is characterised by altered phagocytosis and decreased expression of key macrophage receptor genes, such as *CLEC7A* (C-type lectin domain family 7 member A) and *MSR1* (macrophage scavenger receptor 1) [19].

Metabolic reprogramming is critical for alveolar macrophages to control cell functions and inflammatory status, with a glycolytic shift and modulation of the Krebs cycle being the most important factors [28]. The alveolar environment, rich in oxygen and poor in glucose, appears critical in determining this cellular fate. During severe infections, the lung environment is significantly altered. Inflammation causes the capillaries surrounding the alveoli to become more permeable. This increased permeability causes fluid and proteins to leak into the alveoli, impairing gas exchange. Consequently, there is a significant regional drop in oxygen and an influx of serum rich in glucose. *In vitro* studies of alveolar macrophages in hypoxic conditions demonstrated the stabilisation of hypoxia-inducible factor-1α that leads to increased glycolysis in human or murine alveolar macrophages [29, 30]. Alveolar macrophages have been reported to exhibit distinct cytokine profiles depending on whether their metabolism is centred around oxidative phosphorylation or glycolysis [30]. During *in vivo* influenza infection, it is thus complicated to decipher whether the viral infection induces a glycolytic shift *per se* or if the environmental changes (oxygen deprivation, glucose enrichment) are primarily responsible for the metabolic changes. In alveolar macrophages studied outside of the alveolar environment, influenza virus infection resulted in an increased accumulation of abnormal mitochondria within the macrophages [31]. Transcriptomic analysis revealed that there was a decreased enrichment of mitochondrial oxidative phosphorylation-related genes in *ex vivo* influenza-infected alveolar macrophages [31]. It is likely that influenza infection may cause direct defective mitochondrial fitness and an indirect metabolic shift due to an altered alveoli environment in the context of infection.

A summary of altered cell functions in influenza-infected alveolar macrophages is presented in figure 1.

The "macrophage disappearance reaction"

During influenza infection, a drastic reduction in the alveolar macrophage population is observed in mice and is referred to as the "macrophage disappearance reaction" [9]. This decrease begins within the first 24 h of infection, peaks at day 7 post-infection and returns to normal levels by day 10 post-infection [32, 33]. Although variations in the extent of alveolar macrophage depletion post-influenza infection have been noted between mice strains, similar results are observed across various virus strains, independently of viral doses [32, 33]. For example, >90% of alveolar macrophages are lost within the first week in BALB/c mice infected with H1N1/PR8 IAV [32]. Studies examining alveolar macrophage pools in both BAL fluid and post-lavage lungs after infection confirm that the depletion of alveolar macrophages is total, suggesting that influenza-induced alveolar macrophage depletion is not due to upregulated adhesion molecules but rather reflects a genuine loss of alveolar macrophages [32].

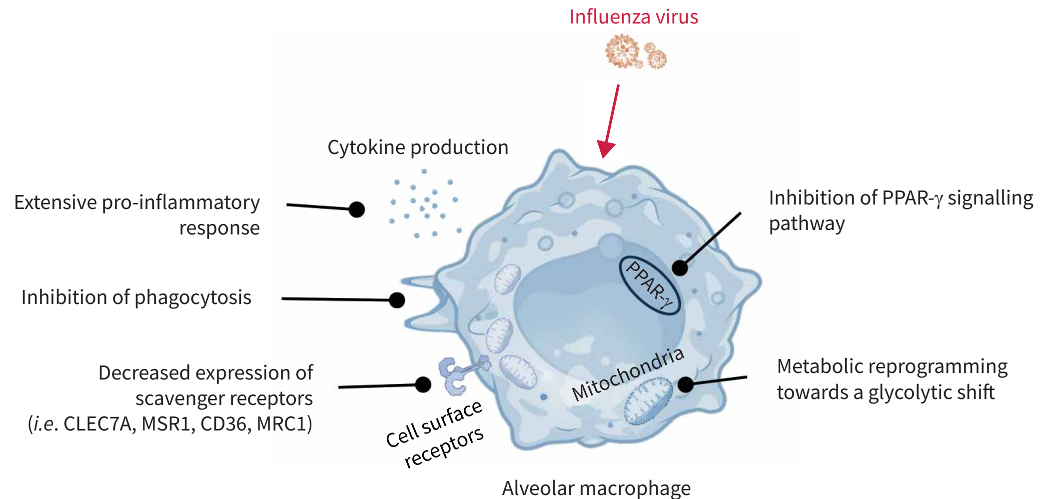


FIGURE 1 Altered cell functions in influenza-infected alveolar macrophages. CLEC7A: C-type lectin domain family 7 member A; MSR1: macrophage scavenger receptor 1; MRC1: mannose receptor C-type 1; PPAR-γ: peroxisome-activated receptor-γ.

