The Immune Response to Influenza A Viruses

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Abstract The influenza A viruses are dangerous pathogens with the potential to provoke devastating disease. The challenge for the medical research community is to design preventive measures and therapeutic interventions that will limit the severe consequences of pandemic influenza A virus infections. Vaccines have long been available, but there is considerable scope for improvement as they target only the prevailing influenza A virus strains, do not give broad immunity, and work poorly in the elderly, the target group that is most at risk of fatal disease. Improved vaccines will only emerge if the development strategy is based on a firm understanding of the host immune response to the virus. Here, we summarize the research to date that details immune mechanisms participating in the control and elimination of influenza A viruses.

1 Introduction

The influenza viruses are *Orthomyxoviruses* with an eight-segmented, negative-sense, single-stranded RNA genome. There are three types: influenza A, B, and C. The influenza A viruses that cause the most serious problems in humans are the subject of this review. These pathogens are classified according to their two major surface glycoproteins: hemagglutinin (HA or H) and neuraminidase (NA or N). Infecting both mammalian and avian species, the highly contagious influenza A

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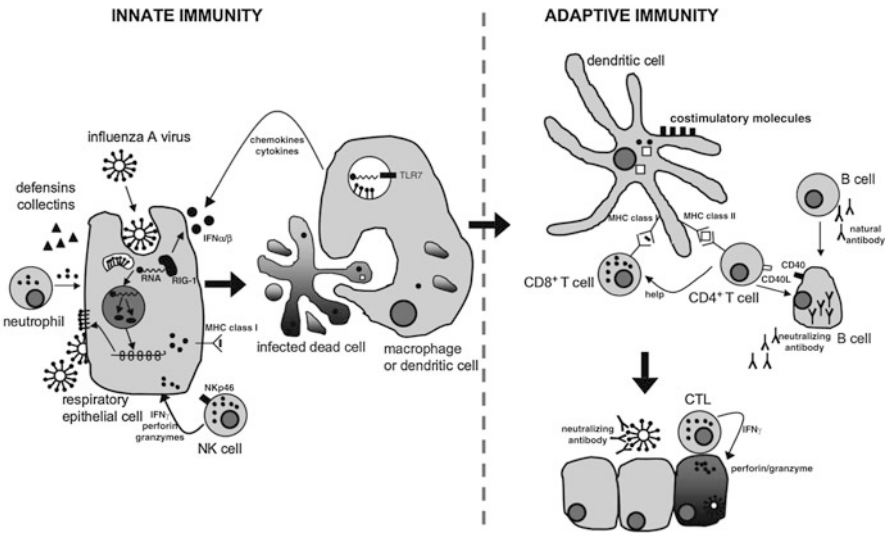

Fig. 1 Summary of the host immune response to influenza A virus

viruses are responsible for widespread morbidity and mortality [1]. In mammals, infection is established in the upper and lower respiratory tracts, provoking an illness that is associated with fever, myalgia, congestion, pharyngitis, and, in severe cases, pneumonia. Early on, some of the very virulent influenza A viruses can induce a "cytokine shock" syndrome mediated via the innate immune response pathway. Fortunately, infection also elicits potent adaptive immunity and long-term memory, though the virus can mutate readily, allowing strains with variant HA molecules to cause successive pandemics. The current killed or subunit vaccines induce effective antibody responses in normal adults, though they do not promote a virus-specific $CD8^+$ T-cell response and memory and they are poorly immunogenic in those who are even marginally immunologically compromised. The major task for immunologists interested in the problem that influenza virus poses is to develop better vaccines. Most of our detailed knowledge about immunity to the influenza A viruses is derived from the murine model that allows rigorous analysis due to the availability of an extensive panel of defined analytical reagents. Here, we provide a comprehensive summary of a large body of research examining the immune mechanisms that act to control influenza A virus infection (Fig. 1). This information should provide a useful basis for the informed design of novel, next generation influenza A virus vaccines.

