Pre-existing immunity to novel pandemic influenza A viruses

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Abstract The recent 2009 influenza A H1N1 pandemic has killed at least 17.000 people and is still ongoing. In addition, the more pathogenic H5N1 avian flu remains cause for concern despite the fact that human cases are rare. Although there are several indications for pre-existing immunity to these novel strains, little is known about underlying mechanisms and the consequences of vaccination to seasonal influenza. Recent studies indicate that pre-existing immunity to 2009 H1N1 and to H5N1 is mediated by B-cell as well as T-cell immunity. Strikingly, neutralizing antibodies to H1N1 are the most abundant in the population >65 years. Furthermore, the development of T-cell immunity to H5N1, and probably also to H1N1, is impeded by vaccination. Together, these findings suggest important considerations are to be made with respect to vaccine design and targeting.

Introduction

The recent emergence of new virulent influenza strains has led to substantial concerns among the public and health care authorities. Given the impact of several pandemic outbreaks in the twentieth century and the immunologically naïve background of the population to these novel influenza strains, it is very probable that they will lead to pandemics with much higher mortality rates than the annually occurring seasonal epidemics. It was estimated that 12%-30% of the population will develop clinical influenza from H1N1 2009, compared to 5-15% in seasonal influenza (1). However, data from the first pandemic wave suggest that this was an overestimation(2). Pre-existing immunity to novel influenza subtypes is a potential factor reducing the impact of pandemic outbreaks.

Genetic shifts and herd immunity

Influenza A virus (IAV) nomenclature is defined by the structure of the two main viral surface glycoproteins H (hemagglutinin) of which there are 16 variants and N (neuraminidase) which comprises 9 variants. Epitopic drift causes these antigens to continuously change slightly in the structure of their immunoepitopes. In contrast, antigenic shifts are sudden major changes in structure which lead to new influenza subtypes and are caused by genetic reassortments or zoönoses. New subtypes originating from antigenic shifts have the potential to cause epidemics or pandemics as has happened with the H5N1 (avian) and the 2009 H1N1 (swine-origin) subtypes. It is generally accepted that the pandemic character of new subtypes is caused by the absence of herd immunity to these new viruses (3). Nevertheless, in certain groups of individuals immunity to novel viruses may be observed due to the occurrence of cross-reactivity induced by previous exposure to other (seasonal) subtypes.

Pandemic 2009 H1N1 influenza

Unlike seasonal influenza epidemics which occur every winter, influenza pandemics are reported only once every few decades. When a new IAV subtype causes a pandemic, it outcompetes the dominant strain that prevails at the given moment, ultimately becoming the

dominant strain itself. In 1918, introduction of a novel H1N1 strain led to the Spanish flu pandemic killing over 40 million people worldwide. Some estimates even range up to a 100 million deaths (4). Subsequently, it became the dominant strain until the subtype prevalence shifted towards H2N2 during the Asian flu in 1957. In 1968 the Hong Kong flu introduced H3N2 as the new dominant strain.

In 2009 a new influenza subtype resembling the 1918 H1N1 emerged, rapidly spreading across the globe. In June 2009 the WHO raised the pandemic alert to phase 6 as sustained community-level transmission of the virus was taking place in more than one region of the world (5). At present, at least 17.000 deaths in more than 214 countries have been reported as a consequence of 2009 H1N1 infection (6). 2009 H1N1 is now the dominant strain in most parts of the world (7) although it is not clear to what extent it banished the circulating seasonal viruses from before 2009.

The reoccurrence of a virus similar to the 1918 type is connected to the ability of IAV to infect, among other species, pigs. At some time in the period of the 1918 pandemic H1N1 was introduced into the domestic swine population (8). Based on genetic analysis, it is assumed that the current pandemic H1N1 strain originated in pigs (9). While influenza infections can cause severe morbidities in humans, analysis of the 2009 H1N1 (CA04) strain revealed that it can pass asymptomatically in swine (10). Pigs are regarded as mixing vessels for IAV conserving the virus as well as providing the opportunity for genetic drift and shifts. (11). Pigs have been the source of several H1N1 reoccurrences that have taken place since 1918. In 1977, a H1N1 virus reemerged which co-circulated with H3N2 until present(12). Currently, the seasonal IAV vaccines are based on H3N2 and the seasonal H1N1 (36).