To date, the mechanism responsible for alveolar macrophage disappearance in mice remains unknown. It is still unclear whether IAV directly infects alveolar macrophages, leading to cellular death, or if alveolar macrophages disappear due to the loss of survival signals triggered by infection of respiratory epithelial cells (*e.g.* granulocyte–macrophage colony-stimulating factor (GM-CSF)). Alternatively, alveolar macrophages may migrate outside the airspace during inflammation. IAV possesses an intrinsic ability to subvert and/or suppress the host immune response. This is facilitated by various virulence factors encoded within the virus [34]. One such factor is PB1-F2 (polymerase basic protein 1-frame 2), which plays a pivotal role in inducing apoptosis in immune cells. PB1-F2 achieves this by targeting mitochondria, leading to significant alterations in their morphology. Consequently, this disrupts the membrane potential, ultimately triggering the apoptotic process [35]. Notably, this effect has been observed in monocytes, although its impact on alveolar macrophages remains uncertain. Interestingly, epithelial cell lines which support productive influenza virus replication do not exhibit this apoptotic process [35].

By targeting critical phagocytic cells such as alveolar macrophages, influenza viruses increase the probability of opportunistic secondary bacterial infections (also called bacterial co-infections), such as those caused by *Streptococcus pneumoniae*. These secondary bacterial infections are the major cause of mortality associated with influenza [36]. Experimental depletion of alveolar macrophages replicates the increased bacterial burden observed during influenza pneumonia [10]. Conversely, administering GM-CSF to mice after the onset of influenza infection has been shown to restore the number and phagocytic function of alveolar macrophages in the lungs, which was associated with more effective protection against *S. pneumoniae* and improved survival [32, 37, 38]. To the best of our knowledge, no randomised controlled trial has investigated the use of GM-CSF as add-on therapy in the context of influenza pneumonia.

The prevailing concept that IAV infection leads to depletion of alveolar macrophages is widely documented in the literature [32, 39–41]. However, the translational significance of this observation is questionable due to notable differences between human and murine alveolar macrophages. Briefly, human alveolar macrophages display considerable heterogeneity, undergoing constant remodelling during the lifespan, a complexity that is poorly recapitulated in laboratory mice. Intriguingly, the influenza-induced depletion of alveolar macrophages was not observed in other animal models such as pigs [42] and ferrets [43]. Thus, the "macrophage disappearance reaction" observed in mice has yet to be confirmed in humans and should be interpreted with caution.

Are alveolar macrophage responses to influenza virus infection deleterious or protective for the host?

The next question is: "Who benefits from the crime?", in this case the disappearance of macrophages (figure 2). On the one hand, alveolar macrophages are important for controlling influenza virus infection and there are clear advantages for influenza viruses in inducing apoptosis in these cells. The recognition of

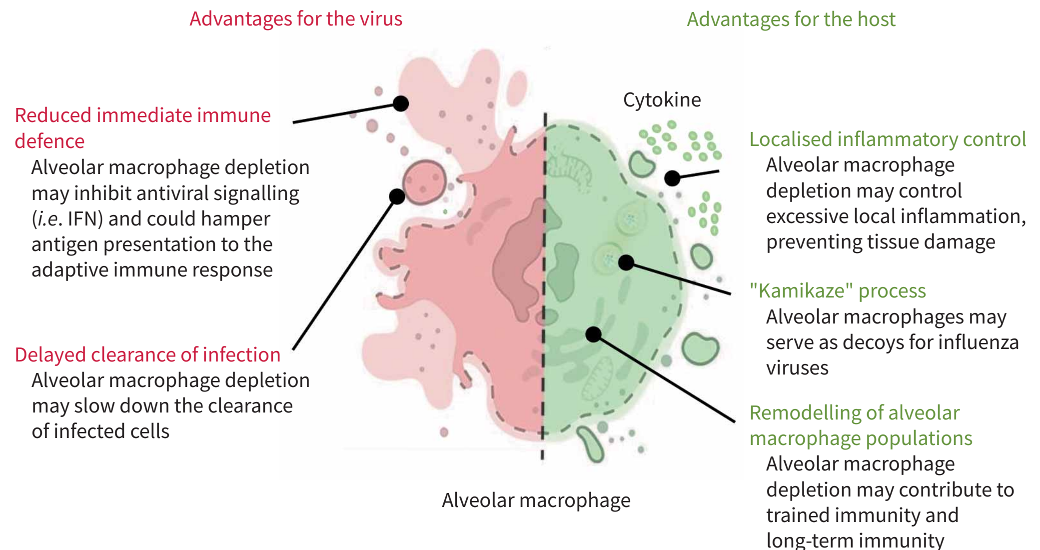


FIGURE 2 Hypothetical trade-offs of the "macrophage disappearance reaction" during influenza infection. IFN: interferon.

