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A MATHEMATICAL MODEL OF ANTIGENIC KINSHIP OF VIRUSES

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Abstract. A combinatorial mathematical model describing the experimentally found diverse antigenic interconnections between different avian paramyxovirus (PMV) serotypes is suggested. According to the model, the whole network of antigenic interconnections is determined by the specific combinatorial sets of antigenic determinants, some of them being serotype-specific and the others being common with some other avian PMV serotypes. The suggested model is based on the postulates concerning PMV virion structure; bifunctional organization of PMV HN glycoprotein, its amount per virion and a mechanism of antibody-caused inhibition of its functional activities; a definition of an antigenic determinant as an elementary unit inducing and reacting only with a homologous type of antibodies.

The suggested model through operating with mathematically expressed different definitions of antigenic kinship describes some experimental phenomena connected with interserotype antigenic relationships, especially, a long-ago-known but unexplainable phenomenon of asymmetric cross reactivity.

Keywords. Viruses, inhibition, antibodies, antigenic kinship, titration.

1. INTRODUCTION

Antigenic relationships between different groups of viruses is an actual problem which is of high theoretical and practical importance. Its theoretical significance is connected with genetic variability, mutagenesis and natural evolution of viruses; association of viral protein three-dimensional structure with antigenic determinants; taxonomy and nomenclature of the viruses. Practical significance of the problem is connected with vaccination and diagnostics — the main aspects of medical and veterinary virology.

The theoretical considerations concerning antigenic kinship are founded mainly on serological tests, most of which are based on titrations of certain viral functional activities (enzymatic, hemagglutinating, infectious, *etc.*), the titers are being expressed as logarithms of end-points of serial dilutions inhibiting those activities. (An antiserum solution induced by the first virus can inhibit the virus. The solution is further diluted several times, every time becoming twice less potent, until the first virus cannot be inhibited any longer. The number of times this dilution is performed is roughly the homologous titer. It is possible that the first virus' antiserum can also inhibit the second virus, which is being compared to the first one. Then again the antiserum can be diluted until it can no loger inhibit the second virus. The number of times these dilutions are performed, each one by a factor of 2, is roughly the heterologous titer.) The antigenic relationship of viruses is connected with their cross reactivity, which is expressed as a difference between homologous and heterologous inhibition titers, i.e. the difference between the logarithms of the minimal potencies of the diluted antiserum of the first virus sufficient to inhibit the first virus and the second virus respectively.

A family of paramyxoviruses (PMV), affecting humans, mammalians, and birds, includes viruses of various antigenic serotypes. The serotypes are classified according to antigenicity of a viral envelope glycoprotein carrying two functional activities — hemagglutinating (HA) and neuraminidase (Nase) — on the same molecule (HN). A group of avian PMVs includes nine different antigenic serotypes (Alexander, 1982; Alexander *et. al.*, 1983). Various antigenic inter-relationships between most of the serotypes have been demonstrated (Lipkind and Shihmanter, 1986). These inter-relationships were shown by both HA inhibition (HI) and Nase inhibition (NI) tests. On the basis of these data the following was suggested:

Each HN molecule contains two sets of antigenic determinants related to two "domains" corresponding to either HA or Nase antigenic sites of the HN molecule. Some of these determinants correspond to the suggested "common-to-all-the-avian-PMVs" portion on the genomic level, meaning that they are common to either all the avian PMVs or, at least, to a part of them. There may be several different sorts of such common determinants. Together with this, there are "serotype-specific" determinants and various combination of the "common" and "serotype-specific" determinants may occur. The assumptions about "conserved" and "variable" portions of the respective gene and various ratios of these portions in different avian serotypes may explain the differences in intraserotype variability between different PMV serotypes.

Together with this, such approach has revealed some additional phenomena (Lipkind and Shihmanter, 1986):

1. Two viral activities (HA and Nase), although associated with the same molecule, may have different patterns of the cross reactivity.
2. Asymmetric cross reactivity when antiserum against the virus X inhibits activity of the virus Y while antiserum against virus Y either does not inhibit such activity or the virus X (one-side asymmetric cross reactivity) or inhibits it to a lesser degree than that of the homologous virus Y (two-side asymmetric cross reactivity) was a frequent case. This phenomenon was observed long ago without any explanation.
3. There may be a situation when two viruses do not differ from each other by certain serological tests ("identity") while they do differ by the same tests in their relationships with other avian PMV serotypes.