2 Detection of Influenza A Virus

Invading influenza A viruses are detected in the host environment by "pattern recognition receptors" (PRRs) [2]. Previously, the molecular target was considered to be double-stranded viral RNA (dsRNA) recognized by the PRR, toll-like

receptor 3 (TLR3) [3, 4]. A role for TLR3 was questioned, however, given that the concentration of dsDNA is unlikely to be sufficient to signal TLR3 [5]. It is now considered that influenza A virus infection does not generate dsRNA at all [6]. Instead, the influenza A virus polymerase generates single-stranded RNA (ssRNA) with an uncapped 5′-phosphate that serves as the molecular signature identified by the immune system [6]. The cytoplasmic RNA helicase, RIG-1 [6, 7], but not MDA5 [6, 8], is responsible for influenza A virus recognition, which occurs independently of viral replication [7]. In addition to RIG-1, TLR7 is implicated in influenza A virus detection. Expressed in the endosomal compartments of plasmacytoid dendritic cells (DCs) and B cells, TLR7 detects influenza A virus ssRNA [9, 10]. The participation of multiple PRRs in the surveillance of influenza A virus may reflect cell type-specific roles [11]. Influenza A virus infection also activates NOD-like receptor-associated inflammasomes that are critical for the processing and release of IL-1β [12–14]. Once influenza A virus is recognized, PRRs initiate multiple signaling cascades that facilitate both innate and adaptive immunity to enable viral eradication.

3 Innate Immunity and the Influenza A Viruses

Innate immunity directed against influenza A virus provides an immediate and rapid response to the pathogen. The pulmonary infiltrate of innate immune cells is comprised mainly of natural killer (NK) cells, neutrophils, and macrophages. The NK cell represents the major innate response element and is detected in the infected lung as early as 48 h following influenza A virus infection [15, 16]. Protection is thought to be mediated by both cytokine production (IFN-γ and TNF-α) and direct cytotoxicity of virus-infected cells [17]. Influenza A virus-infected cells are recognized by NKp46 [18] and NKp44 [19] interaction with HA. The critical role for this pathway in influenza control is illustrated by the fatal infection that occurs in mice that lack NKp46 [20]. Together with NK cells, neutrophils also contribute to influenza A virus clearance through the secretion of an array of proinflammatory molecules that serve to limit viral replication [21–23]. Finally, alveolar macrophages (AMs) are also present in the innate pulmonary infiltrate, although they form only a small contribution early, they are recruited in large numbers later by the T-cell response. AMs represent the major phagocytic cell type resident in the lung [24], acting to scavenge influenza A virus-derived antigen [25]. In addition, AMs secrete proinflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL-1)-β, IL-6, and interferon (IFN)-α/β [26, 27] together with the chemokines macrophage inflammatory protein (MIP)-1α, monocyte chemotactic protein (MCP)-1, RANTES, and IFN-inducible protein (IP)-10 [21, 26, 28–30]. The magnitude and duration of the potent AM inflammatory response are negatively regulated via CD200R/CD200 [31]. The AM can also modulate adaptive T-cell immunity to influenza A viruses [32]. Present in the lung during active viral replication, AMs are fully susceptible to influenza A virus infection [26]. Unlike

in epithelial cells, however, the infection is nonproductive with little, if any, virion release [26, 33], though it does lead to subsequent apoptosis [33]. Depletion of macrophages during influenza A virus infection results in elevated viral titers and increased morbidity and mortality [21]. In contrast, macrophages can elicit damage to the infected respiratory tissue [34]. Therefore, multiple immune cell types participate in the immediate innate response to influenza A viruses.