The exposure of certain population groups to H1N1 during the period of its circulation in 1918 – 1957 and from 1977 until present raises the question whether they have acquired some form of immunological protection. Some findings suggest that previous exposure to 2009-like H1N1 elicited protective responses. This could very well depend on the degree of similarity between H1N1 variants. There are several studies in support of this hypothesis which will be discussed. In addition, previous exposure to other subtypes might invigorate immunity to pandemic IAV as well by inducing heterosubtypic immunity.

Pandemic H5N1

Contrary to 2009 H1N1, the avian IAV H5N1 that was introduced into the human population in 1997 has not spread efficiently. The total number of confirmed cases lies at 493 in 15 countries (37). Nevertheless, of the people that have been infected 59% have died (15) making H5N1 highly pathogenic. As the virus continues to spread among domestic birds and new human cases appear every now and then, the chance that it will cause a pandemic is still present. The transmission of the virus between humans has been very inefficient until now causing human outbreaks to be confined within family clusters. (16) The fear is that this might change. Once a more transmissible variant of H5N1 will come into existence this could initiate a pandemic with substantial mortality rates.

Although no virus similar to the avian H5N1 has circulated in humans in the recent history, memory responses can still be present in the form of heterosubtypic immunity. Apart from hemagglutinin and neuraminidase, which are surface proteins, there are several IAV core and matrix proteins that can be recognized by the immune system when presented on infected cells or antigen presenting cells

(APC). IAV core proteins are more essential than surface proteins for virus functioning. Therefore they are less subject to epitopic alterations hence less variable (17). Less variation between different IAV subtypes implicates that infection by a subtype deviating from the one of interest could very well attribute to immunological memory that is relevant to both subtypes.

Implications of pre-existing immunity

It is clear that strong action should be taken against the new pandemic strains which are threatening to kill millions of people. In order to do this an effective immunization policy is needed. For H5N1 it is desirable to contain the virus within the boundaries of the localized outbreaks that occur now. For H1N1 the emphasis already shifted towards mitigation (21). In either case, it is necessary to understand the nature and molecular basis of pre-existing immunity to these viruses. If we do, we should be able to design and target vaccines more effectively.

Mechanisms of pre-existing immunity

Influenza viruses consist of seven core proteins and the aforementioned HA and NA proteins. Core proteins are conserved within IAV while surface proteins may vary. In the infection mechanism of host cells in the respiratory tract (RT), HA is involved in virus entry by endocytosis and NA in releasing virions produced in the infected cells. This process evokes flu symptoms within a few days while raising an acquired immune response takes approximately one week (22). Memory responses to previous infections or vaccination will act at shorter notice. Therefore, in mitigation as well as in containing IAV, it is crucial to induce neutralizing antibodies to pandemic strains

Heterosubtypic immunity

Immunity to the conserved IAV core proteins, of which nuclear protein (NP) is the most important (23), elicits cross-reactive responses by cytotoxic lymphocytes (CTLs). Antigen presentation of the viral nuclear proteins primarily takes place on the surface of infected RT cells in the context of the MHC-I molecule. This enables $CD8^+$ T-cells specific for these proteins to expand and eliminate infected cells. Since this can only happen after host cell entry, immunity against nuclear proteins is primarily responsible for reducing the severity of an infection (22). This so-called heterosubtypic immunity probably has a minor effect on the rate of disease spread within the human population while it may reduce the mortality of the virus by abating the viral spread in individuals.

Heterosubtypic immunity has been demonstrated in mice that lacked presence of cross-reacting antibodies (aBs) (24). Mice previously infected with IAV were cross-protected against heterosubtypic viral challenge. This cross-protection was still present in knock-out mice missing B-cell immunity but the effect was reversed in $CD4^+$ and $CD8^+$ depleted mice. (25)

Subtype-specific immunity

On the opposite of heterosubtypic immunity stands the ascertainment of subtype specific immunity by creating cross-reactive aBs. Viral particles that are present in the RT mucosal epithelium of the host can be processed by antigen presenting cells. Presentation of HA and NA peptides in the context of MHC-II will ultimately lead to the production of specific antibodies. Anti-HA aBs are responsible for preventing infection since they neutralize the ability of the virus to enter host cells. On the other hand, anti-NA aBs are similar in effect to CTLs in that they reduce

viral spread within individuals (38). aBs specific for core proteins are also produced in response to an infection but they are unsuccessful in protecting from infection (26). In addition, T-cell immunity is involved in subtype-specific responses by reacting to conserved HA and NA epitopes (27).