Those facts, together with maze-like network of the inter-connections, have had no satisfactory explanation. They can be explained by means of a combinatorial mathematical model of antigenic kinship which is presented below.

2. RESULTS

2.1. Postulates of the General Hypothesis

The proposed model and foregoing definitions are based on the following postulates regarding the virion structure, antibody induction, and virus-antibody interaction.

1. Each PMV virion contains a number C of identical HN molecules.
2. Each HN molecule contains two distinct antigenic sites (domains): HA domain and Nase domain.
3. Each HA domain consists of a number D_h of antigenic determinants: some of them are identical to each other while some may be different. Each Nase domain consists of a number D_n of antigenic determinants: some of them are identical to each other while some may be different.
4. An antigenic determinant is an element inducing only one type of antibody which is compatible only with this determinant type and this is the only type of antibody able to bind to this determinant. The pie (percentage distribution) of antibody types in a polyclonal antiserum is proportional to the pie of the determinant types of the antibody-inducing virus. For every antibody type there is one and only one type of determinants that can induce the antibody and react with it. The types of the determinants and antibodies are designated $T_1, T_2, T_3, \ldots, T_n$.
5. There is a universally constant percentage p such that the corresponding HA and Nase activities of the viruses are inhibited when at least $D_h \cdot C \cdot p$ or $D_n \cdot C \cdot p$ of the determinants per virion are bound by antibodies.
6. Sometimes we will assume that the above values D_h and D_n are universally constant, meaning that each of the compared viruses contains the same number of the determinants per HN molecule.

2.2. Definitions of Antigenic Kinship

We suggest four essentially different definitions of antigenic kinship between the compared viruses.

A. An experimental definition

Two viruses are antigenically kin if the antiserum against any one of them inhibits the corresponding (HA or Nase) activities of the other virus.

This kind of kinship is the one which is usually used in serological studies and designated as the cross reactivity. In experimental studies described in [Lipkind and Shihmanter, 1986] the cross reactivity was expressed quantitatively as the ratio between homologous and heterologous inhibition titers in HI and NI tests (practically, as a difference between the titers expressed in $\log_2$). Let us designate this type of kinship as the ***A***-sense kinship.

B. A quantitative theoretical definition based on the determinant pattern

Assume, first, that two compared viruses V_1 and V_2 have the same number of determinants. Let the virus V_1 contain 10 determinants of three types, namely, T_1, T_2, and T_3, in the following quantities: five determinants of T_1 (T_1:5), three determinants of T_2 (T_2:3) and two determinants of T_3 (T_3:2). Let the corresponding pattern of virus V_2 be: $\{T_1{:}0,\ T_2{:}5,\ T_3{:}5\}$. Then there are 3 common determinants of type T_2, and 2 common determinants of type T_3, totaling 5, that is 50 per cent (Figure 1). Let us designate this kind of kinship as the ***B***-sense kinship which can be defined as follows: ***B***-sense kinship between two viruses is the percentage of the determinants which are common in both viruses.

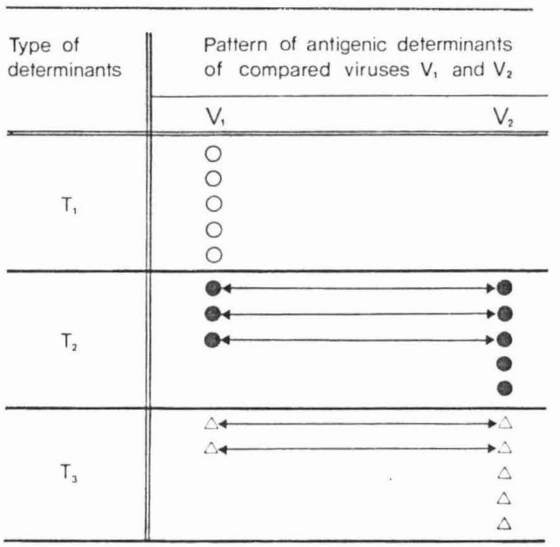

*FIG. 1. Graphic expression of the **B**-sense kinship between two viruses.* Fifty per cent of matching determinants between the two viruses. This antigenic kinship is symmetric.

