Determinants of immunity to influenza

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Neutralising antibody is by far the best protection against influenza virus but, in its absence, do we have any other types of immune counter-measures that can be heightened or exploited to protect us against newly emerging and potentially highly virulent influenza strains?

Virus neutralising antibody is predominantly directed against the major surface glycoprotein of the virus, the haemagglutinin (HA) [1]. The major mechanism of neutralisation is by the binding of antibody to specific sites on the HA surface that surround the receptor-binding pocket, thereby sterically inhibiting the interaction of HA with its sialic acid-containing receptor on the host cell. A second mechanism is by inhibiting the fusion event; the virus enters the cell by endocytosis and the genome escapes the endosome by a process dependent on a low pH-mediated conformational change in the HA required for the viral and endosomal membranes to fuse, reposition and create an exit portal for the viral ribonucleoproteins. Antibodies have been identified that cross-link monomers of the trimeric HA, effectively preventing the conformational change from taking place and aborting endosome escape [2].

The second surface glycoprotein, the neuraminidase (NA), also binds to sialic acid and has a role in removing it from receptors on the host cell from which the virus is exiting by a budding out process. It also removes sialic acids from the carbohydrate side chains attached to HA and NA on nascent virions. Without the action of the NA, virions remain clumped together at the surface of the cell, reducing the efficiency of the virus to infect more cells. Antibodies bound to certain regions of NA can sterically hinder adjacent HA from attaching to its receptor, and others can block the enzyme active site of NA to prevent efficient release of the virus.

Compared to the activity of anti-HA antibodies, anti-NA antibodies are far less potent [3] but history hints at some benefit in individuals having NA-specific antibody on encounter with a newly emerging virus bearing a new HA but retaining the NA. Antibody to the third virion surface protein, M2, is not consistently detected in human convalescent sera [4] and its importance is unclear. Like the HA and NA, the M2 protein is placed in the membrane of infected cells and becomes a target for antibody-dependent cellular cytotoxicity.

Influenza virus is highly immunogenic and antibodies raised in response to infection last for many decades [5]. However, their effectiveness lasts only for a few years because the virus can rapidly evolve to escape antibody-mediated neutralisation. The influenza polymerase complex, which replicates the RNA genome of the virus, does not operate with high fidelity and is constantly inserting base changes to create mutant viruses with single amino acid substitutions. While most of these mutants will be non-viable, a subset with mutations in the regions surrounding the highly conserved receptor-binding pockets of HA and NA, which function largely as scaffold, may remain viable and are selected for under the pressure of pre-existing antibody.