Early virus clearance by subtype-specific immunity had been reported in mice. It is predominantly mediated by anti-HA IgA antibodies in the RT epithelium. This was demonstrated by infecting mice with H3N2 and after recovery challenging them with another H3N2 virus. Mice were protected against RT infection in parallel with anti-HA IgA presence (28). Serum ab's or CTLs did not contribute to early virus clearance in this study.

Kinetics of responses

The temporal segregation of the different elements of the immunological responses involved in heterosubtypic and subtype-specific immunity was investigated in another cross-immunization experiment (29). The presence of virus-Ig complexes and virus specific CTLs was measured. In the first 3 days after re-infecting mice, subtype-specific pre-existing immunity protected them by the formation of viral-Ig complexes. In the virus titres, viral-IgA complexes were much more abundant than viral-IgG complexes. In heterosubtypic re-infection, memory CTLs were produced rapidly from 3 days post infection onwards.

In conclusion, early virus clearance, which is mainly seen in subtype-specific immunity, is mediated by humoral immunity in the first 3 days of the response. Memory CTL responses which mediate heterosubtypic immunity contribute to viral clearance in subsequent days after the virus had passed the mucosal barrier and commenced infecting cells.

Pre-existing humoral immunity to H1N1

It is often assumed that the low incidence of pandemic H1N1 influenza that is observed in the elderly population is caused by previous exposure to similar strains circulating in the early 20th century. Possibly other circulating H1N1 strains of later decades and broader heterosubtypic protection raised by a lifetime of influenza exposure also contribute to this phenomenon. However, data supporting this hypothesis have been very scarce until recently. In recent serological studies, the epidemiological prevalence of protective antibody responses was drawn up (13,14). Furthermore, the role of heterosubtypic immunity to T-cell epitopes was investigated (27,30) and the cross-reactivity between B-cell epitopes was elucidated in a mouse model (31).

Seroprevalence

To asses the seroprevalence of protective antibody to novel H1N1, the CDC conducted a study with serum samples taken from healthy participants in the period of 2007-08 (13). Hemmaglutination inhibition (HI) and microneutralisation (MN) titres to novel IAV were determined. Allthough no reliable estimate for protectiveness is known for MN titres, MN was taken as a measure to predict protectivity by ab's. This was done because of the high number of seroconversions before and after vaccination. A MN titre of 160 or more was interpreted as protective since this correlated to a HI titre of ≥40 which is known to yield a 50% protectiveness. In the population aged over 60 years a MN titre ≥160 was found in 33% of the serum samples as compared to 6%-9% in the age group of 18-64 years.

A similar survey was performed in England where serum samples from different age groups were collected in 2008 and used for

	Microneutralisation titre at or above 1:10	Haemagglutination inhibition titre at or above 1:8	Microneutralisation titre at or above 1:40	Haemagglutination inhibition titre at or above 1:32
0–4 years	7/143 (4·9%, 2·4–9·8)	6/171 (3·5%, 1·6–7·4)	4/143 (2·8%, 1·1–7·0)	3/171 (1·8%, 0·6–5·0)
5–14 years	20/163 (12·3%, 8·1–18·2)	17/188 (9·0%, 5·7–14·0)	7/163 (4·3%, 2·1–8·6)	7/188 (3·7%, 1·8–7·5)
15–24 years	32/110 (29·1%, 21·4–38·2)	30/120 (25·0%, 18·1–33·4)	14/110 (12·7%, 7·7–20·2)	21/120 (17·5%, 11·7–25·3)
25–49 years	41/168 (24·4%, 18·5–31·4)	35/193 (18·1%, 13·3–24·2)	16/168 (9·5%, 5·9–14·9)	19/193 (9·8%, 6·4–14·9)
50–64 years	29/65 (44·6%, 33·2–56·7)	67/182 (36·8%, 30·1–44·0)	12/65 (18·5%, 10·9–29·6)	26/182 (14·3%, 9·9–20·1)
65–74 years	95/167 (56·9%, 49·3–64·2)	68/190 (35·8%, 29·3–42·8)	42/167 (25·1%, 17·6–32·2)	40/190 (21·1%, 15·9–27·4)
75–79 years	101/187 (54·0% 46·9–61·0)	77/193 (39·9%, 33·2–46·9)	33/187 (17·6%, 12·9–23·7)	36/193 (18·7%, 13·8–24·7)
≥80 years	139/166 (83·7%, 77·4–88·6)	105/166 (63·3%, 55·7%–70·2)	78/166 (47·0%, 39·5–54·6)	52/166 (31·3%, 24·8–38·7)
Total	464/1169 (39·7%, 36·9–42·5)	405/1403 (28·9%, 26·6–31·3)	206/1169 (17·6%, 15·5–19·9)	204/1403 (14·5%, 12·8–16·5)

