nature immunology

REVIEW

Influenza and the challenge for immunology

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The continued westward dissemination of H5N1 influenza A viruses in avian populations and the nearly 50% mortality rate of humans infected with H5N1 are a source of great international concern. A mutant H5N1 virus with the capability to spread rapidly between humans could cause a global catastrophe. Governments have reacted by developing national response plans, stockpiling antiviral drugs and speeding up the development and approval of vaccines. Here we summarize what is known about the interaction between influenza A viruses and the mammalian host response, specifically emphasizing issues that might be of interest to the broader immunology community.

Humanity first understood the potential danger of influenza in 1918–1919 (ref. 1), when a disease that seemed to come out of nowhere killed over 40 million people around the globe (**Fig. 1**), many more than died in World War I. The highly contagious nature of the resulting lethal pneumonia was obvious, but the first human influenza A virus[2] was not isolated until 1933 (in ferrets). The discovery that influenza viruses grow in embryonated hen eggs (1940) greatly facilitated research, and the finding that they agglutinate red blood cells (1941) led to a simple inhibition assay that was used to track the antibody 'footprints' of the 1918–1919 pandemic. Very recently, PCR-mediated amplification of paraffin-embedded material and cadaveric lung tissue from a victim buried in the Alaskan permafrost has allowed re-creation of what is believed to be a reasonable replica of the 1918 virus[3–5].

New influenza A viruses caused human pandemics in 1957 and 1968 (ref. 6), although there is serological evidence that something like the 1957 strain may have been circulating in 1888, and regular epidemics result from antigenically 'drifted' influenza strains (**Figs. 1 and 2**). The Geneva-based World Health Organization influenza surveillance network was established 1952; it links four collaborating centers (London, Tokyo, Melbourne and Atlanta) and 112 national laboratories in 83 countries. This effective program constantly monitors the emergence of new influenza strains, is coordinating the global effort to fight H5N1 influenza viruses and was important in the rapid resolution of the 2003 outbreak of sudden acute respiratory syndrome[7].

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Received 17 January; accepted 22 February; published online 18 April 2006; doi:10.1038/ni1343

Basic pathogenesis and evolutionary strategies

All successful viruses evolve strategies to ensure their survival in nature, which in turn determine the pathogenesis and outcome of the associated infectious process. In mammals, replication-competent influenza A viruses are generally recovered only from the superficial epithelium of the respiratory tract. This reflects the anatomical distribution of trypsin-like proteases that cleave the viral hemagglutinin (HA or H (subtype)), an essential step for making new, replicating virus during the infectious process[8]. In contrast, cleavage of the H5 HA tends to be trypsin independent, which may contribute to the tendency of H5N1 viruses to localize to the brain[4].

Influenza can be controlled either by $CD8^+$ T cells or by antibodies, but mice lacking both $CD8^+$ T cells and antibodies succumb[9,10]. Influenza viruses are not natural mouse pathogens, and viral spread between mice is minimal, despite the fact that substantial lung viral titers are achieved within 24 h of infection. Influenza infection in the ferret more closely resembles human influenza infection, but the ferret model has received little attention from immunologists, probably because of the lack of reagents and inbred ferret strains.

Influenza viruses grow rapidly in the human respiratory mucosa, allowing transmission to colleagues and family members via respiratory droplet inhalation (coughs and sneezes) even before the development of obvious symptoms. The net consequence is that influenza outbreaks tend to be 'explosive', moving rapidly through a community, then dying out. Any given influenza A virus variant, however, can be sustained globally for at least 1–2 years in geographically dispersed communities.

The survival of influenza A viruses in human populations is facilitated by poor fidelity of RNA transcription, which allows antibody-mediated selection known as 'antigenic drift' (**Fig. 2**). As a consequence, sequential variants of the same virus circulate for decades[6]. The main mutation target is the surface HA glycoprotein[11]. Such 'drifted' HA genes are readily generated by culture in the presence of HA-specific monoclonal antibodies[2]. The gene encoding neuraminidase (NA or N (subtype)), another viral surface protein, is also subject to mutation.

