Chapter 5

Antigenic Cartography: Overview and Current Developments

Ioannis Sitaras

Abstract

Antigenic cartography is a powerful method that allows for the calculation of antigenic distances between influenza viruses or sera and their positioning on a map, by quantifying raw data from hemagglutination inhibition assays. As a consequence, the antigenic drift of influenza viruses over time can be visualized in a straightforward manner. Antigenic cartography is not only useful in the research of influenza virus evolution but also in the surveillance of influenza viruses. Most importantly, antigenic cartography plays a very important role in vaccine updating decisions, since by calculating the antigenic distances between a vaccine strain and circulating strains, an informed decision can be made on whether the distances are large enough to warrant a vaccine update or not. Recent improvements in antigenic cartography calculations have significantly improved its accuracy.

Key words Influenza, Hemagglutination inhibition, Antigenic cartography, Antigenic distance, Antigenic characterization

1 Introduction

The origin of antigenic cartography lies in the theoretical concept of shape space, first described by Edelstein and Rosen (1978) and Perelson and Oster (1979) [1, 2]. In the shape space concept, antigen and antibodies are considered to be points in an abstract shape space, whose coordinates represent properties related to antigen-antibody binding. Distances between points (or antibodies and antigens) are related to the affinity of one for the other. Therefore, a small distance represents a high binding affinity and vice versa. In more detail, a high binding affinity between antigen and antibody means that the antigen has a similar shape space vector to the antibody; therefore, the Euclidean distance between the two points in shape space describing that antigen and antibody would be close. Almost two decades after it was introduced, shape space took a huge leap forward when Lapedes and Farber developed the theoretical concept of shape space into an explicit concept [3], by using ordinal multi-dimensional scaling

Erica Spackman (ed.), *Animal Influenza Virus: Methods and Protocols*, Methods in Molecular Biology, vol. 2123,
https://doi.org/10.1007/978-1-0716-0346-8_5,

algorithms to analyze hemagglutination inhibition (HI) assay data. These algorithms provide an estimate of dimension, and they construct quantitative coordinates for points in shape space. Since they produce simultaneous coordinates for either antigens or antibodies, they define antigen-antigen and antibody-antibody interactions [3]. Another major advantage of using multi-dimensional scaling algorithms is that they allow for a recovery of high-resolution data, from low-precision data, such as the ones obtained from binding assays like the HI assay.

Only a few years later, and as a natural development of the work of Lapedes and Farber, antigenic cartography was introduced [4]. By applying a modified metric multi-dimensional scaling algorithm, distances between antigens and antibodies were calculated and positioned in a map. Due to the fact that antigens are tested against multiple sera and vice versa, there exist many measurements for each one, all of which can be used to more accurately pinpoint the position of an antigen or serum in a map. As a consequence, the resolution at which antigenic differences are determined and calculated was increased, and the visualization of data in the form of antigenic maps was allowed [4, 5]. In addition, by using modified metric multi-dimensional scaling algorithms, Smith et al. reduced the running time for the necessary distance calculations, thus allowing antigenic distances to be calculated even from very large HI assay datasets [4]. Antigenic cartography therefore could now be used not only for comparison of a narrow spectrum of viruses or sera but also to examine the antigenic evolution of viruses circulating in a large geographic region or over an extended period of time.

For influenza A, antigenic cartography utilizes data from the HI assay. It has already been discussed in Chapter 2 that, compared to other assays, the HI assay is still found to be the gold standard in antigen-antibody interactions, due to its simplicity, accuracy, and cost-effectiveness among other parameters [6]. Data from HI assays are used extensively in epidemiological and mathematical analysis of influenza outbreaks, particularly in measuring outbreak size, levels of herd immunity, calculations of antigenic distances between viruses by means of antigenic cartography, and predicting the success or failure of vaccines in stopping the transmission of circulating challenge strains [7–13].

