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Viewpoint

Rationale for treating human influenza infections by passive transfer of specific antibodies

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The fear of a potential pandemic with a highly pathogenic influenza A virus, such as the avian virus H5N1, has rightly prompted multidisciplinary reflections and calls for better preparedness all over the world. In terms of therapeutic aspects, most of the focus has been on vaccines and antivirals. The present 'opinion paper' intends to discuss a different therapeutic approach, although not mutually exclusive to the two others quoted above. We here propose an approach, based on well-documented experimental evidence in animal models, in which a short series of human monoclonal antibodies adapted to the probable pandemic strain, specific for external antigens of that influenza virus and shown *in vitro* and in experimental models to have neutralizing properties, are prepared and stockpiled for administration to people recently exposed to the pandemic virus.

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Therapeutic efficacy of antibody transfer in experimental influenza infections

It was shown long ago[1] in experimental infection of mice that passive transfer of polyclonal antibody specific to influenza A haemagglutinin (HA) very efficiently protects animals infected intra-nasally (IN) with a virulent strain of influenza virus (A/PR8/33). The main conclusions from this original publication were as follows:

- Protection was complete, as all animals could be protected against IN inoculation able to kill 100% of control mice (inoculated with irrelevant rabbit serum).
- Protection strictly correlated with profoundly diminished virus replication, and with decreased lung consolidation.
- Protection could be afforded by antibody transfer even when transfer was delayed by 48 h , thus occurring after completion of the first cycles of virus replication (deaths were observed between 7 and 12 days in untreated controls).
- Protection could be obtained by transfer of relatively low amounts of rabbit antiserum to homologous HA, 100-fold lower than the antiserum amount providing detectable levels of transferred serum antibody, as measured in a single-radial diffusion test performed 1 h after antiserum injection.
- IgG class antibodies circulating systemically were found to be sufficient for protection against this mucosal infection.

Major protective role of antibodies to the strain-specific determinants of haemagglutinin

In the mouse experimental model described above, the specificity of the antibody transferred was shown to be critical:

- Protection was obtained with antisera to the HA homologous to the virus used for challenge (A/PR8/33), but not antisera to the HA of a strain (A/FM1) having drifted antigenically within the H1 subtype.
- This indicated that only antibodies to strain-specific determinants of HA are protective, a major finding which appears to account for the basic strategy of escape of influenza virus, i.e. a subtle, yet critical antigenic drift within a given subtype, permitting the succession, in human populations, of several epidemics with variant strains of the same subtype virus.
- Thus very few changes in HA sequences are needed to bypass immune memory and permit escape from the protective effects of antibodies induced in human populations by the previous epidemic variant. Better still, such modest changes in sequence remain compatible with a good viral fitness for replication and transmission, another critical condition needed for any virus to maintain its epidemic potential.

Influenza virus strategy of escape to anti-HA protective antibody

Antibodies to HA are by far the most protective providing that there is a good match to the specificity of HA determinants present in the distal (HA1) portion of the molecule. This situation is exploited strategically by the influenza virus to remain epidemic on the planet for tens of years, through subtle antigenic drift. This virus strategy, however, has its own limitations. Indeed the need to maintain a high level of replication fitness is likely not to be compatible with most random HA antigenic changes. Periodically, influenza virus must shift HA altogether, by exchanging (reassorting) its HA genome segment with that of another influenza strain from the varied genome repertoire of the avian flu reservoir, or create a new pandemic virus by adaptation to man of a pathogenic, avian virus with an HA 'novel' to human populations.

The immune system, confronted by such a 'moving target', is thus successfully bypassed by influenza antigenic drift and shift. However, protection is provided in a matter of a few days, in each infected individual, by the early production of antibody to the novel strain-specific determinants encountered. The few days needed for such antibody-mediated virus eradication in a given individual is nevertheless sufficient for efficient, aerosol-mediated dissemination of the virus to other susceptible individuals, thus permitting persistence of the epidemics.