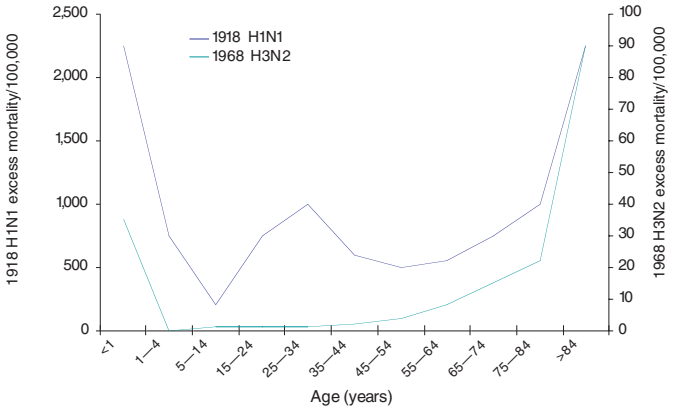

Figure 1 Age-related susceptibility profiles for the 1918–1919 H1N1 and 1968 H3N2 pandemics.

Even so, these 'drifted' viruses emerge in nature less often than might be expected. Influenza-specific $CD8^+$ T cells target more conserved influenza proteins, such as the viral nucleoprotein (NP), and generally minimize the transmission of new HA or NA variants by eliminating infected cells[10]. However, occasional mutations are found in the genes encoding NP peptides recognized by human $CD8^+$ T cells[12].

Although 3 HA proteins and 2 NA proteins have dominated human influenza infections over the past 100 years, 16 different influenza A virus HA types and 9 distinct NA types are now circulating in nature, infecting species as diverse as ducks, pigs, leopards, seals and even whales[2]. This raises the possibility that a new influenza A virus may enter the human population at any time as a consequence of the reassortment process known as 'antigenic shift' (**Fig. 2**). The influenza A virus genome is organized into eight separate segments[2]. The emergence of human H2N2 infections in 1957 is though to have occurred after a human H1N1 and an avian H2N2 virus coinfected the same (presumably mammalian) host. One 'shifted' progeny virus expressed the HA, NA and basic polymerase 1 (PB1) genes from the avian virus and the other five genes from the human virus. Birds, particularly ducks, constitute the greatest risk, as they contain an enormous diversity of viruses, many of which cause protracted, asymptomatic infection of the gastrointestinal tract. Apart from the H5N1 viruses, H9N2 and extremely virulent H7N7 viruses are also prevalent in domestic poultry and cause occasional human disease[13,14]. Antigenic shift is not a problem with related influenza B viruses that, although they can 'drift', are mainly human pathogens. Furthermore, it seems that simple mutation without prior reassortment can allow influenza A viruses to spread to new species (**Fig. 2**).

An H5N1 virus, for example, has been shown to cause systemic disease and transmit horizontally in cats[15]. Although the 1918 virus seems to be an exception, a chief reason for the failure of avian viruses to spread horizontally in humans is that 'human' (and pig) HA proteins bind the α(2-6) variant of sialic acid, whereas the 'avian' HA proteins 'prefer' the α(2-3) form[16]. Adaptation from the 'avian' to the 'human' recognition pattern can happen very rapidly, however, as the virus disseminates and evolves[16]. The H5N1 viruses have been mutating to greater lethality in wild bird populations[17]. Could such random changes contribute to the generation of a variant capable of spreading among humans?

Innate response and cytokine shock

Type 1 interferons have a chief protective function in the early stages of influenza virus infection. Interferons induce expression of '*Mx*' genes, which inhibit primary transcription of, at a minimum, the viral PB2 polymerase. $Mx1^{-/-}$ mice are more susceptible to influenza[18,19]. In contrast, the viral '*NS*' gene encodes a type 1 interferon antagonist that promotes virus growth[20–23]. Although interferon-γ (IFN-γ) is produced by peptide-stimulated, influenza-specific $CD4^+$ and $CD8^+$ T cells, a chief function for this cytokine in influenza control has not been demonstrated. Similarly, no involvement of IFN-γ-producing natural killer cells has been identified. However, some evidence indicates that α-galactosylceramide-stimulated natural killer T cells may be of some benefit[24].