2 Overview of Antigenic Cartography Methodology

In order to calculate antigenic distances between strains or sera, HI assays must first be performed. Ideally, sera must be available for all viruses to be compared, so that the homologous scenario (virus 1 and serum 1 against virus 1) is also available for comparison. In most cases, the HI titer between a strain and its homologous serum would also be the highest, compared to the titer of the same

Table 1
Representation of a simple 3 × 3 matrix in which HI titers are recorded. Titer 1-1 is the HI titer obtained when virus 1 is cross-checked against serum 1 (homologous), titer 2-1 is the HI titer obtained when virus 2 is cross-checked against serum 1, etc. Titers highlighted in gray show homologous titers

Sera	Viruses		
	1	2	3
1	Titer 1-1	Titer 2-1	Titer 3-1
2	Titer 1-2	Titer 2-2	Titer 3-2
3	Titer 1-3	Titer 2-3	Titer 3-3

Table 2
Representation of a 3 × 3 matrix with numerical HI titers in HI units. Titers highlighted in gray show homologous titers

Sera	Viruses		
	1	2	3
1	2048	1024	512
2	512	2048	128
3	128	512	256

Table 3
Outcome of Table 2 titers when using the largest titer per matrix line to subtract all other titers of the same line from. Note that subtracting all titers from the largest titer does not always result in the homologous titer being 0. This means that the antigenic dissimilarity between one virus and itself is larger than 0, which is a paradox. Titers highlighted in gray show homologous virus and sera

Sera	Viruses		
	1	2	3
1	0	1024	1536
2	1536	0	1920
3	384	0	256

strain against other heterologous, sera. All HI titers (i.e., all strains against all sera) are inputted in a spreadsheet in the form of a matrix. Table 1 shows a simple matrix in which three strains are compared against three sera.

The highest HI titer per matrix line or column (depending on whether distances for strains or sera need to be compared) is then used to subtract from it the lower titers. Taking a simple 3 × 3 matrix as an example (*see* Table 2), if the highest obtained HI titer per matrix line is 2048 HI units (or $\log_2 11$) and the other two HI titers in the same line are 1024 and 512, then the result from subtracting all HI titers from the highest titer would be 0, 1024, and 1536, respectively. Table 3 shows the outcome of all these

calculations. After these simple calculations, distance equations and multi-dimensional scaling algorithms are applied, and distance matrices are generated. From these distance matrices, map coordinates can be assigned in order to represent each strain or serum in a map. There exist several web pages in which users can input their HI data and generate maps of their strains or sera. However, it is necessary to point out that each web page often uses its own algorithms to calculate distances and generate maps and oftentimes gives only fairly accurate distance calculations. Although this may not be very important for standard research purposes, for vaccine updating decisions, accuracy is paramount.

3 Advantages of Antigenic Cartography

Perhaps the greatest advantage of antigenic cartography is that by allowing the quantification of distances between viruses or sera, it becomes a powerful and indispensable tool in the research of antigenic evolution, in the surveillance of influenza viruses, and in vaccine updating decisions. If it is demonstrated that the antigenic distance between an influenza vaccine strain and circulating influenza strains is sufficiently large, then in order for vaccination to be effective, the vaccine needs to be updated using a seed strain that is as antigenically close as possible to the circulating strains. This holds true for any influenza virus, either of human or animal origin. For example, in countries that use vaccination as a routine measure against avian influenza, vaccine updating has become much more important, especially since the introduction and diversification of Asian H5N1 lineages. Antigenic cartography allows the analysis of the magnitude, breadth, and focus of vaccination-induced immune response, thus providing additional information on the level of success of a particular vaccine [5, 14]. For this reason, antigenic cartography is routinely used by the World Health Organization (WHO) in its biannual assessment of human vaccines.

Another advantage of antigenic cartography is that it allows the quantitative interpretation and visualization of antigenic data and increases the resolution at which HI data can be interpreted [4, 15]. Finally, antigenic cartography is not only limited to influenza viruses but can also be applied to other antigenically-variable pathogens, such as HIV, rabies virus, and *Campylobacter jejuni*. Nor is the HI assay the only binding assay whose data can be used in antigenic cartography. Data from virus neutralization, enzyme-linked immunosorbent assays (ELISA), and complement fixations can also be used [5, 14].

4 Current Developments in Antigenic Cartography

Recent research has led to a significant improvement in the accuracy of antigenic cartography. When taking a closer look at the currently used antigenic cartography technique, there are some shortcomings which have significant effects on the accuracy of antigenic distance calculations of viruses and sera [16, 17].