The pulmonary infiltrate releases a torrent of innate immune molecules that are considered to limit influenza A virus infection. A long list of cytokines and chemokines are potentially involved. A major player is type I IFN, representing the most potent cytokine attack against the virus [35]. So potent is the IFN response that the influenza A viruses encode a protein (NS2) to disable this pathway (described in Sect. 6). Nasal and pulmonary IFN-α and -β rise rapidly following influenza A virus infection [36] and act to directly limit viral replication and induce further cytokines and/or chemokine secretion that enhances recruitment and activation of multiple immune cell types. Type I IFN serves to enhance macrophage function, promote antigen presentation by antigen-presenting cells (APCs), and modulate adaptive immunity. The importance of this pathway is exemplified by the severe pulmonary disease that develops following influenza A virus infection of mice with disrupted type I IFN signaling [37, 38]. Plasmacytoid DCs are the major producers of type 1 IFN in response to many viruses, including influenza A virus [39–42]. Other cytokines implicated in influenza A virus immunity include TNF-α [43], IL-6 [44, 45], IL-1 [46], IL-18 [47], and IL-12 [48, 49]. In contrast, mice that lack functional IFN-γ can efficiently clear influenza A viruses, suggesting only a minor or redundant role for IFN-γ in the response [50–52]. Chemokines with defined roles in influenza A virus immunity include MIP-1α [53] and CCR5 [54], as illustrated by the elevated disease burden following infection of the chemokine-deficient mice. Finally, while cytokines and chemokines are important in the immune control of influenza A virus infections, their contribution can be detrimental as they elicit potentially fatal "cytokine shock" [55]. Recent studies dramatically illustrate the devastating impact of increased inflammatory infiltrates on viral-induced pathology. In animal models, infection with the reconstructed 1918 influenza A virus promotes massive inflammatory infiltrates with significantly higher levels of cytokines (IFN-γ, TNF-α, IL-1, IL-6, IL-12, IL-18, and granulocyte-colony-stimulating factor) and chemokines (MIP-2, MIP-1α/β, MCP-1) [21, 56–58]. Therefore, particularly early on, potent inflammatory antiviral activity may be dangerous, rather than protective, to the host due to the deleterious impact on lung pathology.

Collectins are collagen-like lectins that participate in innate immunity to viral pathogens [59]. Collectin family members, the surfactant proteins A (SP-A), and SP-D, are constitutively present in the fluids that line the respiratory tract [60]. Together with the mannan-binding lectin (MBL), SP-A and SP-D contribute to influenza A virus clearance via a number of mechanisms. Hemagglutination and viral infectivity are inhibited by SP-A [61, 62], SP-D [61, 63], and MBL [61, 64, 65]. In addition, complement-mediated lysis of influenza A virus-infected cells is enhanced by MBL [66], while SP-A and SP-D promote the binding and uptake of

influenza A viruses by neutrophils [61, 67] and SP-A promotes opsonization and phagocytosis of influenza A virus by the AM population [68]. The sensitivity of different influenza A viral strains to collectin-mediated defense correlates with the degree of glycosylation of the HA glycoprotein [66, 69].

Defensins are cationic peptides produced by both leukocytes and epithelial cells. Defensins can exert direct microbial activity or promote immunity by acting as chemotactic agents. Examples of defensin-mediated anti-influenza A virus activity include retrocyclin-2 (*o*-defensin) and human β defensin 3 inhibition of HA-mediated membrane fusion [70]. The human neutrophil peptide (HNP) 1 (α-defensin) directly inactivates influenza A virus [65, 71].