An early influx of neutrophils into the infected lung, along with a concurrent increase in the number of alveolar macrophages, may be protective during the initial stage of infection with 'mild' virus strains but can be detrimental during infection with the virulent 're-created' 1918 virus[25]. The same correlation applies to the various chemokines and cytokines produced early in the course of influenza infection. In fact, it seems that a 'cytokine storm'[26] contributes to the bronchiolitis and alveolar edema characteristic of severe influenza pneumonia, with at least some of these secreted factors being made by virus-infected lung epithelium[23,25]. That may partly explain the rapid deaths in young, otherwise healthy adults during the 1918–1919 pandemic (**Fig. 1**) and is also a principal factor influencing the course of human H5N1 infection[26].

Antigen presentation for adaptive immunity

There is no detectable viremia and little evidence of virus replication in lymphoid tissue. The main source of antigen is thought to be dendritic cells (DCs) that exit the respiratory tract during the first 36 h after virus challenge and travel either via afferent lymph to the regional cervical and mediastinal lymph nodes or via the blood to the spleen[27–30]. At least some of these DCs will be nonproductively infected. How much of the antigen-specific response is generated by direct exposure to migrated DCs or by cross-presentation on resident lymphoid tissue DCs is not clear[31,32]. What is known, however, is that when DCs, B cells and macrophages are recovered from recently infected mice, only the $CD8\alpha^+$ DCs are able to stimulate influenza peptide–specific T cells or hybridoma cell lines[33,34].

Analysis of the $CD8^+$ T cell response has indicated that all antigen-presenting cells are eliminated within 10–14 d of primary virus infection[35]. However, experiments using T cell receptor (TCR)–transgenic $CD4^+$ T cells have suggested that antigen-presenting cells capable of stimulating influenza-specific $CD4^+$ T cells, albeit with lower efficiency, are still present 3 weeks after initial viral exposure[36]. No evidence indicates that either viral RNA or antigen persists for longer periods.

Effector mechanisms and protective immunity

Influenza A viruses are eliminated[9], although generally with some delay, from the lungs of $CD8^+$ T cell–deficient (β_2-microglobulin-deficient) mice, $CD4^+$ T cell–deficient (major histocompatibility complex class II–deficient) mice and immunoglobulin-deficient (μMT) mice. Mice with severe combined immunodeficiency do not survive influenza infection, but such mice receiving intraperitoneal injection of a substantial dose (200 μg) of an HA-specific neutralizing monoclonal antibody as late as 24 h after virus challenge survive infection[11]. Some poorly neutralizing monoclonal antibodies provide a good measure of control via, at least in one instance, a complement C1q–dependent process[11,37]. Monoclonal antibodies specific for NA and matrix protein 2 (M2) proteins can also reduce virus titers[38]. Complement is not essential for the protection conferred by high-quality neutralizing monoclonal antibodies, however, as Fab fragments also work when infused directly into an infected lung[39].

Influenza-specific $CD4^+$ T cells function mainly to promote high-quality antibody responses (**Fig. 3**) but have not been shown to operate directly as chief effectors of virus control[40]. IFN-γ enzyme-linked