Data are number of individuals with antibody/number of individuals tested (%, 95% CI).

Table 1: Serum samples obtained in 2008 with antibody titre equal to or more than the minimum detection limit and with antibody titre at least four times higher than the minimum detection limit, by age group and test method. Adopted from Miller et al.

MN and HI titre determination (14). For MN, a ≥1:40 titre was interpreted as protective and for HI a titre of ≥1:32 was used. Results are depicted in Table 1 with 95% confidence intervals. Although the presence of cross-reactive antibodies in the groups aged over 64 is markedly higher than in younger age groups, the population ≥80 years stands out with 31.1% deemed protected.

Accordingly, comparison of the baseline HI titres and those obtained in August and September 2009 after the first pandemic wave showed substantial differences by age (14). In the people aged ≥65 the difference between post pandemic wave and baseline was only 0.9% whereas the difference in those aged 5-14 years was 42.0%. This corresponds with the expectation that those who have pre-existing antibodies to 2009 H1N1 will have a smaller chance to be infected and acquire matching immunological responses. Ross et al. found a similar age distribution bias in a survey performed in the US Pittsburgh area (32).

B-cell epitope similarity

As cross-reactivity between the 2009 and 1918 strain should be reflected by the presence of shared immunoepitopes, sequence data was used to make alignments of the HA protein (31). In a setup using mAb escape mutants all mAbs appeared to bind to one conserved antigenic site. Sequence similarity of this site was much smaller when 2009 H1N1 was aligned to the strain that circulated from the late seventies. This might provide an explanation for the fact that immunity to the 1977 H1N1 confers little cross-protection. Others have also found low conservation in B-cell epitopes between novel influenza and seasonal strains (30).

Another approach by Xu et al. led to the assumption that absence of glycosylation of HA may be the key to cross-reactivity (33). By the 1940's human influenza viruses appeared with amino acid residues on the surface of HA that can undergo glycosilation. Sugar residues that were bonded to these sites shielded the HA protein from the human immune system (34). However, both 1918 H1N1 and the 2009 strain lack these protective residues. Allegedly, the increasing immunity in the human population to novel IAV possibly exerts a selection pressure. This selection pressure might stimulate the process of acquiring glycosilation sites de novo. It is not known what the effect of vaccination might be. It is not unthinkable that vaccination will add to the selection pressure hence speeding the process. Consequently, Wei et al. suggest to incorporate glycosilated virus into future vaccines to limit further spread of 2009 H1N1 and its transformation into a seasonal influenza (34).

