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Relationship between haemagglutination inhibition titre and immunity to influenza in ferrets

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ABSTRACT

Our understanding of the antigenic evolution of the human influenza virus is chiefly derived from experiments in which serum from influenza infected ferrets is tested against panels of virus isolates in the haemagglutination inhibition (HI) assay. The interpretation of these results has been much aided by the development of antigenic mapping techniques, which suppose that the antigenic distance between two different influenza viruses is directly proportional to their fold-difference in titre in this assay. Yet, antigenic distance is not necessarily the same as cross-protection, and high levels of protection have been observed in humans against strains to which they have low HI titres. However, no study has previously addressed the relationship between HI titre and cross-protection in ferrets: the standard animal model. This study fills this gap by analysing published data where pre-challenge HI titres are available for individual ferrets, and post-challenge outcomes have been recorded. Ultimately, this work confirms that it is the absolute, rather than relative, HI titre that determines the extent of immunity and that there is a threshold HI titre beyond which ferrets are completely protected from infection. Nevertheless, this titre is much higher in ferrets than has been suggested for humans. Further, we are consequently able to show that using distance between strains within an antigenic map to predict cross-protection between influenza viruses can be misleading.

1. Introduction

Antigenic evolution is driven by selection for onward transmission: pathogens that can vary their antigenic properties may create opportunities to re-infect immunologically experienced hosts. Influenza is one such pathogen. Many mutants are generated as it replicates, enough of which have sufficiently altered antigenic properties to drive recurrent influenza epidemics in humans. The burden of these epidemics is such that we take care to vaccinate those most at risk from infection, but the pace of antigenic evolution means that we must expend exhaustive and expensive efforts to keep the vaccine relevant.

Attempting to understand past, and hence perhaps predict future, trajectories of influenza within antigenic space is of interest, both theoretically and practically. The primary (but by no means only) tool for this is the haemagglutination inhibition (HI) assay, a two-fold dilution assay in which the ability of serum to prevent the agglutination of red blood cells by a particular influenza virus is measured. HI titres are generally reported as the reciprocal of the highest dilution at which agglutination was prevented, with increasing dilutions indicating higher degrees of antigen binding. The assay can be criticised because it is often poorly reproducible between different laboratories [1–3], can only measure the ability of antibodies to prevent the binding of influenza to cells that the virus does not target *in vivo*, and assumes that ferrets and humans develop identical cross-reactive immune responses when challenged with the virus (for more detail see [4–6]). Nevertheless, it remains the gold standard within influenza research. Its results are of critical importance in the process of annual vaccine strain selection: does the serological response of a ferret (the standard animal model for human influenza) vaccinated with strain X suggest that they would be protected against the apparently emergent strain Y? If not, then the vaccine strain may need to be updated.

A seminal paper in this area introduced the concept of antigenic cartography [7]. The authors based their work on the reasoning that if a ferret that has been previously infected by strain X has an HI titre of P against X, and a titre of Q against another strain, Y, then the difference between P and Q tells us something about the proximity

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of strains X and Y within antigenic space. A two-fold difference in titre is taken to correspond to 1 unit of distance within this space.

The method employs multi-dimensional scaling to represent the past antigenic evolution of the virus within a two-dimensional space and it is now exceedingly rare for antigenic data to be presented without an accompanying antigenic map. The original application of these methods to 35 years of human A/H3N2 evolution illustrated that the antigenic evolution of human influenza is more punctuated than continuous: viruses cluster together in antigenic space for a number of years before suddenly jumping to a new point and the average distance between the identified antigenic clusters of H3N2 was 4.5 units. Subsequent applications to A/H1N1 and influenza B have revealed broadly similar patterns, although both appear to 'jump' somewhat less energetically [8].

There is, however, a potential difference between antigenicity (literally: the ability to bind to an antigen) and immunogenicity, or cross-protection. Indeed, a previous analysis of pre-challenge HI titre and its effect on the probability of a serological response in humans has suggested that a titre against a challenge strain as low as 17 may provide a 50% chance of protection against infection [9,10]. Not only is this titre notably below the normal threshold for detection in the assay (a titre of 40), but the results also suggest that any titre in excess of 200 is almost completely protective. Thus, for example, if antiserum raised against strain X has a homologous HI titre of 5120, but HI titres of only 1280 against strain Y and 80 against strain Z, then strains Y and Z are said to be 2 and 6 units of antigenic distance from X. Contrastingly, in terms of cross-protection, the above results suggest not only that infection with strain X provides complete protection against strain Y, but also that infection with X provides significant protection against strain Z.