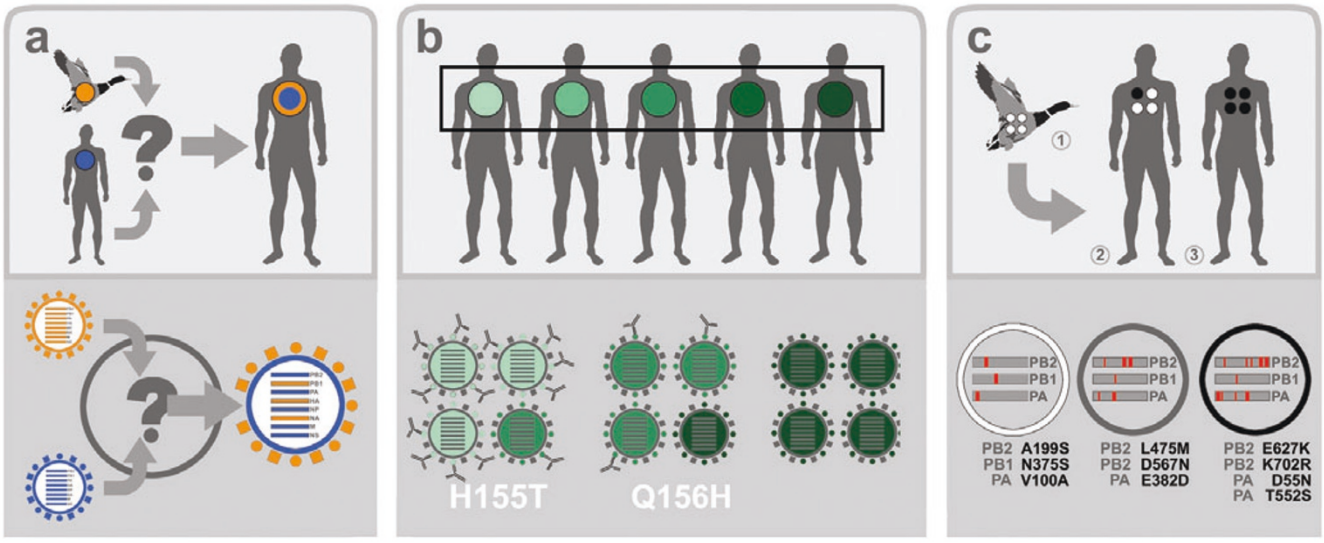

Figure 2 Mechanisms of influenza A virus variation. (**a**) Antigenic shift, representing what is thought to have occurred in the 1957 pandemic, in which an H1N1 virus reassorted with an H2N2 virus. (**b**) Antigenic drift, the process by which circulating viruses in the human population mutate under pressure from antibody to HA. (**c**) Host adaptation. Analysis of the reconstructed 1918 H1N1 virus indicates that it is of avian origin and that the 'jump' into humans was associated with alterations in the viral HA and the polymerase complex (PA, PB1 and PB2). The polymerase substitutions (bottom) show changes from the typical avian profile to residues more commonly associated with human viruses.

immunospot and intracellular cytokine staining assays have suggested that influenza-specific CD4$^+$ T cell clonal proliferation is both smaller in magnitude and more diverse than concomitant CD8$^+$ T cell responses[41,42]. However, lifelong influenza-specific CD4$^+$ T cell memory is maintained in mouse models, and the presence of previously primed CD4$^+$ T cells is thought to promote a more effective antibody response.

Evidence suggests that although cytokine responses may contribute, CD8$^+$ effector T cells[43] eliminate influenza virus–infected lung cells mainly by CD69 (Fas)–mediated or perforin-and-granzyme-mediated cytotoxicity[44]. Individual epitope-specific CD8$^+$ T cells show considerable diversity in their granzyme expression profiles[45], and perforin is not essential in the presence of an intact Fas-FasL pathway. T cell numbers are controlled by a Fas-mediated editing process evidently operating in the regional lymph nodes of mice infected with virulent influenza A viruses[46], whereas tumor necrosis factor contributes to the elimination of high-avidity effector T cells located in the pneumonic lung[47].

Pre-existing antibody provides the sole mechanism for immediate, sterilizing immunity to influenza virus challenge[11]. Both locally produced immunoglobulin A (IgA) and systemic IgG function to neutralize virus growth and, even if there is only partial cross-reactivity, can exert a good measure of early control[48,49]. Virus-specific IgG can persist in serum for years, and murine studies suggest that persistent IgG is produced by long-lived plasma cells in the bone marrow. Plasma cell responses are optimally generated by virus infection and may be less durable when nonreplicating antigens are used for priming[50].