4 Humoral Immunity and the Influenza A Viruses

Humoral immunity provides host defense through B lymphocyte secretion of antibody. Protective antibodies target antigenic structures exposed on the pathogen surface. Antibody-mediated immunity contributes to defense against the influenza A viruses [72–75] but is not always essential for optimal viral clearance [76, 77]. In any case, the influenza A viruses elicit a diverse spectrum of antiviral antibody responses. Natural antibodies present the first line of antibody-mediated defense [78]. These are low-affinity antibodies that restrict early virus dissemination [78] and promote the recruitment of viral antigen to the secondary lymphoid organs [79]. Natural antibodies reduce the overall load of influenza A virus and, as such, are required for optimal specific IgG antibody responses [75, 80]. Secretion of natural antibodies requires the transcriptional repressor Blimp-1: mice with Blimp-1-deficient B cells are more susceptible to influenza A virus infection [81]. Although natural antibodies are involved in the primary response to influenza A viruses, they are not required for optimal protection from secondary challenge [82]. Furthermore, while natural antibodies clearly display antiviral properties, effective virus clearance requires the induction of neutralizing antibody. Such neutralizing antibodies can be rapidly induced and possess high affinity (or avidity) for viral antigen. Mostly, virus neutralization is thought to be optimally achieved via antibody-mediated interference with viral binding to the host receptors required for cell entry or egress. Consequently, the influenza virus HA is heavily targeted by neutralizing antibodies [83, 84]. Crystallographic examination of HA in complex with neutralizing antibodies shows that antibody binding can occur at the same site as host receptor binding [85] or in distal regions where receptor binding is obstructed by steric hindrance [86]. Anti-HA neutralizing antibodies can also interfere with HA-mediated membrane fusion [87]. Similar to HA, NA is also targeted by neutralizing antibodies [88]. Neutralizing antibodies represent the major target of current influenza A virus vaccine strategies. While most neutralizing antibody strategies target HA or NA [89], the matrix protein 2 (M2) represents an interesting potential vaccine candidate [90]. M2 is a transmembrane protein expressed at the infected cell surface [91], but in contrast to HA and NA, is highly

conserved among influenza A virus strains. Unfortunately thus far, M2-targeted vaccine strategies have elicited only weak immunity that does not protect mice from lethal challenge [92].

$CD4^+$ T-helper cells contribute to humoral immunity by promoting B-cell differentiation into immunoglobulin class-switched, antibody-secreting cells. In most studies, the production of anti-influenza A virus antibody is $CD4^+$ T-cell dependent [74, 93–95], although exceptions are reported [73, 74]. Classically, $CD4^+$ T-cell help involves (1) the recognition of viral antigen and (2) the delivery of an activation signal to the B cell via the TNFR family member, CD40. Mice deficient in CD40 generate significantly impaired influenza A virus-specific antibody responses [93, 96]. Of interest, $CD4^+$ T cells can help B lymphocytes by noncognate interactions that do not require specific influenza A virus antigen recognition [93].

5 T-Cell Immunity and the Influenza A Viruses

5.1 Dendritic Cells

DCs enable pathogen-derived antigens to be presented in a context that facilitates successful T-cell immunity [97]. Specialized in antigen presentation, the DCs facilitate (1) the acquisition of antigen, (2) processing and presentation of antigenic peptides in the context of host major histocompatibility complex (MHC) molecules, and (3) the provision of costimulatory signals. Immunity to influenza A virus infection requires DCs for both primary [98] and secondary T-cell responses [99, 100]. Many DC subsets are involved including the CCR2-dependent "inflammatory" DCs [101, 102], while plasmacytoid DCs are dispensable for influenza A virus clearance [103]. DC can control the magnitude of influenza A virus-specific T-cell immunity via FasL-mediated apoptosis [104]. In the respiratory tract, an extensive network of DC populations is present both in the lung [105] and in the draining lymph node [106]. Furthermore, pulmonary infection recruits additional DC populations into the lung [107–109]. To acquire influenza A virus antigen, DC may simply be directly infected with the virus. Infection induces the maturational changes (upregulation of costimulatory molecules and MHC class II) that are necessary for DC stimulation of T cells [110–112]. Infection can result in the expression of influenza NA at the DC surface, with NA-mediated removal of sialic acids serving to both enhance and inhibit DC function depending on the multiplicity of infection [113, 114]. DCs can also acquire influenza A virus-derived antigen released following the apoptotic lysis of infected respiratory cells [115, 116]. Once antigen is acquired, lung DCs migrate to the lymph node that drains the respiratory tract [107, 117, 118]. Migration occurs early after infection (24–48 h), and then the DCs display a refractory state to further inflammatory stimuli [107]. The lymph node also contains a resident DC set that has no direct access to the airways. Despite