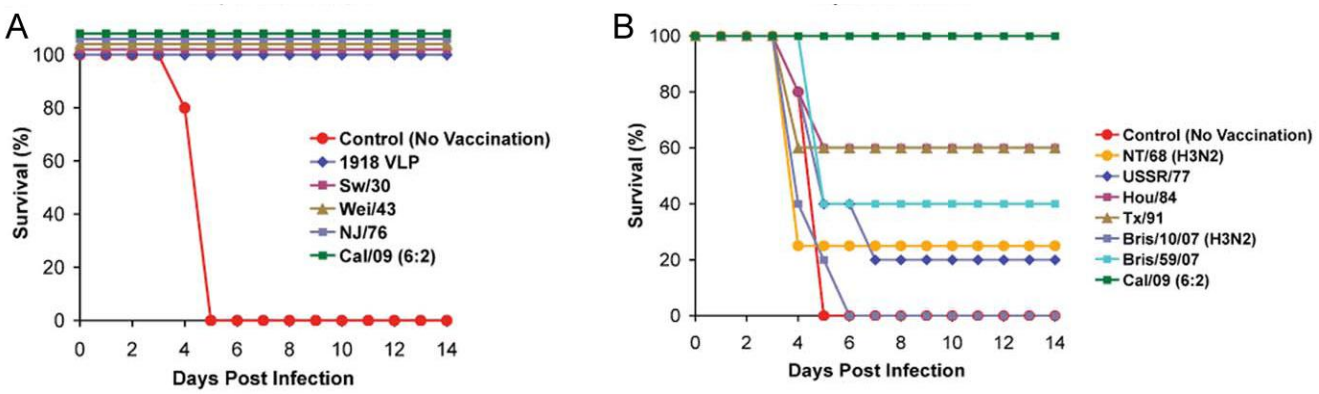

Figure 1: Survival curves of mice immunized with different H1N1 isolates and challenged with Neth/09 infection four weeks post immunization. Figure **A:** Inactivated classical swine viruses (Sw/30 or NJ/76) , 1918 VLP, virus isolated in 1943 (Wei/43) and pandemic 2009 H1N1 completely protect from lethal challenge. **B:** Contemporary viruses offer only partial protection. Adopted from Manicassami et al.

Memory B-cells from 1918 pandemic are still present in survivors

In order to reveal the longevity of B-cell reactions raised against influenza, Yu et al. investigated antibody responses in survivors of the 1918 pandemic (35). By using the 1918 virus sequence they produced recombinant HA with the 1918 phenotype. Each of the tested individuals that were born in or before 1915 showed seroreactivity to the recombinant HA. Monoclonal antibodies derived from their circulating B-cells showed high reactivity with the 1918 virus and to the genetically similar 1930 strain but not with more contemporary strains. Since this study was performed in 2008, cross-reactivity with H1N1 2009 was not investigated. In conclusion, neutralizing antibodies to the 1918 pandemic virus still circulate in survivors nearly a century later but the implications of this study to 2009 H1N1 are unclear.

Neutralizing activity

More conclusive research about the protective ability of 1918 derived aBs has been performed in cell cultures and in vivo. Addition of human sera and IAV onto MDCK cells and examination of the cytopathological effects of 2009 H1N1 infection led to the observation that only sera from those born before 1920 conferred protective ability (10). Interestingly, Manicassami et al. showed in a mouse model that vaccination with inactivated virus to 1940's and late 1970's H1N1 strains was also protective (31). Active immunization based on several isolates of viruses that circulated between 1918 and the 1950's offered complete protection from lethal challenge by H1N1 Neth/09 (figure 1A) although infection with a 1943 virus led to higher pathogenicity reflected by weight loss (not shown). However, immunization with more contemporary isolates from after 1977 only led to partial protection (figure 1B). Partial protection was also observed in immunization with one of the H3N2 strains suggesting that this is caused by heterosubtypic CTL responses rather than by cross-reacting antibodies.

In the CDC seroprevalence study, the effect of vaccination with seasonal TIV was assessed by comparing the microneutralization titers of cross-reacting antibodies pre- and post vaccination (13). In the collected sera of those aged 18-64 years modest increases in cross-reactivity were found. The group aged ≥60 years hardly profited from vaccination although they did show a high percentage of seroconversions indicating that vaccination did raise a protective response to seasonal IAV. Supposedly, in the elderly population immunity raised against seasonal AIV as well as the new H1N1 is overruled by pre-existing

immunity to viruses other than those contained in the vaccine.

In conclusion, it can be said that exposure to pandemic flu in the period following the 1918 pandemic led to immunologic memory protecting older population groups from infection with novel pandemic H1N1. Infection with the more cotemporary influenza strains offers far less (B-cell) protection and thus, vaccines based on these types will not be effective. In addition, observations made on this subject provide justification for focusing immunization policy on younger population groups in stead of the elderly population which is usually considered to be at the highest risk of being infected.

