Review Article

Alveolar Macrophages in Influenza A Infection Guarding the Castle with Sleeping Dragons

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Influenza A is a Worldwide Burden and Recurring Threat

Despite major advances in influenza vaccination and flu-prevention community awareness campaigns, Influenza A virus (IAV) remains a worldwide and recurring threat [1, 2]. Seasonal influenza causes 3-5 million cases of severe respiratory and systemic illness and upwards of 650,000 deaths annually, particularly among the elderly, very young, and chronically ill [3]. Countless hours of missed school and work have significant economic consequences [4, 5]. In pandemic years, morbidity and mortality soars, especially among the young [6, 7].

The Influenza A Viral Infection Cycle

In humans, influenza A targets epithelial cells of the respiratory tract via droplet inhalation. Viral Hemagglutinin (HA) protein binds to sialic acid receptors decorating the surface of polarized respiratory epithelial host cells. If the patient has repeated exposure to the same IAV strain, or one with similar HA antigenic structure, IAV-specific IgG and secretory IgA antibodies may neutralize and thereforeeliminate the virus.Circulating CD4+ helper T-cells directed against influenza proteins may hasten the effectors T cell response; as well as provide some direct antiviral function by targeted destruction of infected cells, limiting viral spread [8, 9]. Antigen-specific effector CD8+ T cells primed against internal viral antigens, when present, clear virus and destroy infected cells, thus limiting severity of disease [9, 10]. If the virus is not cleared by these mechanisms,or if the host response is impaired (as with smokers and other conditions), retrograde infection can proceed from the upper respiratory tract to the lower.Following virion uptake in the cell endosome and uncoating viral RNA is transported to the host cell nucleus, where the virus begins replication and transcription, utilizing host cell mRNA cap-stealing mechanisms to induce viral mRNA synthesis [11]. New viral RNAs are transcribed and viral proteins translated, and new virions are assembled in the infected host cell [12, 13]. This cellular hijacking turns the host into a virus-manufacturing machine, shutting down host cell protein synthesis while simultaneously inhibiting infection-induced apoptosis [14, 15]. Viral nucleic acids and proteins are,

are transported to the cell surface, packaged into new virionswhich bud off the plasma membrane and released via the action of viral neuraminidase (NA) enzyme. Virions can retain host cell membrane sialic acid receptors for the HA,enabling virions to clump. These large viral clusters may spread more easily through the lower respiratory tract; NA cleaves these domains, allowing virions to disperse in the distal airway [13, 16].

Alveolar Macrophages: The Primary Defensive Line and Cleanup Crew of the Lower Respiratory Tract

Lung macrophages derive from multiple lineages, and play different roles in the lung. Alveolar macrophages (AMΦ), thought to derive fromprogenitors present in fetal liver [17] move to the lung interstitium during development then migrate to the air-tissue interface after birth, where they maintain and repopulate locally through life. AMΦ are tightly adherent to alveolar epithelial cells. This cell-cell contact plays a key role in homeostasis and function. Under the direction of GM-CSF, these macrophages primarily remove surfactant and cellular debris, preventing Pulmonary Alveolar Proteinosis (PAP). They are also responsible for phagocytosis of foreign pathogens that have overcome or bypassed the mechanical defenses and immune defenses of the upper airway [18]. AMΦ regenerate locally, as demonstrated by long-term persistence of donor macrophages in patients who undergo lung transplant [19, 20]. If there is complete loss of alveolar macrophages, as in irradiation, AMΦ will regenerate from circulating monocytes [21].

By contrast, interstitial pulmonary macrophages, which make up a smaller proportion of lung macrophages derive from bone marrow precursors, and play a different role in lung immune defense: namely antigen-presentation and modulation of tissue injury [22]. A third, smaller subset of primitive macrophages, derives from yolk sac progenitors, and resides in the mesothelium adjacent to the vasculature [19, 23]. AMΦ play myriad roles in the lungs, including localized homeostasis, injury repair and remodeling, and innate defense. Perhaps their most remarkable feature is the capacity toselectively regulate induction of the adaptive immune response to foreign

pathogenswhich invade the terminal airways at the air-tissue interface [24]. Under homeostatic conditions AMΦ, like microglia, primarily exist in a resting state controlled by interaction between the OX-2 membrane glycoprotein CD200 and the CD200 receptor through TGFβ signaling [25]. In this state, AMΦ downregulate expression of macrophage CD11b, a surfaceintegrin protein critical for phagocytosis [24, 26], thus phagocytic activity is suppressed. AMΦ adhere tightly to alveolar type I and type II cells. In this quiescent state macrophages induce low localized levels of αvβ6 integrinin alveolar epithelial cells, which binds to the latency associated peptide (LAP) of TGFβ to form latent-TGFβ on AMΦ [27]. This complex suppresses AMΦproduction of proinflammatory and cellular recruiting cytokines interleukin 1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) [28].Suppression of phagocytosis and inhibition of inflammatory cytokine release regulates activation of the adaptive immune response and mitigates unchecked inflammation and edema that might otherwise impair alveolar gas exchange [29].