this, these resident DCs can also present influenza A virus-derived antigen [117]. Therefore, antigen transfer between the resident and migratory lung DC subsets must occur [119, 120]. Most experiments indicate that MHC class I presentation of influenza A virus-derived antigen in the lung draining lymph node ceases beyond 12–14 days [121, 122], although recently it has been suggested that antigen presentation can occur for up to 2 months following infection [123]. MHC class II presentation is also reported to persist for as long as 4 weeks after infection [124]. This is surprising given that infectious virus is cleared by day 10 [125]. Therefore, it has been postulated that the respiratory lymph node DCs can serve as a reservoir for antigen, with a depot being maintained well beyond the clearance of pathogen from the infected respiratory tissue [123, 126]. This, however, remains a contentious issue as the presence of an influenza A virus antigen depot was not detected in a separate independent study [127].

5.2 Costimulation

The participation of DCs in adaptive immunity is critical due to the rich array of costimulatory molecules expressed at the cell surface. A growing list of costimulatory molecules has been identified, most of which belong to either CD28/B7 [128] or TNFR [129] families. Costimulation serves to enhance the antigen-specific signals that are delivered through the T-cell receptor (TCR). As such, costimulation is required for optimal T-cell immunity in many viral infections [130]. The major pathway of costimulation is via the CD28/B7 interaction that plays an important role in influenza A virus immunity. This signal contributes to the generation of influenza A virus-specific T-cell immunity at multiple levels. For $CD8^+$ T cells, CD28/B7 contributes to expansion [131–133], cytotoxicity, and/or effector cytokine production [131, 134, 135], recruitment to the infected airways [134], and survival [135]. In contrast, the hierarchy of T-cell response magnitude to individual influenza A virus-derived epitopes (a phenomenon termed immunodominance [136, 137]) is not altered in the absence of CD28/B7 signaling [138]. Mice deficient in CD28/B7 also display impaired influenza-specific neutralizing antibody responses [133]. While CD28/B7 plays a prominent part early in response to influenza A virus infection, 41BB/41BBL is important for sustained $CD8^+$ T-cell expansion and is critical for optimal recall responses [131, 133, 139]. Effective $CD4^+$ T-cell immunity during influenza A virus infection also requires CD28/B7 [133], OX40/OX40L [140], and ICOS/ICOSL [141]-mediated costimulation. The accumulation of T cells in influenza A virus-infected lungs depends on CD27/CD70 signaling [132, 142]. This is due to its impact on T-cell survival and/or migration to the infected respiratory tract [132]. Together, multiple costimulatory signals are delivered via the DCs to promote optimal adaptive immunity and, in turn, influenza A virus elimination.

5.3 $CD8^+$ T Cells

Effector $CD8^+$ T cells, also known as cytolytic T lymphocytes (CTLs), are important in the normal clearance of influenza A viruses [143]. Mice deficient in $CD8^+$ T cells show delayed influenza A virus clearance, though they eventually control infection with all but the most virulent viruses [144]. The influenza A virus-specific $CD8^+$ T-cell response has been extensively characterized utilizing murine models of infection, particularly with the HKx31 (H3N2) and PR/8 (H1N1) influenza A viruses. $CD8^+$ T cells are primed, are activated, and expand in the lung draining lymph nodes during the first week or so after primary infection [121, 145]. Activated $CD8^+$ T cells then traffic to the respiratory airways and the infected lung to mediate viral clearance [146]. The trafficking [147] and retention of $CD8^+$ T cells in the lung [148] are dependent on LFA-1 expression. At the site of infection, $CD8^+$ T cells target virus-infected cells that express peptide derived from influenza A virus protein associated with major histocompatibility complex class I (MHC I). An array of epitopes is recognized in the C57BL/6 (B6) mouse model, with the dominant epitopes (in terms of response magnitude) seen by $CD8^+$ T cells being provided by the viral polymerase A ($PA_{224-233}$) [149] and nucleoprotein ($NP_{366-374}$) [150, 151]. Subdominant epitopes are derived from the basic polymerase subunit 1 ($PB1_{703-711}$) [152], the mitochondrial protein $PB1\text{-}F2_{62-70}$ [152, 153], nonstructural protein 2 ($NS2_{114-121}$) [151], and matrix protein 1 ($M1_{128-135}$) [154]. In the absence of the dominant epitopes, subdominant epitope-specific $CD8^+$ T cells account for a compensatory response, although a slight delay in viral clearance is observed [155, 156]. Depending on the experimental model, 30–90% of $CD8^+$ T cells recovered from the respiratory tract are influenza A virus specific at the peak of the primary response, illustrating their enrichment in the pneumonic lung [137, 151, 152, 157]. Epitope-specific $CD8^+$ T cells can be found widely dispersed throughout various body organs, including the lung, spleen, bone marrow, blood, liver, and nondraining lymph nodes [157, 158]. Once their target antigen is recognized, $CD8^+$ T cells exert multiple effector functions. Cytokines such as IFNγ, TNF-α, and IL-2 are secreted by influenza A virus-specific $CD8^+$ T cells [159]. In addition, $CD8^+$ T cells mediate direct cytolysis of influenza A virus-infected target cells by the exocytosis of cytolytic granules that contain perforin and granzymes [160–163] and/or through the expression of Fas-ligand (FasL) [164–166]. $CD8^+$ T cells also exert regulation of the inflammatory process via the production of IL-10 [167].