H1N1-primed mice with relatively modest numbers of influenza virus–specific memory CD8$^+$ T cells (less than 1% of total CD8$^+$ cells) clear intranasal H3N2 infection 1–2 d earlier than do naive mice (**Fig. 3**). At least a proportion of these CD8$^+$ T cells proliferate locally in the virus-infected lung[51]. Furthermore, mice primed with H1N1 and boosted with H3N2 have large numbers of cross-reactive CD8$^+$ memory T cells, demonstrate only transient weight loss and survive respiratory exposure to extremely virulent H5N1 and H7N7 viruses[10,52,53]. Comparable CD8$^+$ T cell responses have been noted in chickens[54]. The fact that mutations in genes encoding NP peptides recognized by human CD8$^+$ T cells allow viral 'escape'[12,55] emphasizes the idea that CD8$^+$ T cells can potentially be involved in controlling influenza in humans.

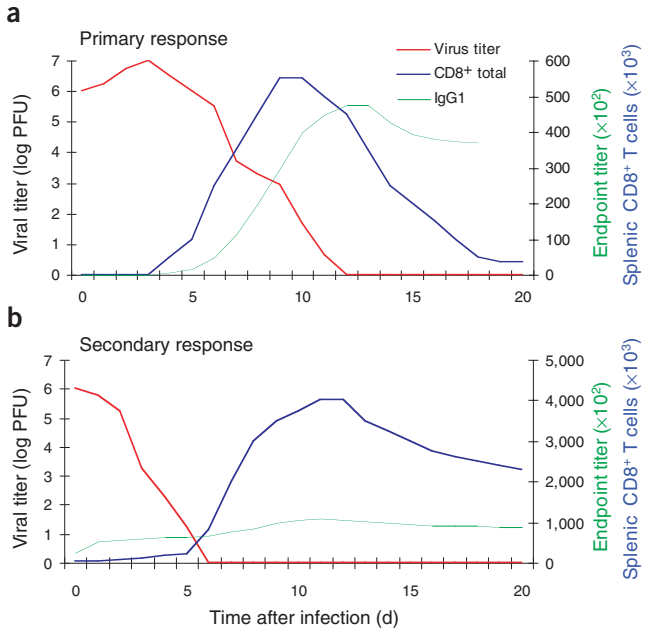

Figure 3 Characteristics of primary and secondary anti-influenza CD8$^+$ T cell and serum IgG responses. Both antibody and CD8$^+$ T cell responses contribute to viral clearance in primary (**a**) and secondary (**b**) infections. Secondary infections are cleared several days earlier as a result of cross-reactive T cell responses. PFU, plaque-forming units.

CD8$^+$ T cell immunodominance hierarchies

The availability of peptide–major histocompatibility complex class I tetramers and the development of intracellular cytokine staining assays has allowed investigation of response characteristics of