Alveolar macrophages become activated when AMΦ pattern recognition receptors (PRRs) interact with pathogen-associated molecular patterns (PAMPs) in the respiratory tract [30]. Activation induces conformational changes resulting in loss of contact with alveolar epithelial cells. Interruption of cellular contact abruptly halts αvβ6 integrin binding with LAP binding and results in loss of latent-TGFβ. Without latent-TGFβ production, the suppression of AMΦ phagocytic activity and cytokine production is lost, priming AMΦ to produce TNF-α and IL-6, becoming cell-recruitmentand phagocytic machines [31]. Activated macrophages recruit neutrophils and inflammatory monocytes to the airways (and interstitium) which replace resident alveolar macrophages over the course of several days [32, 33]. Once activated, these macrophages have astonishing phagocytic and pro-inflammatory activity. MacLean and Kradin's in vivo rat model demonstrated that AMΦare able to engulf greater than 10^9 intratracheally-injectedListeria organisms before macrophage spillover occurs [34]. Fine particulate activation of AMΦ induces high levels of reactive oxygen species (ROS), 8-isoprostane, and Arachidonic Acid (AA) metabolites including prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4) [35]. Within days of insult, T lymphocytes and natural killer cells are recruited to the site of injury, where they secrete interferon gamma (IFN- γ) [36]. IFN- γ stimulates matrix metalloproteinase (MMP-9) production by AMΦ, which alternatively activates latent-TGFβ on macrophages. This induces macrophages to re-adhere to epithelium, restoring suppression of inflammation and phagocytosis, and returning AMΦ to their homeostatic inflammation-suppressing state [24].

The Alveolar Macrophage Arsenal against Influenza A

AMΦ employ several pattern recognition receptors against influenza. The primary pathogen-associated molecular patterns generated by influenza A are cytoplasmic viral RNAs produced during cellular viral replication [37, 38]and viral M2 protein.AMΦ exposed to IAV have marked upregulation of type I interferons (especially α1, 4, 7, 8, 13, 17, and 21),chemokine CXC motif ligands 5, 9, 10, and 11; fibroblast activation protein α (FAP);TNF-α, and members of the IL-1 family [39]. As AMΦ endocytose virions, viral membrane degradation releases viral ssRNA into the macrophage cytoplasm. Viral ssRNA is recognized as foreign by AMΦ toll-like receptor 7 (TLR7), inducing the NF-κB inflammatory signaling pathway expression. AMΦ phagocytose dying IAV-infected alveolar epithelial cells and cellular debris. As cells are degraded, viral dsRNA is recognized by TLR3 [40],further inducing NF-κB inflammatory signaling, and producing type I interferons (IFN-I) [38, 41], and inducing expression of monocyte-recruiting chemokines like CCL2 [42]. In Infected AMΦviral RNA released in cytoplasm is recognized by retinoic acid-inducible gene I (RIG-I), which activates mitochondrial antiviral signaling protein (MAVS) [43]. The viral matrix ion channel protein M2 induces formation of the NOD-LRR-pyrin domain-containing inflammasome 3 (NLRP3), activating caspases, and releasing IL-1β [44].

Alveolar Macrophages Play a Unique Role in the Protection Against Influenza A Infection

Cardani and Braciale et al(2017) demonstrated that AMΦ are critical in the protection of type I alveolar epithelial cells (AEC-I) against lethal influenza infection [45].The authors developed a novel mouse model in which there is a cellular deficiency of mature alveolar macrophages. AMΦ-deficient mice infected with sublethal doses of intranasal IAV developed acute respiratory distress syndrome and death 8-12 days post-infection. In these mice, IAV spreads unchecked throughout the lower respiratory tract, resulting in massive inflammation upon effector T-cell elimination of infected cells. They further demonstrated that intranasal administration of AMΦ up to 24 hours post-infection rescued these mice from lethal infection by limiting IAV spread, illustrating the time-dependent role of AMΦ in IAV respiratory infection. The authors determined that AMΦ suppress autocrine production of cysteinyl leukotriene D4 in AEC-I, and that protection against IAV by AMΦ could be replicated with drugs targeting the same downstream metabolites of the arachidonic acid pathway inhibiting production of cysteinyl leukotrienes. These finding demonstrated a previously unappreciated, protective role of AMΦ in Influenza A infection.

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