viral pathogen-associated molecular patterns and nucleic acids by alveolar macrophage receptors triggers early and strong IFN signalling. Within 4 h post-infection, seven of the top 10 upregulated genes in IAV-infected alveolar macrophages belong to the type I IFN family [19], essential for combating viral replication [44]. Consequently, macrophage-depleted animal models such as mice [44], ferrets [43] and pigs [42] subsequently infected with influenza virus exhibit high virus replication in the airways, more severe respiratory symptoms and a higher mortality rate compared to infected control animals. Moreover, the influenza-mediated depletion of macrophages could hamper antigen presentation to the adaptive immune response, thus reducing the overall ability of the immune system to perform viral clearance.

On the other hand, this temporary disappearance of alveolar macrophages could also confer advantages to the host. First, during influenza infection, the immune response can become overly aggressive or unregulated, leading to immunopathogenicity [45]. This can damage lung tissues and contribute to acute respiratory failure [46]. Given that alveolar macrophages are known to sustain persistent inflammation, their absence could help limit excessive inflammation. Second, alveolar macrophages may serve as decoys for influenza viruses, restricting virus release and spread by undergoing abortive infection, in contrast to epithelial cells [47]. Therefore, the alveolar macrophage depletion might result from a "kamikaze" process aimed at preventing infection of surrounding cells. Lastly, there is an extraordinary heterogeneity among alveolar macrophages in adult humans, including mixed populations of resident and newly recruited cells [48]. The disruption of homeostasis during influenza infection contributes to the remodelling of alveolar macrophage populations by recruiting more efficient cells, such as monocytes that differentiate locally into macrophages or by driving evolution of resident alveolar macrophage within the lung niche. This process, known as trained immunity, involves the innate immune system "remembering" past encounters with pathogens, leading to an enhanced response upon subsequent exposure [49], a phenomenon observed in alveolar macrophages [10]. Interestingly, recent findings suggest that influenza-trained alveolar macrophages confer long-term antitumour immunity in the lungs [50].

Conclusions

The pathophysiological significance of alveolar macrophages in IAV infection remains largely unexplored in humans. This gap in knowledge contrasts sharply with the recognised importance of alveolar macrophages in lung biology and the significant public health impact of influenza infection. It is probable that alveolar macrophages play a substantial protective role during IAV infection, which has likely been underestimated thus far. However, direct analysis of alveolar macrophage functions during IAV infection *in vivo* presents several challenges. These include the difficulty in accessing human lung tissues, which necessitates invasive procedures, as well as the limited quantity of alveolar macrophages in IAV-infected mice. Additionally, the situation in human lungs may differ considerably from that of laboratory mice, owing to factors such as repetitive infections and airborne insults, which can influence alveolar macrophage heterogeneity.

Questions for future research

- Do influenza viruses infect human alveolar macrophages and, if so, does the viral replication result in infective viral particles?
- What causes the peculiar disappearance of alveolar macrophages, unique among lung leukocytes, during the initial days of influenza infection in mice?
- Is the immune response of alveolar macrophages beneficial or deleterious to the host during influenza infection?

Provenance: Submitted article, peer reviewed.

Author contributions: All authors contributed to the writing of this mini-review and approved the final version.

Conflict of interest: All authors have nothing to disclose.