Nevertheless, this analysis considers protection from infection in natural human infection, as opposed to experimental infection in ferrets, which is the actual source of almost all antigenic data and inference. Whether the relationship between HI titre and cross-protection is the same in ferrets as it is in humans is therefore important. Although this question has not been previously considered, relevant data has been presented in a number of different papers and is analysed together here. In particular, we focus on the results of a series of experiments in ferrets in the 1970s using the 1934 H1N1 and 1968 H3N2 viruses.

An advantage of the present study is that previous work on cross-protection in humans [9,10] could only try to infer if an individual had been infected by whether they had a four-fold or greater rise in HI titre over the course of the flu season (this is the definition of seroconversion [11], but see [12]). Yet cross-protection between strains must be related to the reduction in transmission of one following prior exposure to the other: something about which a serological response may itself tell us little. Fortunately, data on both the change in HI titre against a particular strain upon challenge and the viral titre in nasal wash taken after challenge has been presented for individual ferrets [13–19].

We therefore use this data to estimate the relationship between HI titre and (i) prevention of a serological response and (ii) reduction of virus production (and hence transmission) in ferrets. We then compare our results to those of [9] and consider the consequences for our understanding of the antigenic evolution of influenza.

2. Materials and methods

Using the keywords "immunity", "influenza" and "ferret" we identified papers in PubMed that might contain appropriate data. We then included data from studies where ferrets had pre challenge HI titres against a specific strain recorded, with post challenge measurements of HI titre and/or the amount of virus produced in nasal wash but excluded those ferrets that had been previously vaccinated or challenged with a strain that was HA-mismatched but NA-matched with the subsequent challenge strain (e.g. previously challenged with H1N2, measurements provided for subsequent challenge with H3N2). This resulted in 162 observations from 7 papers [13–19] (Supplementary Table S1).

2.1. Probability of a serological response

Typically, a four-fold rise in HI titre following challenge is taken as a positive serological response and is indicative of a successful infection; this was the measure used in the studies analysed by Coudeville et al. [9]. Following their lead, we start with a baseline probability (P) that an individual naïve ferret will exhibit a serological response when challenged with an influenza strain. To estimate the reduction in this probability in the presence of HI antibodies, this baseline probability is combined with a function ($0 \leq \rho(T_j, \eta) \leq 1$, where T_j is the HI titre and η is the associated vector of parameters) such that the probability of a serological response in a ferret upon exposure is

$$R\left(P, T_j, \eta\right) = P * (1 - \rho(Tj, \eta))$$

In line with Coudeville et al., we will specify the functional form associated with $\rho(T_j, \eta)$ as a two parameter inverse logit function ($\eta = \{\chi, \phi\}$) applied to log-transformed HI titre values:

$$\rho\left(T_j, \eta\right) = \frac{e^{\phi(\log_2(T_j)-\chi)}}{1+e^{\phi(\log_2(T_j)-\chi)}} = 1 - \frac{1}{1+e^{\phi(\log_2(T_j)-\chi)}}$$

and so

$$R(P, T_j, \chi, \phi) = \frac{P}{1+e^{\phi(\log_2(T_j)-\chi)}}$$

Coudeville et al. point out that ϕ corresponds to the titre that halves the amount of virus produced (it is a location parameter for the curve) whilst χ determines its steepness [9]. The main reason for choosing a two-parameter inverse logit function, as opposed to a different smooth increasing function, is that it leads to a straightforward method of constructing confidence intervals for the probability of protection. It is also the standard 'link function' for binary data so represents a natural choice here. For more information see [20].

2.2. Reduction in virus production in undiluted nasal wash

In these papers, the amount of virus produced in undiluted nasal wash is reported 3 days after challenge as 10^X EID50/ml (50% egg infectious dose per ml). In this paper we will model the effect of HI titres on reducing the size of this exponent.

The model estimates the amount of virus, V, which an individual ferret will produce when inoculated with influenza. In the absence of HI antibodies, the average amount, μ, corresponds to a baseline, $\gamma \log_{10}$ EID50/ml. This value may be reduced in the presence of HI antibodies however, and is therefore combined with a function describing the effect of HI titre ($0 \leq \pi(T_j, \theta) \leq 1$, where T_j is the HI titre and θ is the associated vector of parameters). Thus, the average amount of amount of virus produced by an exposed ferret is

$$\mu(\gamma, T_j, \theta) = \gamma(1 - \pi(T_j, \theta))$$

As above, we will specify the functional form associated with $\pi(T_j, \theta)$ as a two parameter inverse logit function ($\theta = \{\alpha, \beta\}$) applied to log-transformed HI titre values:

$$\pi\left(T_j, \alpha, \beta\right) = \frac{e^{\beta(\log_2(T_j)-\alpha)}}{1+e^{\beta(\log_2(T_j)-\alpha)}} = 1 - \frac{1}{1+e^{\beta(\log_2(T_j)-\alpha)}}$$