Pre-existing T-cell immunity to H1N1

The emphasis in IAV research is largely on serological work and subtype-specific antibodies (30). Although heterosubtypic T-cell immunity to IAV strains does not prevent infection, it is known to blunt disease severity and therefore it is an important factor In reducing pandemic impact. Investigation of T-cell responses revealed that large fractions of T-cell epitopes are conserved in novel IAV (27).

Subtype-specific T-cell reactions

In parallel with the low conservation of B-cell HA and NA epitopes in 2009 H1N1, only a small percentage is conserved in sites recognized by T-cells (30). Greenbaum et al. concluded from sequence comparison in the Immune Epitope Database that overall, only 12% of epitopes were conserved. On the other hand, De Groot et al. state that a more than 50% conservation in HA epitopes is present between novel IAV and proteins contained in conventional influenza vaccine (27) thus vaccination to divergent seasonal H1N1 might still contribute to helper and cytotoxic T-cell responses. However, de Groot et al. did not perform in vitro or in vivo experiments to validate this estimate.

Heterosubtypic reactions

The conservation of T-cell epitopes contributing to heterosubtypic immunity is notably higher than that of B-cell epitopes. Whereas overall conservation of B-cell epitopes is predicted to be 31%, the conservation of CD4+ and CD8+ T-cell epitopes is 41% and 69% respectively with high conservation of core proteins and low conservation of HA and NA (30). These epitopes were functionally tested for pre-existing CD4+ and CD8+ reactivity in an ex vivo essay using peripheral blood mononuclear cells (PBMC) from donors taken before the 2009 pandemic outbreak. Data obtained from this study indicate that T-cell memory responses to conserved epitopes in 2009 H1N1, in parallel with the expression of memory markers, were present. Furthermore, the level of pre-existing immunity was higher in CD8+ T-cells than in CD4+ T-cells (30). Similar results were obtained by Ge et al. in ex vivo functional analysis (39).

Overall, it can be concluded that T-cell responses raised against either seasonal influenza or conventional vaccine can cross-react with 2009 H1N1 epitopes. As a consequence, population groups that have acquired immunity to H1N1 are likely to benefit from this. Whether they also benefit from exposure to other subtypes like H3N2 is less clear. High conservation of nuclear proteins between different subtypes make this a viable hypothesis. It is hard to predict the significance of T-cell reactions in relation to humoral immunity. The age distribution found in 2009 H1N1 population prevalence is consistent with the presence of cross-reactive aBs but also with the probable increase of specific and heterosubtypic memory that accumulates with age. Possibly the two factors

are reinforcing each other. At least, both should be taken into account in designing and targeting new vaccines.

Pre-existing immunity to H5N1

Although prior infection with circulating IAV strains probably does not raise protective aB responses to avian flu, heterosubtypic immunity plays a significant role in evading the threat of a pandemic. Recent studies have stressed the importance of careful consideration of vaccination policies by demonstrating the effect of vaccination on the induction of heterosubtypic responses (18,19,20). These studies indicate that perhaps, it is not as self evident to vaccinate certain risk groups to IAV as often is assumed.

Pre-existing CTL responses

To asses the population presence and characteristics of pre-existing immunity to H5N1, Lee et al. performed ex-vivo analysis on healthy subjects without prior exposure to the virus (40). PBMC were tested for reactivity with the H5N1 proteome. Of the participants, approximately 87% exhibited broadly cross-reactive memory T-cell responses against H5N1 internal proteins. The cross-reactive responses were predominantly directed against matrix protein M1 and nuclear protein NP. Lee et al. extrapolate these findings to hypothesize that vaccines stimulating cross-reactive T-cell populations may enhance immunity to potentially pandemic strains. Bodewes et al. showed that natural influenza infection may be as effective in inducing heterosubtypic immunity (19,20).