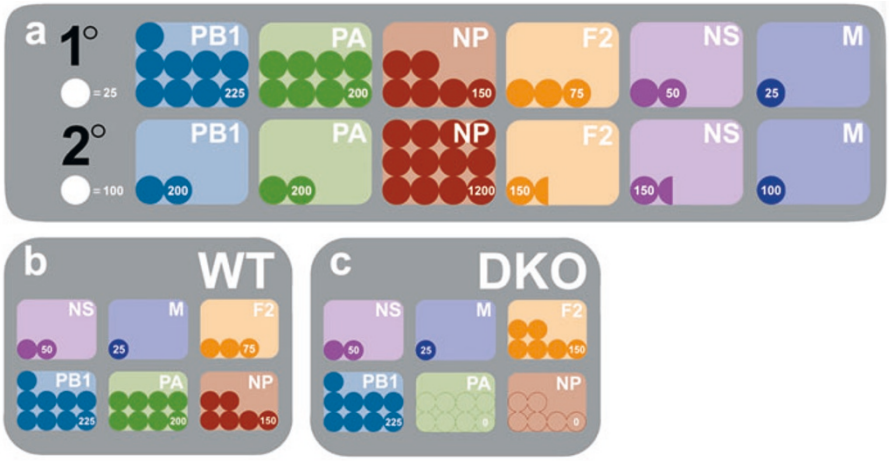

Figure 4 Immunodominance hierarchies of influenza epitope–specific CD8+ T cells. Filled circles represent 2.5×10^4 ('25') or 1×10^5 ('100') spleen cells in the primary (1°) and secondary (2°) responses, respectively. (**a**) Typical CD8+ T cell epitope immunodominance hierarchy in influenza-infected H-2^b mice (**b**,**c**) Disruption of the prominent NP-specific responses (**b**) and PA-specific responses (**c**) allows proliferation of less-prominent PB1-F2-specific CD8+ T cells after primary and secondary virus challenge. WT, native, wild-type virus; DKO, double knockout of NP and PA.

influenza-specific CD8+ T cells (**Fig. 3**). Much of this analysis has used C57BL/6 mice[56–58], whose CD8+ T cells proliferate in response to at least six viral epitopes (**Fig. 4**). Most investigation has focused on CD8+ T cells specific for complexes of H-2D^b and the peptides consisting of NP amino acids 336–374 (NP(336–374)), or acid polymerase (PA) amino acids 224–236 (PA(224–236)). CD8+ T cells specific for both epitopes are prominent during the primary response, but after secondary challenge, the NP(336–374)-specific CD8+ T cells can be more than ten times 'overdominant'[56,59] (**Fig. 4**).

Disruption[60] of the prominent wild-type H-2D^b–NP(336–374)–specific and H-2D^b–PA(224–236)–specific responses (**Fig. 4**) allows expansion of the set specific for a complex of H-2D^b and a peptide of the alternative frame product of PB1 (PB1-F2(62–70))[58] after primary and secondary virus challenge. Even so, that fails to compensate totally for loss of the main epitopes, suggesting that there are inherent size limits to individual virus-specific CD8+ T cell responses. As for protective immunity, experiments using genetically disrupted viruses[58,60] have indicated that the PA(224–236)-specific response that is targeted mainly to the DCs can be beneficial[33], whereas vaccination studies have suggested the contrary[61,62].

Reverse genetics has also been used to disrupt both peptides (NP(336–374) and PA(224–236)) in their native configuration[63], followed by equivalent re-expression by insertion of the nucleic acid sequence in the viral '*NA*' gene (in the stalk region). Normally, much more NP than PA is made, and the structurally 'flatter' H-2D^b–NP(336–374) epitope is recognized by a substantially less diverse and more 'public' (shared by most mice) TCR repertoire than is the 'protruding' H-2D^b–PA(224–236) epitope[64]. Although the more numerous 'private', individualized TCRs specific for H-2D^b–PA(224–236)[65] are detected earlier than the less prevalent public TCRs specific for H-2D^b–NP(336–374)[66], the greater concentrations of NP(336–374) apparently allow the emergence of equivalent numbers of memory CD8+ T cells specific for each epitope. After secondary viral exposure, the larger amounts of NP(336–374) generate a much larger recall response. The size of this response is greatly reduced when the challenging virus expresses NP(336–374) in the NA protein, which is expressed in much lower quantities than is the NP protein. Therefore, this particular immunodominance hierarchy may be a direct consequence of T cell precursor frequency and antigen dose[63]. However, that simplistic dose-response model is clearly not the only mechanism regulating CD8+ T cell immunodominance hierarchies (**Fig. 4**), as proteasomes and other antigen-processing pathways are involved[67].

The correlation between tetramer and intracellular cytokine staining profiles indicates that every influenza epitope–specific CD8+ T cell is capable of producing IFN-γ, a substantial proportion make both IFN-γ and tumor necrosis factor, and a minority produce IFN-γ, tumor necrosis factor and interleukin 2. Intense tetramer staining and a diverse cytokine profile are considered to indicate high-avidity TCR–peptide–major histocompatibility complex class I interactions[68]. At least among the diverse H-2D^b–PA(224–236)–specific CD8+ TCR repertoire, the TCRs with highest avidity (identified by tetramer dilution) undergo the largest clonal proliferation[69].