Support statement: The study was supported by the Agence Nationale de la Recherche (ANR-19-CE15-0032-01). C. Verney was funded by “Poste d'accueil INSERM”. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Mizgerd JP. Lung infection – a public health priority. *PLoS Med* 2006; 3: e76.
2 US Centers for Disease Control and Prevention. Influenza (flu). 2021. www.cdc.gov/flu/index.htm Date last accessed: 7 October 2021.
3 Le Goffic R, Balloy V, Lagranderie M, *et al.* Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog* 2006; 2: e53.
4 Taubenberger JK, Kash JC, Morens DM. The 1918 influenza pandemic: 100 years of questions answered and unanswered. *Sci Transl Med* 2019; 11: eaau5485.
5 Yu Y-RA, Hotten DF, Malakhau Y, *et al.* Flow cytometric analysis of myeloid cells in human blood, bronchoalveolar lavage, and lung tissues. *Am J Respir Cell Mol Biol* 2016; 54: 13–24.
6 Baharom F, Thomas S, Rankin G, *et al.* Dendritic cells and monocytes with distinct inflammatory responses reside in lung mucosa of healthy humans. *J Immunol* 2016; 196: 4498–4509.
7 Desch AN, Gibbings SL, Goyal R, *et al.* Flow cytometric analysis of mononuclear phagocytes in nondiseased human lung and lung-draining lymph nodes. *Am J Respir Crit Care Med* 2016; 193: 614–626.
8 Heron M, Grutters JC, ten Dam-Molenkamp KM, *et al.* Bronchoalveolar lavage cell pattern from healthy human lung. *Clin Exp Immunol* 2012; 167: 523–531.
9 Aegerter H, Lambrecht BN, Jakubzick CV. Biology of lung macrophages in health and disease. *Immunity* 2022; 55: 1564–1580.
10 Guillon A, Arafa EI, Barker KA, *et al.* Pneumonia recovery reprograms the alveolar macrophage pool. *JCI Insight* 2020; 5: e133042.
11 Cardani A, Boulton A, Kim TS, *et al.* Alveolar macrophages prevent lethal influenza pneumonia by inhibiting infection of type-1 alveolar epithelial cells. *PLoS Pathog* 2017; 13: e1006140.
12 Steuerman Y, Cohen M, Peshes-Yaloz N, *et al.* Dissection of influenza infection *in vivo* by single-cell RNA sequencing. *Cell Syst* 2018; 6: 679–691.
13 Weinheimer VK, Becher A, Tönnies M, *et al.* Influenza A viruses target type II pneumocytes in the human lung. *J Infect Dis* 2012; 206: 1685–1694.
14 Friesenhagen J, Boergeling Y, Hrincius E, *et al.* Highly pathogenic avian influenza viruses inhibit effective immune responses of human blood-derived macrophages. *J Leukoc Biol* 2012; 92: 11–20.
15 Lee SMY, Dutry I, Peiris JSM. Editorial: macrophage heterogeneity and responses to influenza virus infection. *J Leukoc Biol* 2012; 92: 1–4.
16 Rodgers BC, Mims CA. Role of macrophage activation and interferon in the resistance of alveolar macrophages from infected mice to influenza virus. *Infect Immun* 1982; 36: 1154–1159.
17 Rodgers BC, Mims CA. Influenza virus replication in human alveolar macrophages. *J Med Virol* 1982; 9: 177–184.
18 Ettensohn DB, Frampton MW, Nichols JE, *et al.* Human alveolar macrophages may not be susceptible to direct infection by a human influenza virus. *J Infect Dis* 2016; 214: 1658–1665.
19 Wang J, Nikrad MP, Travanty EA, *et al.* Innate immune response of human alveolar macrophages during influenza A infection. *PLoS One* 2012; 7: e29879.
20 Yu WCL, Chan RWY, Wang J, *et al.* Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses. *J Virol* 2011; 85: 6844–6855.
21 Lee SMY, Gardy JL, Cheung CY, *et al.* Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages. *PLoS One* 2009; 4: e8072.

22 Short KR, Brooks AG, Reading PC, *et al.* The fate of influenza A virus after infection of human macrophages and dendritic cells. *J Gen Virol* 2012; 93: 2315–2325.

23 Zhang J, Miao J, Hou J, *et al.* The effects of H3N2 swine influenza virus infection on TLRs and RLRs signaling pathways in porcine alveolar macrophages. *Virol J* 2015; 12: 61.

24 Ettensohn DB, Roberts NJ. Influenza virus infection of human alveolar and blood-derived macrophages: differences in accessory cell function and interferon production. *J Infect Dis* 1984; 149: 942–949.

25 Koch CM, Anekalla KR, Hu Y-S, *et al.* Influenza-induced activation of recruited alveolar macrophages during the early inflammatory phase drives lung injury and lethality. *bioRxiv* 2022; preprint [https://doi.org/10.1101/2020.06.08.141309].

26 Huang S, Zhu B, Cheon IS, *et al.* PPAR-γ in macrophages limits pulmonary inflammation and promotes host recovery following respiratory viral infection. *J Virol* 2019; 93: e00030-19.