The design of antibody-based immune intervention against influenza infection (whether based on active immunization through vaccine, or on passive transfer of antibodies) must thus target either HA determinants as close as possible to those of the infecting virus, or determinants conserved among many flu strains over times.

Protection afforded by influenza antigens other than haemagglutinin

Many influenza antigens remain relatively conserved among multiple epidemic variants. Unfortunately, antibodies to these conserved proteins are not protective upon transfer to mice. This is the case for antibodies to nucleoprotein (NP).[1,2] Many authors have worked on 'heterosubtypic immunity' (HSI) to influenza, i.e. protective cross-reactivity to lethal infection with influenza A virus of a serotype different from the virus initially encountered. It was expected that protection would be afforded by cytotoxic lymphocytes, known to respond preferentially (in an 'immunodominant way') to cross-reactive antigens such as NP.[3] Contrary to expectation however, it was found that mice deficient in CD8 molecules performed HSI normally, whereas B cell-deficient animals did not.[4] This and other experiments suggested that heterotypic protection within an influenza subtype relies on humoral, but not CD8-mediated T cell immunity. Clearly, the specificity of the B memory cells involved and that of the corresponding antibodies must be to antigens conserved within a subtype.

The eight structural proteins of influenza virus do not evolve at the same rate. The influenza virus genome segment coding for the neuraminidase protein is less variable than the HA segment. In addition to the major role played by antibodies to HA, some protection can indeed be transferred by antibody to influenza neuraminidase (NA). This was shown by passive transfer experiments in mice[1,2] and was also indicated by epidemiological data in human populations.[5] Vaccines based on NA were reported to be protective.[6]

To protect people against flu after an antigenic shift in both NA and HA, it would of course be useful to target an antigen with very high conservation and expressed on the virus membrane. A very minor, yet potentially important, viral antigen present on the virion surface is the outer part of the trans-membrane M2 antigen (M2e), which is largely invariant. Interestingly, it was shown that transfer of monoclonal antibody to M2 reduces the growth of both H1N1 and H3N2 viruses.[7] However, the possibility of basing a 'universal influenza vaccine' on immunization with M2e should be discussed with prudence, as it was shown that influenza mutants emerge *in vivo* in the presence of antibodies to M2e.[8]

The case of avian flu

Avian influenza is normally a disease of birds. The contribution of bird influenza under normal conditions is to provide a reservoir of 16 different HA (and other viral genes), that influenza virus circulating in human populations uses periodically to undergo antigenic shift through genome reassortment, thus totally escaping protection by specific antibody for the entirely different HA present in the flu variants responsible for previous epidemics.

However, it appears that from time to time that avian flu strains can become pathogenic to man, for molecular reasons not yet fully elucidated. It is likely, in particular, that mutations in some non-structural proteins enable the virus to escape the protective effects of interferon which, together with antibody to HA, is a major host defence mechanism against flu infection.[9] Another major parameter of influenza adaptation of bird strains to human respiratory tract is modifications at the level of HA domains interacting with the sialic acid residues recognized on cell membrane proteins and lipids, used as receptors for virus entry.

This adaptation may permit avian strains to recognize, instead of the avian α 2-3 sialic acid receptors, the α 2-6 type sialidation expressed by the human upper respiratory tract mucosal cells.[10] In addition, the study of the 'Spanish

flu' genome indicated that some mutations in the influenza polymerase gene increase the efficiency of the enzyme *in vitro* and the replicative efficiency of the virus in animal models.[11]

Strains of avian flu with pathogenicity to humans were observed in Asia during the last years. At the present stage, these strains remain poorly infective to man, in that a massive exposure (for example in poultry-associated professions) is needed for infection to occur. Only few human cases are expected to be observed if these avian influenza strains do not mutate and do not acquire the capacity to be transmitted from man to man. However, the latter situation, if it ever happened, would provide conditions for a pandemic with a potential for morbidity and mortality rates comparable to the deadly 'Spanish flu' pandemic of 1918, now suggested to have been caused by direct passage to man of an influenza virus strain with avian characteristics.