Characteristics of CD8+ T cell memory

The massive clonal proliferation[35,65,66] after secondary challenge (**Fig. 3**) results in the distribution of memory CD8+ T cells to both lymphoid and nonlymphoid tissues[64]. These sites include the lung, which obviously supports virus growth, and liver and bone marrow, which do not. The retention of these T cells in nonlymphoid tissues depends on expression of the integrin VLA-1 (ref. 70). After secondary viral infection, further proliferation of virus-specific CD8+ T cells can occur in the regional lymph nodes and spleen and in the bronchus-associated lymphoid tissue of the respiratory tract[71]. Control of the infectious process is never immediate, and even when many effector memory CD8+ T cells are present in the lung, viral titers remain high for about 48 h after virus challenge[53].

The most abundant CD62L^{hi} and CD62L^{lo} memory CD8+ T cell subsets are found in the spleen[41,72]. Large clonal proliferations occurring after primary infection are preserved indefinitely in the memory CD8+ T cell pool. The overall impression is of great stability. That is in accord with earlier indications that once the antigen-driven phase of the response is over, both large and small CD8+ T cell populations contract at equivalent rates. However, although a substantial proportion of the memory CD8+ T cell pool may comprise those clones that dominated primary responses, those clones do not necessarily dominate secondary responses. The selective process seems to be essentially stochastic, with minority (or previously undetected) populations often becoming dominant[65]. Although concurrent primary and secondary responses may occur[73], many memory CD8+ T cells are present at frequencies low enough to render them undetectable in the blood of individual mice sampled repeatedly over a long interval.

Therapeutics and vaccines

X-ray crystallographic analysis of the structure of monoclonal antibody–NA complexes has helped the development of the 'rationally designed' therapeutics zanamivir (Relenza) and oseltamivir (Tamiflu)[74,75]. Both influenza A and influenza B viruses find it difficult to 'escape' by altering the NA site targeted by these drugs. However, escape variants can emerge during severe H5N1 infection[76]. These drugs are being stockpiled by national governments, but the real need is for an effective vaccine.

Flu vaccines are designed by the World Health Organization surveillance network. Each year, members meet well before the Northern and Southern Hemisphere winter 'flu seasons' to decide which strains will be included in the trivalent vaccines produced by commercial suppliers. Those are predictions, not certainties, and their best efforts are sometimes defeated by mutation[77]. In Western nations, influenza is grown *in ovo*. Although *in ovo* growth requires specialized facilities, eggs are still the best source of high-titer influenza virus. It is also feasible to produce vaccines using cell culture–based systems[78], but such vaccines have yet to be approved for wide use in humans.

Russia has long used live attenuated ('cold-adapted') influenza vaccines[79], and a similar product (Flumist) has been approved for use in the US. One advantage of live virus vaccines is their ability to stimulate $CD8^+$ T cell memory. In addition, because these vaccines replicate in the host, lower doses are effective. The disadvantage of live virus vaccines is that pre-existing, cross-reactive antibody is more likely to diminish the value of a live vaccine given by the respiratory route. An initial concern with live influenza vaccines was that they might elicit asthma attacks[80], but studies have indicated that these products are generally safe[81].

The 'Holy Grail' influenza vaccine would target a 'universal' influenza antigen that is essential for virus function. One possible target is the relatively conserved M2 homotetramer that serves as a pH-induced proton channel on the surface of all influenza A viruses[82,83]. M2 is present in low abundance (400 HA/100 NA/10 M2) and does not project far from the surface of the virion. However, some degree of resistance to virus challenge is achieved by priming with an M2 ectodomain peptide in adjuvant[38].

Various other vaccine strategies have been tested experimentally. Both heat-inactivated[84] and β-propiolactone-inactivated whole influenza virus can induce cross-reactive $CD8^+$ T cell responses specific for epitopes derived from the more conserved, internal virus proteins. Perhaps vaccination only against the surface viral HA and NA proteins, which rarely produce peptides capable of stimulating $CD8^+$ T cells, is too 'clean'. In addition, because it is possible to insert up to 45 amino acids into the stalk of the NA protein, fully infectious influenza A viruses have been used experimentally to generate $CD8^+$ and $CD4^+$ memory T cells specific for other viruses[85,86]. That may be of some value if the use of live attenuated influenza vaccines becomes more widespread. Various DNA-priming strategies[87] have also been explored experimentally, but none has been developed for human use. Another approach is to use immune-stimulating complexes made from recombinant influenza virus proteins[88]. The standard alum adjuvant is now being tested with candidate H5N1 vaccines, mainly because the HA of this virus is not a 'strong' antigen[89], either because of heavy glycosylation or as a result of the structure of the HA globular head.