Following influenza A virus clearance, virus-specific $CD8^+$ T cells decrease in number until a plateau is reached approximately 2 months following infection [122, 157]. After primary infection, the codominant $D^bNP_{366-374}$ and $D^bPA_{224-233}$-specific $CD8^+$ T-cell populations contract at the same rate [157] to memory pools that are approximately equivalent in number and represent 10% of the population at the peak of the response [168]. Influenza A virus-specific $CD8^+$ T cells persist as a stable population for the life of a laboratory mouse [157, 169, 170]. Retention of memory $CD8^+$ T cells in nonlymphoid tissue, such as the lung, is mediated by T-cell expression of VLA-1 [171]. Secondary challenge recruits the memory $CD8^+$

T cells that expand in the lymph nodes and promote viral clearance approximately 2 days earlier than after primary infection [157]. During secondary infection, the $NP_{366-374}$ $CD8^+$ T-cell population is clearly dominant representing up to 80% of the virus-specific CTL responses [122, 137, 151, 152]. This dominance is maintained in the memory populations that persist following the peak of the secondary response (day 8) [122]. The skewed immunodominance hierarchy observed in secondary versus primary influenza A virus infection was initially thought to be largely a consequence of differential antigen presentation [172], though it is now considered that T-cell precursor frequency and antigen dose are likely to be important determining variables [173].

5.4 $CD4^+$ T Cells

Virus-specific $CD4^+$ T cells are important participants in influenza immunity [174, 175]. Although, acting alone, these cells do not normally eliminate virus [176], they exert distinct roles in both humoral immunity (as discussed) and $CD8^+$ T-cell responses. A vigorous, heterogenous $CD4^+$ T-cell response is elicited following influenza A virus infection [175]. Again, the process of clonal expansion and differentiations is initiated in the lung draining lymph node, with the peak response in the respiratory airways occurring 6–7 days following infection [175]. This is dominated by producers of the Th1 cytokines, such as IL-2, IFN-γ, and TNF-α [177]. $CD4^+$ T cells also secrete IL-10 contributing to the regulation of the inflammatory response [167]. Following influenza A virus clearance, $CD4^+$ T cells demonstrate increased contraction in the respiratory tract compared with influenza A virus-specific $CD8^+$ T cells [178, 179]. A major role for $CD4^+$ T cells is the provision of "help" for optimal $CD8^+$ T-cell immunity. Although $CD4^+$ T cells are not required for primary influenza-specific $CD8^+$ T-cell responses, presumably due to the direct activation of DC by viral infection [180–182], they are critical for the optimal establishment of $CD8^+$ T-cell memory. The absence of $CD4^+$ T cells during primary influenza A virus infections leads to a significant reduction in the size and magnitude of the secondary response and impaired viral clearance [77, 180]. Activation of $CD4^+$ T cells requires antigen-specific signaling via TCR recognition of antigens presented in the context of MHC class II molecules. Until recently, the spectrum of influenza A virus $CD4^+$ T-cell epitopes was much less well characterized than the panel known for the $CD8^+$ subset. Recently however, 20–30 peptides were identified for the influenza-specific $CD4^+$ T-cell response in C57BL/6 mice, with the majority being derived from the NP and HA proteins [183]. There is some evidence that influenza MHC class II epitopes are persisting for a substantial interval after the virus has been cleared from the host [124]. Overall, the adaptive immune response to the influenza A viruses involves complex interactions between a spectrum of functionally different cell types and their secretions.

