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Antigenic Relationships Between Avian Paramyxoviruses

II. A Combinatorial Mathematical Model of Antigenic Kinship

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With 3 Figures

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Summary

A combinatorial mathematical model describing the antigenic relationships found between different avian paramyxovirus (PMV) serotypes (LIPKIND and SHIHMANTER, 1986) is presented. According to the model, the network of the antigenic interconnections is determined by the specific combinatorial sets of antigenic determinants, some of them being serotype-specific and the others being common to certain other avian PMV serotypes. The suggested model is based on certain postulates concerning PMV virion structure; the bifunctional organization of PMV haemagglutinin-neuraminidase (HN) glycoprotein, its amount per virion and a mechanism of antibody-caused inhibition of its functional activities; the definition of an antigenic determinant as an elementary unit inducing and reacting only with a homologous type of antibodies.

The model interprets in specific terms some serological results, in particular the old but mysterious phenomenon of asymmetric cross reactivity.

Introduction

The group of avian paramyxoviruses (PMV) numbers to date 9 serotypes (2, 4) characterized by the antigenic specificities of haemagglutinin (HA) and neuraminidase (Nase) which are antigenic sites on a large glycoprotein molecule (HN) which carries both activities (3, 12, 13). In addition, certain established avian PMV serotypes cross react by HA inhibition (HI) and Nase inhibition (NI) tests (see reviews 1, 2, 7). These cross reactions

between different PMV serotypes, complicate the problem of taxonomy and also raise the question of whether these antigenic relationships reflect antigenic drift of the avian PMV HN glycoprotein. The purpose of the present studies was to carry out a comprehensive comparison of representatives of all the avian PMV serotypes by HI and NI tests in order to reveal the network of interconnections (8) and to elaborate the quantitative characteristics of the antigenic cross reactivity.

In the previous communication antigenic relationships between different avian PMV serotypes studied by means of both HI and NI tests have been described (8). The whole network of the relationships turned out to be diverse and complicated; some of the interconnections appeared to be asymmetric and the interconnections displayed by HI and NI tests did not always go in parallel.

On the basis of these data the following assumptions have been suggested.

a) HA and Nase antigenic sites are topologically distinct entities on the HN molecule and undergo independent antigenic drift.

b) Genomic material coding for the HN glycoprotein consists of a "common-to-all-the-avian-PMVs" portion and a "serotype-specific" portion, on one side, and of a "conserved" portion and a "variable" portion, on the other side; the ratios between the portions may be different in different serotypes.

c) The group of avian PMV can be subdivided into two subgroups, one including PMV- 2 and PMV-6 and the other including PMV-1, PMV-3, PMV-4, PMV-7, PMV-8 and PMV-9 serotypes.

Analysis of the antigenic relationships revealed the following phenomenon: a certain pair of compared viruses may be very close (if not identical) to each other by either (both) HI or (and) NI tests, but may be strikingly different from each other by the pattern of their cross reactivity with the other members of the avian PMV group. This difference may be expressed by either (both) the spectrum of interconnections or (and) by different quantitative patterns of their cross reactivity with the other avian PMVs, including the phenomenon of asymmetric cross reactivity. This led to the conclusion that if two viruses seemed to be identical by mutual cross reactions but were different in the spectrum or quantitative pattern of their cross reactions with other avian PMN serotypes, then they were not identical but antigenically different and this requires an adequate explanation.

In the previous communication (8) the following suggestion was put forward: Each HN molecule contains two sets of antigenic determinants (we use "determinant" to mean the same as an "epitope" in relation to monoclonal antibodies). These determinants are related to two "domains" corresponding to the HA and Nase antigenic sites. Some of these determinants correspond to the suggested "common-to-all-avian-PMVs" portion at the genomic level, meaning that they are common to either all the avian PMVs,

indeed, or, at least, to one of the two proposed subgroups of avian PMVs. There may be not merely one sort but several different sorts of such common determinants. Together with this, there are "serotype-specific" determinants and various combinations of the "common" and "serotype-specific" determinants may occur. The assumptions about "conserved" and "variable" portions of the respective gene and the various ratios of these portions in different avian serotypes may explain the differences in intraserotype variability between different PMV serotypes, e.g. the stability of PMV-1 (NDV) and the variability of PMV-2 (Yucaipa-like viruses).

In the present communication a mathematical combinatorial model is suggested to describe the phenomena, including the phenomenon of one way asymmetric cross reactivity.

Results

I. Postulates of the General Hypothesis

The elements of the proposed model can be defined as follows.

[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 as well as Nase domain consists of number D_h and D_n, respectively, of antigenic determinants: some of them may be 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 determinant that can induce the antibody and react with it. The types of the determinants and antibodies are designated as T_1, T_2, T_3, ..., 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 $D \cdot C \cdot p$ of the determinants per virion are bound by antibodies.

[6] Sometimes we will assume that the above values D_h and D_n are constant meaning that each compared viruses contains the same number of the determinants per HN molecule.