Antibody transfer as a therapeutic measure in cases of human contamination by influenza virus strains of avian origin

Thus strong evidence supports the concept that transfer of specific antibodies could efficiently protect people who were recently in close contact, professionally or not, with influenza virus-infected patients. Other therapeutic interventions can be used, such as the taking of antiviral drugs, particularly drugs able to inhibit neuraminidase activity. However, it should be stressed that strains resistant to presently available anti-neuraminidase drugs have been reported. Moreover, it was shown that these drugs are efficient only when taken within a few hours after exposure.

Interestingly, observations in the few people who were infected with the H5N1 bird virus indicate that the incubation period is relatively long,[12] a delay which would give time to administer antibodies to H5, with hopefully protective effects obtained even after the first virus replication cycles, as may be extrapolated from the experiments in mice described above.[1,2] Of further interest is the observation that the H5N1 virus can be found in the digestive tract at autopsy,[12] which provides further incentive to treat contaminated people systemically with highly diffusible IgG antibodies to H5N1 virus.

Conclusion and prospective

The evidence described and discussed in the present article strongly support the concept of designing and producing monoclonal antibodies aiming at protecting people recently exposed to highly pathogenic influenza virus. It is important to note that therapeutic transfer of antibody should be protective even against highly pathogenic influenza viruses. Indeed, recent evidence indicates that mice immunized against the highly virulent 1918 'Spanish' flu are efficiently protected against lethal challenge. This strong protection is not broken by T-lymphocyte depletion, but can be transferred by IgG from protected mice.[13] This is in keeping with recent suggestions that polyclonal antibodies from convalescent people could have an important preventative effect on severe influenza infection, as indicated in a meta-analysis of pneumonia in the Spanish influenza pandemic.[14]

Lu *et al.*[15] and Renegar *et al.*[16] recently reported that whereas circulating IgG antibodies do protect against disease, they do not completely prevent infection of the nose. If these observations were valid in people, they would be compatible with the idea that transferred antibody would protect against flu disease but might not prevent some transmission of the virus in the population in contact with the individuals treated. Although this may appear to be a limitation of the therapeutic approach consisting in transferring protective IgG to influenza virus, that phenomenon may in fact have important, positive consequences. Indeed, the giving of IgG antibodies might actually act as a surrogate vaccine allowing those who would get infected despite antibody transfer to become immune without becoming sick.

Technical aspects

In addition to financial aspects, which are beyond the scope of the present, short opinion paper, numerous questions remain to be discussed about the technical aspects of the approach discussed here. Main questions would concern:

- The route of administration, with the probability that subcutaneous injection will be efficient, and more readily accepted by people than intravenous administration.
- The nature of the Mab to be used: IgG Mab of human origin (rather than secondary 'humanization' of animal Mabs) should be used. The biotechnology for large scale production of human immunoglobulins in different systems, including plant technology, has enormously progressed in these last years, ensuring a reasonable feasibility for the development phase. If plant technology was used, the lack of animal virus or prion contaminants would facilitate the acceptance of administration in humans by regulatory authorities.
- The specificity of the Mabs to be raised: HA (H5 if H5N1 virus were to be treated) would obviously be a priority.
- However, administration of antibodies with more than one specificity in the same injection(s) should be considered: the evidence reviewed above points to the potential interest of including (in addition to the anti-HA Mab) other Mabs, in particular those specific for the neuraminidase of the pandemic virus, or even to M2e. Synergism in terms of protection, and prevention of antibody-induced resistance to neutralization would be expected.

– The sub-specificity and protective capacity of the antibody preparation to the pathogenic avian strain targeted should be screened by studies of its neutralizing activity *in ovo* and/or in cell cultures, and its protective efficiency verified in animal models, as performed by Kong *et al.* in the case of the 1918 virus in mice.[13] If, as suggested above, more than one Mab was present in the preparation to be administered, the likelihood of obtaining a protective effect on influenza strains despite mutations occurring during the pandemic dissemination of the virus should remain very high.

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