For the above example the ***B***-sense kinship is symmetric. If the Postulate 6 concerning the constant number of the determinants per HN molecule is wrong, then the definition is not always symmetric: it may depend on what one considers as the first comparand and thus takes

its percentage of common determinants. We will see that from the quantitative point of view this intrinsic definition has nothing to do with the cross reactivity values obtainable experimentally.

C. A qualitative theoretical definition based on the determinant pattern

Taking into account the universal constant p (Postulate 5), we can obtain another kind of B-sense kinship. This kind of kinship can be defined qualitatively as holding if and only if $B \geq p$, where B is the percentage of the common determinants. In this case there is a certain indirect correlation between this qualitative definition and the experimentally defined A-sense kinship. Let us designate this kind of kinship as the C-sense kinship.

D. A quantitative theoretical definition forced to correlate with experimentally measurable data

Let us, first, designate the D-sense kinship between viruses V_1 and V_2 as the percentage among V_1's determinants of those types which are represented by at least one determinant in V_2.

Let us compare the virus V_1 to the virus V_2. Consider the subset of the V_1 antigenic determinants each of which can be found in the virus V_2 even though any matching determinant in the V_2 has already been taken into account for any other determinant. In the above-described example for the definition of the B-sense kinship there is no such determinant of T_1 type (Figure 1) because there is no T_1 in V_2. But all the rest of the determinants are separately matchable. Thus, there are 5 matchable determinants, *i.e.*, 50 per cent. This is the D-sense kinship between V_1 and V_2 viruses but only in this one-way direction (Figure 2). In the opposite direction the kinship is 100 per cent because each determinant of V_2 can be separately found in the virus V_1 (Figure 3).

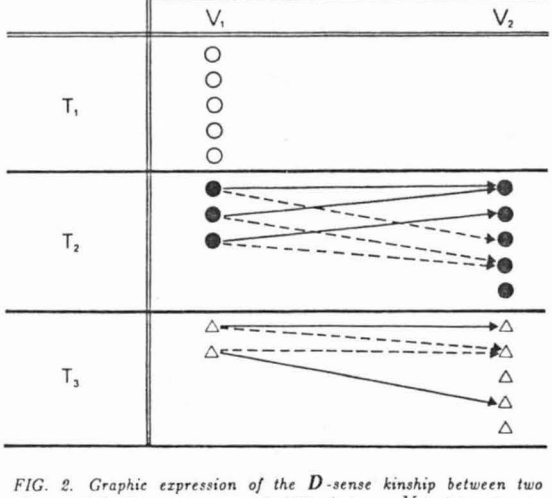

FIG. 2. Graphic expression of the D-sense kinship between two viruses. Solid lines express matchability between V_1's determinants and V_2's determinant types; the broken lines present graphically one of the alternative pictures of the matchability which is essentially the same, according to the D-sense definition of antigenic kinship. Fifty per cent of determinants of the virus V_1 have matching determinants in the virus V_2.

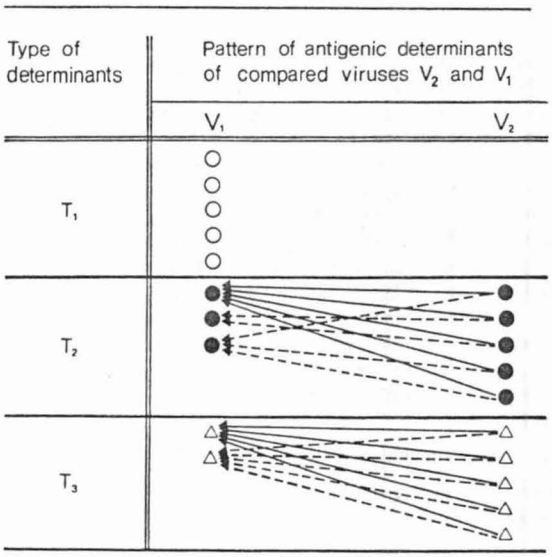

FIG. 3. Graphic expression of the D-sense kinship in the opposite direction: between the viruses V_2 and V_1. Designations are the same as those for Figure 2. All the determinants of the virus V_2 (100 per cent) have matching determinants in the virus V_1. This antigenic kinship is asymmetric.