First, when in the process of distance calculations the differences in HI titers for different strains or sera are standardized, current antigenic cartography calculations use the highest obtained HI titer to subtract all lower HI titers from. Although in many cases the highest HI titer also corresponds to the interaction between a strain and its homologous serum, this is not always so, due to artifacts and differences that are inherent in HI assays, such as the ones described in Chapter 2 (i.e., difference in protocols, materials, origin of sera, operator-specific differences, etc.). An approach to eliminate these artifacts is to always consider the HI titers between homologous strain and serum as the highest HI titers, which is biologically sound. Then, when subtracting all other HI titers from the homologous titers, the difference is standardized to be ≥ 0. This means that in case a nonhomologous HI titer happens to be higher than the homologous one, the difference between them is set to 0 (since a negative distance between strains or sera cannot exist) [16]. Bearing in mind the example titers of Table 2, this standardization would yield the following results shown in Table 4.

Another caveat of current antigenic cartography methods that has a huge significance in the accuracy of distance calculations and how they are represented in an antigenic map is that all current methods rely on multi-dimensional scaling algorithms. These algorithms are used to reduce a mathematical problem that exists in multiple dimensions (from dozens to hundreds depending on how many strains or sera are compared at the same time and how many measurements are available for the comparisons) to two or three dimensions, in order for the distances to be visualized in an

Table 4
Outcome of Table 2 titers when using the homologous titer per matrix line to subtract all other titers of the same line from. In the third matrix line, because the difference between the homologous HI titer (256 HI units) and titer 2–3 (512 HI units) is negative (−256 HI units), it is standardized to 0, since a negative distance does not exist. Note that all homologous titers are now 0 (i.e., the antigenic dissimilarity between a virus and itself is 0). Titers highlighted in gray show homologous virus and sera

Sera	Viruses		
	1	2	3
1	0	1024	1536
2	1536	0	1920
3	128	0	0

antigenic map. However, such multi-dimensional scaling distorts the geometry of shape space that exists between the compared strains or sera, the very concept on which antigenic cartography relies.

To demonstrate the geometric distortion that occurs when a higher dimension is scaled down to a lower one, consider a standard geographic map. In that map, all locations, be they flat or elevated (such as mountains), are projected onto a two-dimensional plane. However, since mountains are three-dimensional, they are "flattened" in order to be depicted on the map. As a consequence, covering a distance of 5 miles (8 km) over a flat area is not the same as covering the same distance but by having to climb Mount Everest. This is an example of the distortion that occurs when reducing a mathematical problem by only one dimension (from three-dimensional to two-dimensional). It follows that reducing the problem from dozens, or even hundreds of dimensions, to two dimensions (such as is the case when applying multi-dimensional scaling algorithms) results in even greater distortion, which in turn leads to greater inaccuracies in distance calculations and consequently construction of antigenic maps. Sitaras et al. have addressed this problem by constructing a master distance matrix that contains true distances between strains or sera and then depicting the distance between three or four strains in a two-dimensional or three-dimensional map, respectively, by extracting the corresponding 3×3 or 4×4 submatrices from the master distance matrix. This method increases the accuracy of positioning of viruses or sera in an antigenic map, by eliminating geometric distortion and preserving the original geometry between these viruses and sera while at the same time allowing for simultaneous visualization of all viruses or sera by extracting all the relevant submatrices [16, 17].

The final drawback with current antigenic cartography methods is that, for each strain to be compared, sera are often raised in one animal only. In the case of human influenza viruses, this animal is usually a ferret, while for avian influenza viruses ferrets or chickens are used. However, raising sera in only one animal disregards inter-individual variation in vaccination-induced immune response, a well-documented and ever-present phenomenon in both animals and humans for every type of vaccination [9, 10, 16, 18–25]. Indeed, individual variations in HI titers of a magnitude of 3–4 $\log_2$ or even greater are common [9, 10, 16, 26, 27]. The consequence of this is that differences in the immune response of individual animals can become confused with antigenic differences between the strains compared, since there is no way of distinguishing whether the immune response obtained from one animal only is indeed a representative one or is higher or lower than usual. It follows that raising sera against a strain in more than one animal should minimize the effect of inter-individual variation in immune response in distance calculations. The effects of inter-individual