27 Roquilly A, Jacqueline C, Davieau M, *et al.* Alveolar macrophages are epigenetically altered after inflammation, leading to long-term lung immunoparalysis. *Nat Immunol* 2020; 21: 636–648.

28 Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? *Trends Immunol* 2017; 38: 395–406.

29 Woods PS, Kimmig LM, Meliton AY, *et al.* Tissue-resident alveolar macrophages do not rely on glycolysis for LPS-induced inflammation. *Am J Respir Cell Mol Biol* 2020; 62: 243–255.

30 Woods PS, Kimmig LM, Sun KA, *et al.* HIF-1α induces glycolytic reprograming in tissue-resident alveolar macrophages to promote cell survival during acute lung injury. *eLife* 2022; 11: e77457.

31 Gao X, Zhu B, Wu Y, *et al.* TFAM-dependent mitochondrial metabolism is required for alveolar macrophage maintenance and homeostasis. *J Immunol* 2022; 208: 1456–1466.

32 Ghoneim HE, Thomas PG, McCullers JA. Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections. *J Immunol* 2013; 191: 1250–1259.

33 Califano D, Furuya Y, Metzger DW. Effects of influenza on alveolar macrophage viability are dependent on mouse genetic strain. *J Immunol* 2018; 201: 134–144.

34 Lowy RJ. Influenza virus induction of apoptosis by intrinsic and extrinsic mechanisms. *Int Rev Immunol* 2003; 22: 425–449.

35 Chen W, Calvo PA, Malide D, *et al.* A novel influenza A virus mitochondrial protein that induces cell death. *Nat Med* 2001; 7: 1306–1312.

36 Sethi S. Bacterial pneumonia. Managing a deadly complication of influenza in older adults with comorbid disease. *Geriatrics* 2002; 57: 56–61.

37 Steinwede K, Tempelhof O, Bolte K, *et al.* Local delivery of GM-CSF protects mice from lethal pneumococcal pneumonia. *J Immunol* 2011; 187: 5346–5356.

38 Umstead TM, Hewage EK, Mathewson M, *et al.* Lower respiratory tract delivery, airway clearance, and preclinical efficacy of inhaled GM-CSF in a postinfluenza pneumococcal pneumonia model. *Am J Physiol Lung Cell Mol Physiol* 2020; 318: L571–L579.

39 Robinson KM, Kolls JK, Alcorn JF. The immunology of influenza virus-associated bacterial pneumonia. *Curr Opin Immunol* 2015; 34: 59–67.

40 Rynda-Apple A, Robinson KM, Alcorn JF. Influenza and bacterial superinfection: illuminating the immunologic mechanisms of disease. *Infect Immun* 2015; 83: 3764–3770.

41 McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. *Nat Rev Microbiol* 2014; 12: 252–262.

42 Kim HM, Lee Y-W, Lee K-J, *et al.* Alveolar macrophages are indispensable for controlling influenza viruses in lungs of pigs. *J Virol* 2008; 82: 4265–4274.

43 Kim HM, Kang YM, Ku KB, *et al.* The severe pathogenicity of alveolar macrophage-depleted ferrets infected with 2009 pandemic H1N1 influenza virus. *Virology* 2013; 444: 394–403.

44 Tate MD, Pickett DL, van Rooijen N, *et al.* Critical role of airway macrophages in modulating disease severity during influenza virus infection of mice. *J Virol* 2010; 84: 7569–7580.

45 Tisoncik JR, Korth MJ, Simmons CP, *et al.* Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 2012; 76: 16–32.

46 Guillon A, Brea-Diakite D, Cezard A, *et al.* Host succinate inhibits influenza virus infection through succinylation and nuclear retention of the viral nucleoprotein. *EMBO J* 2022; 41: e108306.

47 Meischel T, Villalon-Letelier F, Saunders PM, *et al.* Influenza A virus interactions with macrophages: lessons from epithelial cells. *Cell Microbiol* 2020; 22: e13170.

48 Mould KJ, Moore CM, McManus SA, *et al.* Airspace macrophages and monocytes exist in transcriptionally distinct subsets in healthy adults. *Am J Respir Crit Care Med* 2021; 203: 946–956.

49 Netea MG, Domínguez-Andrés J, Barreiro LB, *et al.* Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020; 20: 375–388.

50 Wang T, Zhang J, Wang Y, *et al.* Influenza-trained mucosal-resident alveolar macrophages confer long-term antitumor immunity in the lungs. *Nat Immunol* 2023; 24: 423–438.