This definition is asymmetric and it will help us later to interpret the phenomenon of asymmetry in the antigenic cross reactivity. In order to expose the asymmetry of D-sense kinship for any given pair of viruses V_1 and V_2, it is necessary, but not sufficient, that either there is a determinant type of which one of the viruses has determinants and the other does not, or that the Postulate 6 does not hold.

2.3. A Model of Cross-Reactivity

Let us first try to understand the mechanism of serologically measurable cross reactivity in view of the above-described postulates. Let the anti-V_1 anti-serum inhibit virus V_2. Knowing hypothetically the determinant pattern, let us try to predict the values of the cross reactivity which is defined as the ratio between homologous and heterologous inhibition titers.

Consider such a concentration of the anti-V_1 antiserum that just fully inhibits V_1. This means that p percent of V_1's determinants have the matching antibodies. If in order to bind one determinant a certain efficient number k of antibodies are needed, then there are kp antibodies per virion provided by this antiserum. This is the number of the matching antibodies, which is proportional to p. They are partitioned into types according to the pie of the determinants of the virus V_1 which has been used to induce the antibodies.

We designate the patterns of the viruses as follows: for the virus V_1 the pattern is $\{T_1:t_{11},\ T_2:t_{12},\ T_3:t_{13},\ \ldots,\ T_n:t_{1n}\}$, where t_{1i} is the quantity of the i-th type (T_i) of determinants in the virus V_1; for the virus V_2 the corresponding pattern is $\{T_1:t_{21},\ T_2:t_{22},\ T_3:t_{23},\ \ldots,T_n:t_{2n}\}$. The antibody pattern in anti-V_1 antiserum is: kpt_{11} of the 1st type, kpt_{12} of the 2nd type, ..., kpt_n of the n-th type (T_n).

Consider the reaction between the anti-V_1 antiserum and the virus V_2 having t_{21} determinants of the 1st type,

$\ldots, t_{2n}$ determinants of the n-th type. (Some of these variables t can be equal to zero.) We wish to find a minimal concentration u of the antiserum which fully inhibits the corresponding activities of the virus V_2. (The concentration u is relative to the antiserum's present state when it just fully inhibits the virus V_1.) We now have $kupt_{11}$ antibodies of the 1st type, $kupt_{12}$ antibodies of the 2nd type, ..., $kupt_{1n}$ antibodies of the n-th type. When reacting with the virus V_2, the anti-V_1 antibodies of the 1st type fully inhibit upt_{11} determinants of the 1st type but not more than t_{21} because that is what we have in the virus V_2. Or, we may say, $\min(t_{21}, upt_{11})$ of the virus V_2 determinants of the 1st type are bound. Totally, the number of the bound determinants is

$$\min(t_{21}, upt_{11}) + \min(t_{22}, upt_{12}) + , \ldots, + \min(t_{2n}, upt_{1n})$$

In order to bind the virus V_2, this should be no less than the percentage p of all V_2 determinants, namely

$$\sum_{i=1}^{n} \min(t_{21}, upt_{1i}) \geq p \sum_{i=1}^{n} t_{2i} \qquad (1)$$

Knowing the exact pattern of both viruses and wishing to find the concentration coefficient u, one just has to solve the equation (1) extracting the minimal solution for u. We expect that the logarithm of this value ($\log_2 u$) is the cross-reactivity indicator which would appear in the experimental tables. The reciprocal of this value ($\frac{1}{u}$) is the indicator of ***A***-sense kinship between the viruses.

Example 1:

Let the determinant pattern of the virus V_1 be (5, 0, 5) and the determinant pattern of the virus V_2 be (0, 0, 10).

Then the equation (1) becomes

$$[\min(0,5up) + \min(0,0up) + \min(10,5up)] \geq 10p \qquad (2)$$

Hence, here $\min(10,5up) \geq 10p$.