Infection of mice induces protective immunity

Bodewes et al. investigated the protective efficacy of heterosubtypic immunity to H5N1 virus induced by prior H3N2 infection in mice (20). After priming with non-lethal H3N2 infection and a recovery period, mice were challenged with lethal H5N1. Controls were either mock-infected or infected with RSV in the priming step. Whereas H3N2 primed mice showed rapid recovery and a high survival rate, mock infected mice and RSV infected mice showed significantly more pathogenicity and a lower survival rate. The beneficial effect of natural infection was reversed when mice were immunized in advance to the priming infection.

This was shown in a second mouse study performed by Bodewes et al. (19) In this study, mice were divided into groups that received either vaccination to H3N2 (adjuvanted or not adjuvanted) or mock vaccination. They were further divided into groups that were infected with H3N2 IAV (HK/68) several weeks later and groups that were not. Mice that developed immunity to H3N2 as a consequence of vaccination showed no weight loss and mild clinical symptoms correlating to increased antibody titers. Interestingly, after a recovery period, the frequency of H3N2 specific CD8+ memory T-cells was significantly lower in previously immunized mice than in mice that were not vaccinated prior to H3N2 infection. Hereafter, all groups were challenged with lethal H5N1 IAV (IND/05) infection. Mice that developed clinical influenza during the H3N2 infection recovered more rapidly and showed higher survival rates than mice from other groups. This difference was also significant between the not-immunized, H3N2 exposed group and the immunized, H3N2 exposed group. Summarizing, non-lethal IAV infection prior to infection with the more lethal pandemic H5N1 virus leads to the development of heterosubtypic T-cell mediated immunity protecting mice from lethal challenge.

Discussion

Serological data indicate that there is a relation between IAV immunity acquired in

the early 20th century and immunity to novel H1N1. Nevertheless, a part of the biased age distribution in prevalence can also be contributed to differences in social contacts among different age groups. School-going children share the highest number of social contacts and therefore spread IAV at a higher rate among their contemporaries (41). On the other hand, adults have an important role in spatially distributing the virus (basal 63). Based on mathematical modeling it is assumed that differences in contact patterns can contribute to the age bias significantly and furthermore, this bias can shift in following pandemic waves (41). Thus, the epidemiological prevalence of H1N1 should be monitored scrupulously in order to account for possible shifts.

The epitope similarity that is found in T-cell epitopes is notably higher than in B-cell epitopes. Due to the fact that B-cells and T-cells act through different mechanisms and target different proteins, it is difficult to compare these two reactions. The fact that both contribute to immunity significantly does raise the question whether or not vaccines to novel IAV should aim to induce broader cross-protection, not only by the concept of trivalent vaccines but also by incorporating viral nuclear proteins. It would be interesting to investigate pre-existing T-cell immunity to H1N1 with a lethal challenge model to get a clearer picture of its protective ability. If the protective ability of this heterosubtypic immunity would be comparable to the one seen in H5N1, it could be questioned whether or not healthy individuals should be vaccinated to seasonal viruses. Natural infection of non-immunocompromised individuals with seasonal influenza raises heterosubtypic immunity to more pathogenic novel strains. Since vaccination prevents infection with the seasonal strains, the build up of heterosubtypic immunity is also prevented. The unwanted for effect could be that the impact of novel viruses increases within certain population groups.

Pointing out target groups for vaccination is subject to conflicting interest. One the one hand, concentrating on school-going children seems to be the most sensible in the context of disease transmission. On the other hand, concentrating on older population groups will probably protect immunologically vulnerable individuals from a seemingly pathogenic virus. It is tempting to say that, because there is pre-existing immunity to 2009 H1N1 in the elderly population, vaccination to IAV should solely aim at younger individuals. Apart from the fact that vaccination may have adverse effect in healthy, often younger, individuals, it is well known that T-cell immunity decreases with age (). Therefore, excluding older population groups entirely from vaccination would be irresponsible.

In conclusion, pre-existing immunity to influenza is a key player in epidemiology of the virus as well as being of interest in reducing virulence within individuals. Therefore it is of great importance to incorporate knowledge on this phenomenon into mitigation and vaccination strategies involving novel pandemic strains. With this knowledge we can not only pinpoint target groups for vaccination with greater accuracy, but we can also apply it in the design of novel vaccines. Although it is impossible to predict when a new pandemic virus will appear, when it does, lessons learned from the current pandemic with respect to pre-existing immunity might be a key factor in timely taking control of the situation.

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