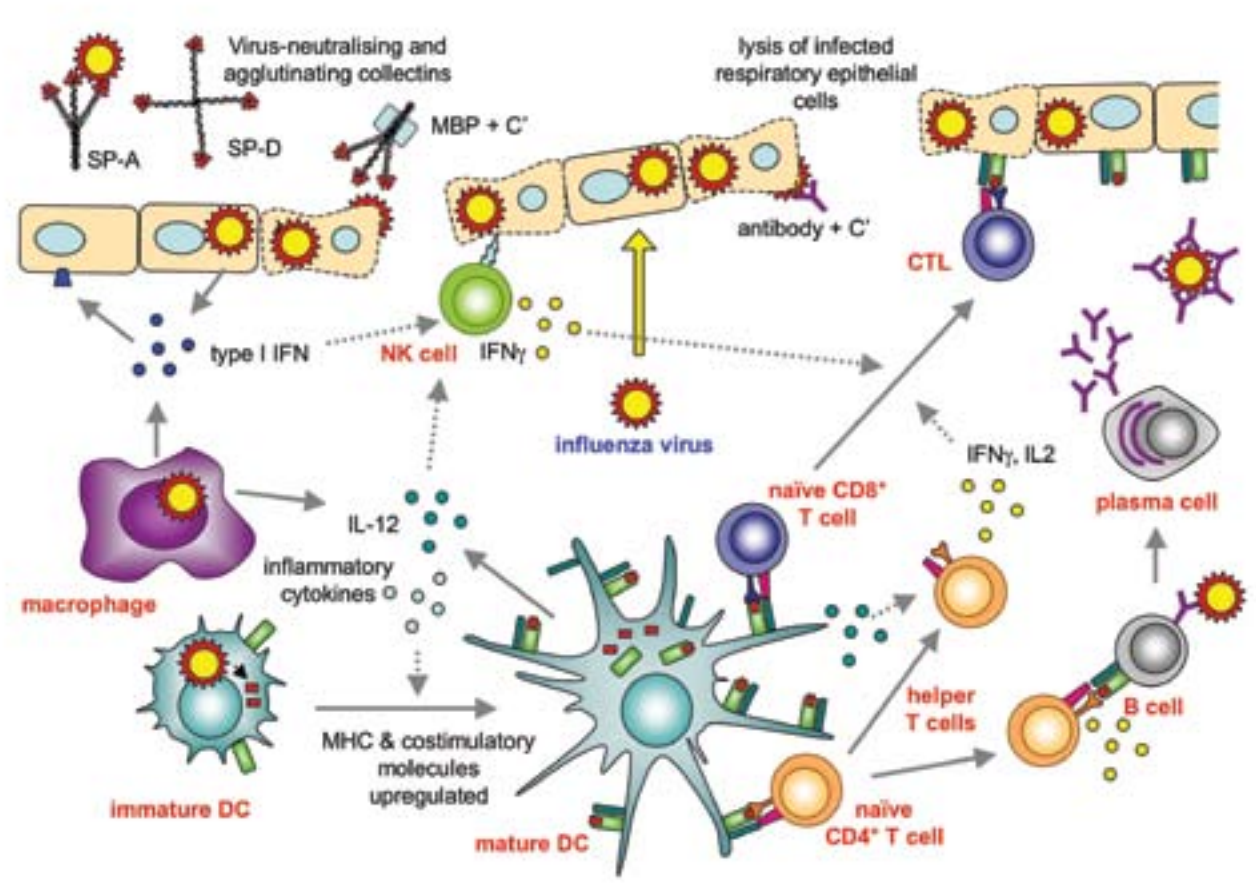
The mechanism of this selection is not well understood. For example, there are four or five main sites on the HA head that contain epitopes for neutralising antibodies and selection of a mutant with a change in a particular site would only be expected to occur in a person with a very restricted antibody repertoire because antibodies to other sites would rapidly neutralise the virus; the probability of a virus arising spontaneously with a mutation in all sites simultaneously is far too low to happen readily, if at all. Perhaps children experiencing their first infection with influenza have a predominance of antibody to a single site [6] and shed escape mutants with changes at that site that go on to mutate further in other children. Once all sites have changed, the population at large sees this as an antigenically distinct strain and the lack of effective pre-existing antibody leads to an epidemic. This is the basis for the need for regularly updating the vaccine strains that are used.

Both the mouse model of respiratory tract infection and immune correlates in humans have shown that IgG is an important class of antibody functioning in protection. During inflammation, IgG can enter the respiratory tract as a transudate from the serum to protect the vulnerable lung tissue. Complement-fixing subclasses of IgG are prevalent and thought to be of particular importance. There is also evidence that mucosal IgA can prevent influenza virus gaining a hold and progressing down the respiratory tract, and it has been claimed that IgA may be more broadly cross-reactive against different viral strains than is IgG.

Other mediators also act to protect the respiratory tract during infection. A very early defence is the type 1 interferon response which can have a role in determining the rate of virus replication in the initial stages of infection and in shaping the initial inflammatory and downstream adaptive immune responses. However, the virus encodes a countermeasure in the form of the non-structural protein NS1 that appears not to inhibit interferon production but rather sequester the dsRNA genome to prevent it triggering many of the antiviral pathways induced in cells on interferon encounter [7].

Members of the collectin family of proteins, made up of multimers of a basic unit composed of a collagenous domain linked to a globular C-type lectin domain,

also function as innate inhibitors of the virus. Mannose-binding protein (MBP), found in serum, and the lung-associated proteins, surfactant proteins A (SP-A) and D (SP-D), are present in humans and have been shown to neutralise influenza *in vitro* [8]. MBP and SP-D bind via their lectin domains to the high mannose-type carbohydrate side chains of the viral surface glycoproteins while the sialic acid on SP-A is bound by HA and NA. On binding of the collectin, access of cell-surface receptors to the receptor-binding site on HA is blocked and infection inhibited [9]. The binding of MBP to virus-infected cells can also trigger activation of the complement pathway, leading to lysis of the infected cell [10].



Overview of the major effector cells and molecules induced in response to influenza infection.

When influenza virus [centre] enters the respiratory tract, several early defence systems come into play [left]. Lung surfactant proteins act to neutralise and agglutinate virus particles. Type 1 interferon (IFN) is secreted by infected epithelial cells and macrophages and binds to receptors on uninfected cells to induce a refractory state. Type 1 IFN also activates NK cells that lyse infected cells. Inflammatory cytokines produced by macrophages and dendritic cells (DC) attract more effector cells to the site of infection.