I.e., either $10p \leq 5up \leq 10$ or $5up \geq 10 \geq 10p$. *I.e.*, either $2 \leq u \leq \frac{2}{p}$ or $u \geq 2 \geq \frac{2}{p}$. Since $p \leq 1$, the minimal solution for u is $u=2$.

The values of the cross reactivity which were registered experimentally as the difference between the homologous and heterologous inhibition titers expressed in $\log_2$ would be in this case equal to $\log_2 2 = 1$. Note, that the reciprocal of this value $u = 2$, *i.e.*, 50 per cent. is exactly the kinship between the viruses V_1 and V_2, according to the ***D***-sense kinship definition.

Example 2:

For the viruses of *Example 1* let us compute the inverse: the corresponding values of the cross reactivity between the anti-V_2 antiserum and the virus V_1:

$$\min(5,0 \cdot up) + \min(0,0 \cdot up) + \min(5,10 \cdot up) \geq 10p \qquad (3)$$

This equation is satisfied if and only if either

$$10p \leq 5 \leq 10up \qquad (4a)$$

or

$$10p \leq 10up \leq 5 \qquad (4b)$$

In the case (4a) we have:

$$p \leq 0.5, \; u \geq \frac{1}{2p} \qquad (5a)$$

In the other case (4b) we have:

$$1 \leq u \leq \frac{1}{2p} \qquad (5b)$$

which can be satisfied only if $p \leq 0.5$.

Extracting the minimal u from both cases we get: $u = 1$ if $p \leq 0.5$, and no solution, *i.e.* no cross-reactivity, if $p > 0.5$.

Thus, in this example, the expected cross-reactivity $V_2 - V_1$ is different from the cross-reactivity $V_1 - V_2$ evaluated in *Example 1* (where $u=2$, i.e. 50%). This is an obvious asymmetry.

Example 3:

For more complex determinant patterns we shall skip the way of calculation and present here only the results.

Let the pattern of V_3 be (6,2,2) and of V_4 — (0,2,8).

If $p=0.6$ then for the cross-reactivity between V_3 and V_4 the minimal u is 3.4. If $p=1$ then $u=4$. In the opposite direction (between V_4 and V_3) there is no solution for u either for $p=0.6$ or (consequently) for $p=1$, *i.e.* no cross-reactivity is expected in the opposite direction for these p.

Example 4:

Let (11,4,17,0.9,6,2,0,0,7,1,8,15,5.3,12) be the pattern of V_5 and (1,2,8,10,9,0,15,6.5,5,6.9,6,0,5,13) be the pattern of V_6. (16 types of determinants are considered. Each virion has a total of 100 determinants of these types.)

For the cross-reactivity model between V_5 and V_6: $u=1.15$ if $p=0.1$, $u=1.95$ if $p=0.6$, $u=5.8$ if $p=0.7$, $u=10$ if $p=0.789$, and there is no solution if $p \geq 0.79$.

In the opposite direction, $u=1.9$ if $p=0.6$, $u=3.05$ if $p=0.8$, $u=12.5$ if $p=0.889$, and there is no solution if $p \geq 0.9$.

Example 5:

Let (911,4,17,0,9,6,2,0,0,7,1,8,15,5.3,12) be the pattern of V_7 and (3,900,8,10,9,0,15,6,5,5,6,9,6,0,5,13) be the pattern of V_8. (16 types of determinants are considered. Each virion has a total of 1000 determinants of these types.)

For the cross-reactivity model between V_7 and V_8: $u=65$ if $p=0.1$, $u=229$ if $p=0.9$.

In the opposite direction, $u=75$ if $p=0.1$, $u=305$ if $p=0.9$.

Example 6:

This example shows what may happen if Postulate 6 is not valid. Let us compare V_7 (1000 determinants) and V_6 (100 determinants).

For the cross-reactivity model between V_7 and V_6: $u=10$ if $p=0.789$. In the opposite direction, $u=915$ if $p=0.889$.