6 Influenza A Virus Escape

The major influenza A virus escape mechanism rests in the inherent genetic variation of these RNA viruses, combined with the selective pressure exerted by HA-specific neutralizing antibody [184–186]. This process is known as "antigenic drift." Lacking proof reading capacity, the influenza A virus RNA polymerase promotes the accumulation of nucleotide point mutations. Such mutations generate approximately 3.5 amino acid substitutions per year [187]. Circulating viral subtypes are then selected where substitutions have occurred and maintain viral fitness [188] but abrogate immune recognition. For example, virus escape mutants are poorly recognized by neutralizing antibody due to (1) introduced steric interference with antibody binding [85], (2) virus conformational changes that render antibody binding energetically unfavorable [86], or (3) the introduction of new oligosaccharide attachment sites to surface glycoproteins that obscure antibody binding [189, 190]. Retention of amino acid substitutions at the HA membrane distal surface, an area targeted by antibodies, is favored over those buried within the protein [83]. Virus-specific CTL immunity can also be targeted by antigenic drift [191]. Here, viruses are selected with mutations that interfere with epitope binding to MHC class I or with epitopes that are no longer recognized by the TCR. Both $NP_{388-391}$ [192, 193] and $NP_{418-426}$ [194, 195] CTL peptides have shown evidence of antigenic drift. Hypervariability within a CTL epitope correlates with the functional avidity of the TCR [196]. Such antigenic drift can function to limit cross-protective immunity against multiple influenza A virus strains and, as a consequence, contribute to seasonal epidemics.

While antigenic drift represents a subtle mode of immune escape, influenza A viruses can also undergo major antigenic variation to outmaneuver the immune system. This takes place by "antigenic shift," where infection of the same cell with two distinct influenza A virus strains allows reassortment of the viral genomic segments, generating a new hybrid influenza A virus. Reassortment can occur following infection with different species-adapted viruses. For example, pigs can be infected with both human and avian influenza A viruses. Simultaneous infection may thereby generate a reassortment virus where the "human" pathogen acquires an "avian" virus HA or NA gene. In this case, for the HA and NA in particular, there would be no prevailing immunity in the human population, leading to the possibility of a human pandemic [197, 198]. Such antigenic shift involving avian and human strains has been implicated in two of the influenza A virus pandemics that have occurred in the twentieth century; the 1957 H2N2 [199, 200] and 1968 H3N2 [187, 200] infections. Of interest, the influenza A virus that provoked the 1918 pandemic did not arise through antigenic shift. Instead the 1918 H1N1 virus, which was responsible for millions of deaths worldwide, is believed to be an entirely avian viral strain that mutated in a way that allowed it to infect humans [201, 202].

The nonstructural protein 1 (NS1) encoded by influenza A virus provides a mode of immune escape that does not require manipulation of the genome. NS1 inhibits the host cell IFNα/β response [203, 204], a major pathway of immune defense

against the virus (as discussed). Type 1 IFN induction is antagonized by NS1-mediated suppression of IFN-induced proteins dsRNA-activated protein kinase, 2′–5′-oligo (A) synthetase [205–207], the transcription factors NFκB [208], and the IFN regulatory factor-3 [209]. Containing an RNA-binding domain at its N-terminus [208], it was previously considered that NS1 sequestered influenza A virus dsRNA [210]. Instead, NS1 forms a complex with RIG-1, the cellular sensor of influenza A virus uncapped ssRNA [6]. Therefore, NS1 acts to disable the host mechanism for detection of viral-derived RNA and the induction of the IFN response. Influenza A viruses lacking the NS1 protein are good vaccine candidates as the absence of this immunomodulatory protein greatly enhances the immunogenicity of the virus [211].