MBP from the serum enters the lung and, in the presence of complement (C'), kills cells expressing influenza glycoprotein on their surface. Immature DC become activated and undergo a maturation process that involves the upregulation of molecules necessary for T cell 'priming' as well as their migration to the site of induction of adaptive immunity, the draining lymph nodes.

Virus that has been previously processed by DC in their immature state is now efficiently presented to naïve T cells to trigger the adaptive immune response [right]. Encounter with viral antigen presented on MHC molecules on DC leads to priming of T cells having the appropriate receptors. After priming, naïve $CD8^+$ T cells differentiate into cells that have cytolytic function upon encounter with the same processed viral antigen-MHC complex on epithelial cells. Priming of naïve $CD4^+$ T cells allows differentiation into one of two types of helper T cell that either acts to promote the expansion of activated $CD8^+$ T cells or acts to trigger the differentiation of B cells into antibody-secreting plasma cells.

Antibody can directly bind and neutralise the virus or bind to infected cells expressing surface viral glycoproteins and lyse these in the presence of C'. Differentiation and secretion pathways are shown as solid lines; the action of some of the key cytokines in promotion of particular pathways are shown as dotted lines.

Another mediator of innate defence is the natural killer or NK cell, which is a large granular lymphocyte, distinct from the T and B cell lineages. NK cells can lyse virally infected targets *in vitro* and secrete proinflammatory and antiviral cytokines, especially interferon-γ. These cells may be relevant in the early stages of pulmonary infection; in human volunteers infected with influenza, a rise in lytic activity of blood NK cells was noted 1-3 days following infection [11]. Their role in clearance of influenza virus is, however, much less crucial than T cells or antibody which appear later.

It is well established in the mouse model that $CD8^+$ T cells are a major effector in the clearance of virus [12]. These cells, which start to appear from day 3 onwards, aid the innate defences in keeping virus titres from escalating to uncontrollable levels until the developing antibody response has reached a sufficient strength to eliminate the remaining virus. As predicted from their mode of action in killing infected cells, $CD8^+$ T cells do not prevent infection but lead to a more rapid viral clearance.

Studies in humans [13] also suggest that these cells contribute to recovery from influenza. Clinical observations on volunteers intranasally infected with a re-emerging 1979 strain of live influenza virus showed that all subjects with demonstrable $CD8^+$ cytotoxic T lymphocyte (CTL) responses cleared virus effectively with reduced virus shedding. This was observed in all volunteers, including those born after 1956, who did not have specific antibody and therefore had probably not been exposed to this subtype of influenza A virus before. This seminal study showed, not only that CTL-mediated viral clearing responses existed in man, but that these could reduce viral shedding in the absence of pre-existing antibody. Of equal importance was the confirmation of ability of CTL to be recalled by virus of a heterologous subtype, presumably because these cells can respond to processed fragments of the internal conserved proteins of the virus presented on class I MHC molecules.

Can we exploit this cross-reactive arm of the immune system to provide some protection against morbidity and mortality due to a newly emerging and serologically distinct influenza virus? CTL responses are not induced or boosted by current detergent-split inactivated virus vaccines where the viral antigens do not access the class I processing pathway efficiently, but natural exposure to endemic strains does recall this type of immunity [14].

So why aren't we protected against severe influenza disease (as opposed to infection) from our prior exposure to different strains of virus? The answer is that we probably are. Only very rarely will unvaccinated individuals suffer from debilitating influenza during their lifetime and this may occur when CTL levels decline. In humans, the rate of decline has been shown to be quite rapid in years of low prevalence of influenza A in the community [14].

The key may be to find a safe means of regularly boosting the memory pool of $CD8^+$ T cells to obtain sufficiently high numbers such that no further expansion is necessary when a new virus is encountered, allowing for rapid control of infection. Such a strategy has been demonstrated in mice by sequential infection with two different serologically distinct subtypes of influenza virus, resulting in high levels of protection against a subsequent infection with a highly virulent third subtype of virus [15]. Even though the virulent virus initially grew to similar high titres in the double-primed mice as it did in naïve mice, the infection was controlled within 3 days and the virus-induced damage greatly limited.

The challenge therefore is to achieve similar high numbers of memory $CD8^+$ T cells by vaccination. This may not only increase the efficacy of sub-optimal antibody responses in the elderly and other target groups but, more topically, may reduce severe disease and death in the face of a pandemic in the interval before highly specific antibody-inducing vaccines can be manufactured. Perhaps some of the experimental approaches being currently developed will show promise in achieving this goal [16, 17].

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