2.4. The Relationship Between the Kinship Definitions and the Cross-Reactivity Model.

Analyzing the equation (1) for the general case we conclude that when V_2 is kin to V_1 according to the ***C***-sense kinship definition, then the modeled reciprocal value of the cross-reactivity ($1/u$), *i.e.* the expected ***A***-sense kinship, can roughly be estimated by the value the ***D***-sense kinship, provided the determinant patterns are sparse, *i.e.* most determinant types of one virus have no counterparts in the other virus. This estimation was precise in *Examples 1* and *2*. Furthermore, we can conclude that when the ***A***-sense kinships between V_1 and V_2 in both

directions exist (symmetric qualitative cross-reactivity) then the viruses are kin in the *C*-sense. Nevertheless, there is no direct quantitative dependence between *B*-sense kinship and *A*-sense kinship, *i.e.* the experimental cross-reactivity should not be indicative quantitatively of any "straight-forward topological resemblance" between the viruses.

2.5. On the Phenomenon of Asymmetric Cross Reactivity

The phenomenon of asymmetric cross reactivity has been met with quite often without any explanation or comment. The usually used formula of cross reactivity is that suggested by [Archetti and Horsfall, 1951], namely, $r = \sqrt{r_1 \cdot r_2}$, where r_1 is the ratio obtained by dividing the heterologous titer of V_2 virus by the homologous titer of V_1, and r_2 is the ratio obtained when the heterologous titer of V_1 virus is divided by the homologous titer of V_2 virus. Such a formula levels out the possible asymmetric cross reactivity.

In [Lipkind and Shihmanter, 1986] the phenomenon of asymmetric cross reactivity was often found. It consisted in that the anti-V_1 antiserum inhibited the activities of the virus V_2, while the anti-V_2 antiserum either did not inhibit at all the activities of the virus V_1 (one-side asymmetry), or the titer of heterologous inhibition by the anti-V_2 antiserum was significantly lower than that shown by the anti-V_1 antiserum (two-side asymmetry).

In view of the above-presented cross-reactivity model and definitions of the antigenic kinship the asymmetry is normal and expected. All the hypothetical pairs of viruses in examples 1-6 exhibited two-sided asymmetry. For some high values of p most of the pairs exhibited also one-sided asymmetry. It can be easily proved that there should be one-sided asymmetry (according to our model) for some high values of p if and only if the *D*-kinship is asymmetric for the given pair of viruses (provided Postulate 6 is valid.)

3. DISCUSSION

The aim of these studies was to develop a hypothesis describing the experimentally found diverse antigenic relationships between avian PMV antigenic serotypes using the universal principles of a combinatorial mathematical model. The hypothesis is based on the principle that both HA and Nase antigenicity of any PMV serotype detected by HI and NI test, respectively, is determined by specific sets of distinct antigenic determinants as elementary antigenic units. Accordingly, the antigenic relationships between avian PMVs are attributed to the presence of the same determinants in the sets concerning different serotypes, and the whole network of the antigenic relationships is determined by respective combinatorial mosaics of the determinant types in each serotype. Of the six postulates of the general hypothesis, the first one reflects a well established fact (Choppin and Scheid, 1980). The second postulate has a solid experimental ground (Lipkind and Shihmanter, 1986; Portner, 1981; Smith and Hightower, 1980, 1982). The third as well as the fourth postulates, which are not unreservedly established facts, form the central body of the general hypothesis. The fifth postulate is a logical inference from the accepted mechanisms of inhibition of the viral HA and Nase activities. The sixth postulate is a certain simplification made for the sake of convenience of the mathematical model but this is a simplification of the conceivable picture rather than that of certain established facts.

Our model explains the following phenomena: (a) asymmetric cross reactivity, and (b) the phenomenon of the difference in cross reactivity between two "identical" PMVs (with no difference in HI and (or) NI titers between them) which was expressed either (both) by a spectrum of the interconnections or (and) by different quantitative patterns of their cross reactivity with the other avian PMVs (Lipkind and Shihmanter, 1986). The phenomenon of asymmetric cross reactivity (a) has been observed very often, mainly with influenza viruses, but also with PMVs (Numazaki, *et al.*, 1968; Rybinskaya, 1976; Starke, *et al.*, 1977; Tumova *et al.*, 1979; Yamane *et al.*, 1982). The only explanation (if any) of the asymmetric cross reactivity was connected with the notion of the "avidity". However, the experimental results of (Lipkind and Shihmanter, 1986) excluded such possibility. Phenomenon (b) has never been explained before.

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