Another difficulty encountered during H5N1 vaccine development efforts is that these viruses are so virulent that they kill the eggs in which they are grown. The application of reverse-genetics technology[90] to engineer the 'avian' HA and NA proteins into one of the standard influenza A virus vaccine strains has now overcome that problem[91]. This strategy will probably allow the rapid production of seed stocks for new inactivated vaccines, but the fact that a genetically modified organism will be used for the first time means that the initial constructs are now progressing through phase I–III clinical trials before they can be licensed for human use in some countries.

There have been some indications that the extent of antigenic drift for the H5N1 HA glycoprotein in birds may be sufficiently limited that vaccine produced against an earlier variant can elicit a measure of immunity to a later strain[92]. Unlike the situation with a persistent infection (such as with human immunodeficiency virus), partial control that limits the severity of lung damage (and the possibility of transmission) will allow early virus clearance and host survival. Stockpiling of a monovalent H5N1 vaccine specific for an HA type now circulating may be justified, even if it is only modestly protective. Yet, the present annual global production capacity is less than 0.5×10^9 doses of trivalent vaccine for a world of 6.5×10^9 people[93].

Insights and opportunities

Over the years, studies of influenza A virus immunity contributed to the idea[11] of 'original antigenic sin' (coined by Thomas Francis, Jr., in 1953), to the antibody-NA structures that led to the design of Relenza and Tamiflu[74], to the initial evidence (after major histocompatibility complex restriction) that virus-specific $CD8^+$ T cells and neutralizing antibodies have very different specificity profiles[94,95], and to the consequent discovery that $CD8^+$ T cells recognize processed peptides associated with major histocompatibility complex class I glycoproteins[96]. The last finding sparked the field of antigen processing, which is important in viral and tumor immunity. In addition, the recognition that 'cytokine shock' is a chief factor influencing influenza lung pathology[26] has emphasized potential therapeutic targets for treating severe acute viral pneumonia.

The fact that reasonably good influenza virus vaccines have long been available contributes to a general lack of interest from the immunology community. After all, how many successful vaccines have been made by professional immunologists? However, real challenges of interest to immunologists exist in this field. The adoption of reverse-genetics strategies will allow relatively simple insertion of small genetic elements (encoding fewer than 45 amino acids) into influenza viruses[85,90]. Perhaps that technique will allow the investigation of new strategies[97] that enhance the antigenicity of these viruses and/or help to minimize the effect of antigenic drift. In addition, improved adjuvants[89] and increased understanding of innate immunity[98] are essential to the development of better priming regimes. The simplest way to increase the global production of inactivated vaccines would require a smaller antigen dose. More research is also needed to improve influenza vaccine efficacy in the elderly, a population that is particularly at risk from this disease[99,100].

Experimentally, the mouse model that is so familiar to immunologists provides a well characterized system for analyzing acute and memory responses in this localized, nonpersistent infection. A further advantage for immunologists is that the standard laboratory viruses are safe for humans and do not spread in animal facilities. The active involvement of immunologists with diverse expertise and insights has the potential to improve the capacity to deal effectively with this very dangerous family of pathogens.

ACKNOWLEDGMENTS

Supported by the National Institutes of Health (AI29579 to P.C.D.; AI95357 to R.G.W.; and AI065097 to P.G.T.); the Australian National Health and Medical Research Council (P.C.D. and S.J.T.); Science, Technology, Innovation funds from the State of Victoria, Australia; and private donations to American Lebanese Syrian Associated Charities at St. Jude Children's Research Hospital.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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