variation in immune response on the calculation of distances between strains or sera and on the construction of antigenic maps have been quantified by examining how the positions of strains in the map are affected when distance calculations are based on sera raised in 1, 2, 8, and 12 animals [17, 28]. The results showed that distance calculations based on raising sera in only one animal per strain can under-estimate or over-exaggerate the antigenic distance between strains. Thus, even strains that are very antigenically distant may appear similar and vice versa. This can have substantial consequences for vaccine updating decisions, since errors like this may lead to either a false sense of security regarding the vaccines currently used, or an unnecessary and costly vaccine update. However, by raising sera in at least two animals per strain, the accuracy of antigenic distance calculations significantly improves, since the variance in immune response is considerably smaller.

References

1. Edelstein L, Rosen R (1978) Enzyme-substrate recognition. J Theor Biol 73 (1):181–204
2. Perelson AS, Oster GF (1979) Theoretical studies of clonal selection: minimal antibody repertoire size and reliability of self-non-self discrimination. J Theor Biol 81(4):645–670
3. Lapedes A, Farber R (2001) The geometry of shape space: application to influenza. J Theor Biol 212(1):57–69. https://doi.org/10.1006/jtbi.2001.2347
4. Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF, Osterhaus AD, Fouchier RA (2004) Mapping the antigenic and genetic evolution of influenza virus. Science 305(5682):371–376. https://doi.org/10.1126/science.1097211
5. Fouchier RA, Smith DJ (2010) Use of antigenic cartography in vaccine seed strain selection. Avian Dis 54(1 Suppl):220–223
6. Comin A, Toft N, Stegeman A, Klinkenberg D, Marangon S (2013) Serological diagnosis of avian influenza in poultry: is the haemagglutination inhibition test really the 'gold standard'? Influenza Other Respir Viruses 7(3):257–264. https://doi.org/10.1111/j.1750-2659.2012.00391.x
7. Noah DL, Hill H, Hines D, White EL, Wolff MC (2009) Qualification of the hemagglutination inhibition assay in support of pandemic influenza vaccine licensure. Clin Vaccine Immunol 16(4):558–566. https://doi.org/10.1128/CVI.00368-08
8. Zhao X, Fang VJ, Ohmit SE, Monto AS, Cook AR, Cowling BJ (2016) Quantifying protection against influenza virus infection measured by hemagglutination-inhibition assays in vaccine trials. Epidemiology 27(1):143–151. https://doi.org/10.1097/EDE.0000000000000402
9. Sitaras I, Rousou X, Kalthoff D, Beer M, Peeters B, de Jong MC (2016) Role of vaccination-induced immunity and antigenic distance in the transmission dynamics of highly pathogenic avian influenza H5N1. J R Soc Interface 13(114):20150976. https://doi.org/10.1098/rsif.2015.0976
10. Sitaras I, Rousou X, Peeters B, de Jong MCM (2016) Mutations in the haemagglutinin protein and their effect in transmission of highly pathogenic avian influenza (HPAI) H5N1 virus in sub-optimally vaccinated chickens. Vaccine 34(46):5512–5518. https://doi.org/10.1016/j.vaccine.2016.10.002
11. Kumar M, Chu HJ, Rodenberg J, Krauss S, Webster RG (2007) Association of serologic and protective responses of avian influenza vaccines in chickens. Avian Dis 51 (1 Suppl):481–483. https://doi.org/10.1637/7605-041706R1.1
12. Swayne DE, Suarez DL, Spackman E, Jadhao S, Dauphin G, Kim-Torchetti M, McGrane J, Weaver J, Daniels P, Wong F, Selleck P, Wiyono A, Indriani R, Yupiana Y, Sawitri Siregar E, Prajitno T, Smith D, Fouchier R (2015) Antibody titer has positive predictive value for vaccine protection against challenge with natural antigenic-drift variants of H5N1 high-pathogenicity avian influenza viruses from Indonesia. J Virol 89 (7):3746–3762. https://doi.org/10.1128/JVI.00025-15
13. Tian G, Zeng X, Li Y, Shi J, Chen H (2010) Protective efficacy of the H5 inactivated

vaccine against different highly pathogenic H5N1 avian influenza viruses isolated in China and Vietnam. Avian Dis 54 (1 Suppl):287–289

14. Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Jones TC, Rimmelzwaan GF, Osterhaus AD, Fouchier RA (2004) Mutations, drift, and the influenza archipelago. Discov Med 4 (24):371–377
15. Smith DJ (2006) Predictability and preparedness in influenza control. Science 312 (5772):392–394. https://doi.org/10.1126/science.1122665
16. Sitaras I, Kalthoff D, Beer M, Peeters B, de Jong MC (2014) Immune escape mutants of Highly Pathogenic Avian Influenza H5N1 selected using polyclonal sera: identification of key amino acids in the HA protein. PLoS One 9 (2):e84628. https://doi.org/10.1371/journal.pone.0084628
17. Sitaras I, Duijzer M, Peeters B, de Jong MC (2020) Influence of inter-animal variability of HI titers on antigenic cartography in the study of avian influenza viruses: towards making a better map. Heliyon Submitted Manuscript
18. Henn AD, Wu S, Qiu X, Ruda M, Stover M, Yang H, Liu Z, Welle SL, Holden-Wiltse J, Wu H, Zand MS (2013) High-resolution temporal response patterns to influenza vaccine reveal a distinct human plasma cell gene signature. Sci Rep 3:2327. https://doi.org/10.1038/srep02327
19. Pannuti CS, Morello RJ, Moraes JC, Curti SP, Afonso AM, Camargo MC, Souza VA (2004) Identification of primary and secondary measles vaccine failures by measurement of immunoglobulin G avidity in measles cases during the 1997 Sao Paulo epidemic. Clin Diagn Lab Immunol 11(1):119–122
20. Sawitri Siregar E, Darminto WJ, Bouma A (2007) The vaccination programme in Indonesia. Dev Biol 130:151–158
21. Leach RJ, Craigmile SC, Knott SA, Williams JL, Glass EJ (2010) Quantitative trait loci for variation in immune response to a foot-and-mouth disease virus peptide. BMC Genet 11:107. https://doi.org/10.1186/1471-2156-11-107
22. Tan PL, Jacobson RM, Poland GA, Jacobsen SJ, Pankratz VS (2001) Twin studies of immunogenicity—determining the genetic contribution to vaccine failure. Vaccine 19 (17–19):2434–2439
23. Roome AJ, Walsh SJ, Cartter ML, Hadler JL (1993) Hepatitis B vaccine responsiveness in Connecticut public safety personnel. JAMA 270(24):2931–2934
24. Wang ML, Skehel JJ, Wiley DC (1986) Comparative analyses of the specificities of anti-influenza hemagglutinin antibodies in human sera. J Virol 57(1):124–128
25. Tsang JS, Schwartzberg PL, Kotliarov Y, Biancotto A, Xie Z, Germain RN, Wang E, Olnes MJ, Narayanan M, Golding H, Moir S, Dickler HB, Perl S, Cheung F, Baylor HC, Consortium CHI (2014) Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell 157 (2):499–513. https://doi.org/10.1016/j.cell.2014.03.031
26. van der Goot JA, Koch G, de Jong MC, van Boven M (2003) Transmission dynamics of low- and high-pathogenicity A/Chicken/Pennsylvania/83 avian influenza viruses. Avian Dis 47(3 Suppl):939–941. https://doi.org/10.1637/0005-2086-47.s3.939
27. van der Goot JA, Koch G, de Jong MC, van Boven M (2005) Quantification of the effect of vaccination on transmission of avian influenza (H7N7) in chickens. Proc Natl Acad Sci U S A 102(50):18141–18146. https://doi.org/10.1073/pnas.0505098102
28. Sitaras I, de Jong MC, Spackman E (2020) Selection and antigenic characterization of immune-escape mutants of H7N2 low pathogenic avian influenza virus using homologous polyclonal sera. Virus Research Submitted manuscript