7 Heterotypic Influenza A Virus Immunity

Heterotypic immunity in this system is defined by cross-reactive, protective responses between serologically different (HA-distinct) influenza A viruses. It would obviously be advantageous if, for example, prior infection with a human influenza A virus could generate immune memory that provides at least some resistance to a highly pathogenic avian virus that suddenly adapted to transmit between people [212, 213]. Clearly, promoting heterotypic immunity is a desirable strategy for influenza A virus vaccine development. Described many decades ago [214], heterotypic immunity has now been shown for many influenza A virus combinations [215–218]. At least in mice, heterotypic immunity can both be long lasting and provide protection against otherwise lethal virus challenge. The best understood component of such responses is CTL immunity directed at generally conserved, internal viral proteins [215, 217, 218]. However, there is also evidence for the retention of a measure of heterotypic immunity in mice lacking $CD8^+$ T cells [216, 219]. In addition to the $CD8^+$ T effectors, $CD4^+$ T cells, nonneutralizing IgA antibody, NKT cells, and γδ T cells have all been considered as possible players [217]. Immunization with a low dose of a cold-adapted, attenuated influenza A virus provides one vaccination strategy that has the potential to induce at least some degree of long-term, heterotypic immunity [220]. The promotion of such responses is clearly a worthwhile focus for future vaccination strategies.

8 Influenza A Virus Immunity and Vaccination

Ultimately, studies of the immune response to influenza A virus aim to provide the foundation for strategies that will combat influenza-mediated disease. Vaccination is the major weapon to enable reduced morbidity, mortality, and economic damage associated with widespread influenza A virus infection. The 2009 HINI pandemic highlights the urgency of developing safe and effective vaccines to emerging

influenza A virus strains. H5N1 avian influenza A virus is another immediate concern. H5N1 is a highly pathogenic virus that possesses the capacity to provoke a debilitating pandemic of greater severity than that of H1NI. As such, much effort has been employed to design a suitable H5N1 vaccine. Eliciting high titer neutralizing antibody is a major priority of any vaccination strategy, although cell-mediated immunity is also considered important. Cell-mediated immunity is powerful in that it has the potential to provide universal protection against divergent viral strains [221, 222]. Many vaccine formulations have been tested to date, but the most widely utilized platform is the inactivated, attenuated H5N1 virus (whole virion, subvirion, or surface antigen). Studies indicate that two doses of this vaccine, together with an adjuvant such as MF59, elicit cross-protective immunogenic responses in healthy subjects [223–225]. Mechanisms underlying protection include the expansion of antigen-specific $CD4^+$ T cells, which serves as a reliable correlate of vaccine protection [226]. H5N1 vaccination studies provide valuable lessons that are currently being harnessed for a swift and rapid response to the 2009 HINI pandemic.

9 Conclusion

The influenza A viruses pose intriguing challenges for vaccine design [227]. Moving beyond the currently available products will depend on exploiting our understanding of immune defense mechanisms against this important and potentially very dangerous group of human pathogens. Here, we have briefly summarized a current view of how these viruses are controlled by elements of both innate and adaptive host response, together with the escape strategies that influenza A viruses exploit to survive in nature and to maintain transmission at the species level. An ideal vaccine could be thought to induce high levels of neutralizing antibody and CTL memory. This might optimally be achieved by promoting more effective DC vaccination, perhaps via the pathway of driving the innate response in ways that enhance T-cell immunity. An important caveat is, though, that much of our understanding of (particularly) the innate and T-cell responses to the influenza A viruses is based on mouse experiments. As we go forward to develop vaccine candidates, it is important that the analysis of influenza virus cell-mediated immunity, in particular, should be greatly extended in human subjects.

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