II. Definitions of Antigenic Kinship

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

A. An Experimental Definition — [A]-sense Kinship

Two viruses are antigenically kin if antiserum against any one of them inhibits the corresponding (HA or Nase) activities of the other virus. This definition is bidirectional and, thus, symmetric. Let us designate this kind of kinship as [A]-sense kinship.

This kind of kinship is, in fact, the one which is usually used in serological studies and designated as cross reactivity. In our studies described in the previous communication (8) the cross reactivity was expressed quantitatively as the ratio between homologous and heterologous inhibition titres in HI and NI tests (practically, as the difference between the titres expressed in log_2).

B. A Quantitative Theoretical Definition Based on Determinant Pattern — [B]-sense Kinship

[B]-sense kinship between two viruses is designated as the percentage of the determinants which are common to both viruses.

Assume, first that two viruses V_1 and V_2 have the same D number of determinants. Let the virus V_1 contain ten 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 three common determinants of type T_2, and two common determinants of type T_3, totalling five, that is 50 per cent (Fig. 1). For the above example

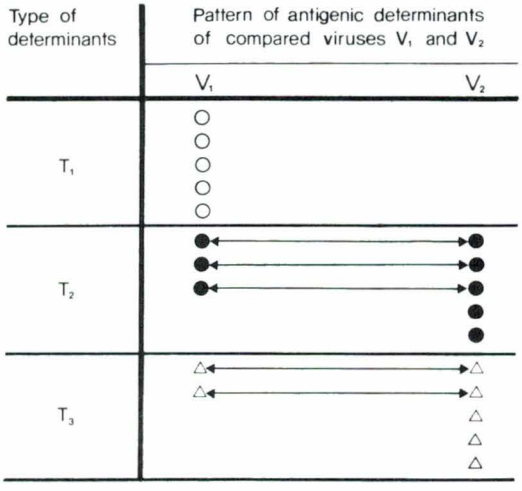

Fig. 1. Graph expressing [B]-sense kinship between viruses V_1 and V_2. The lines express matchability between the determinants which are common in both viruses. 50 per cent of matching determinants between the two viruses. The antigenic kinship is symmetric

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 would depend on what one considers as the first comparand taking its percentage of common determinants. We will see that from the quantitative point of view (see Appendix) this intrinsic definition has nothing to do with the cross reactivity values obtainable experimentally. For the example given in the Appendix the [B]-sense kinship is symmetric.

C. A Qualitative Theoretical Definition Based on the Determinant Pattern — [C]-sense Kinship

Taking into account the universal constant p from the 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 $[B] = p$, where [B] is the number 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 [C]-sense kinship.

D. A Quantitative Theoretical Definition Forced to Correlate with Experimentally Measurable Data — [D]-sense Kinship

[D]-sense kinship between viruses V_1 and V_2 is designated as the percentage of determinants on V_1 of which at least one is found on V_2.

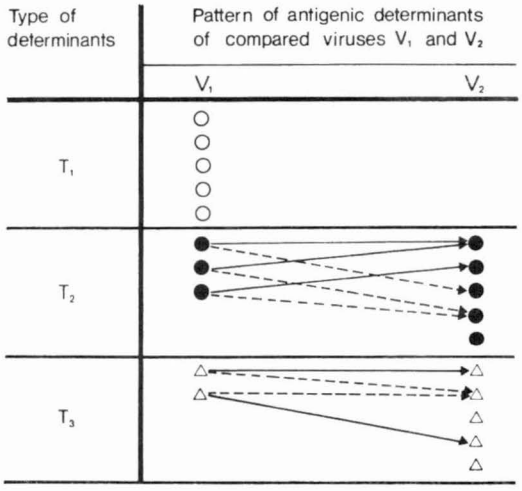

Fig. 2. Graph expressing [D]-sense kinship between viruses V_1 and V_2. Solid lines express matchability between V_1 and V_2 determinant types; the broken lines present graphically one of the alternative pictures of the matchability which is essentially the same, according to the [D]-sense definition of antigenic kinship. 50 per cent of determinants of the virus V_1 have matching determinants in the virus V_2. The antigenic kinship is asymmetric

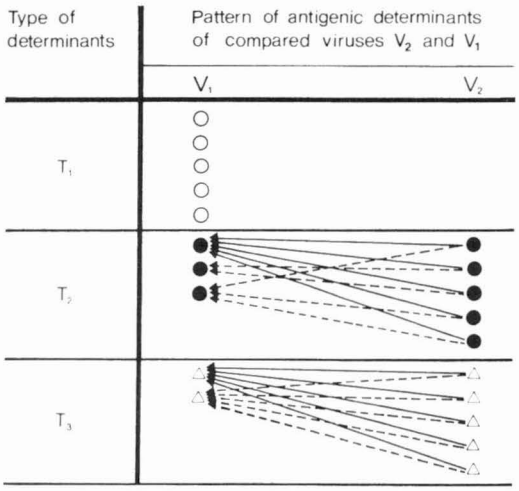

Fig. 3. Graph expressing [D]-sense kinship between viruses V_2 and V_1. Designations are the same as those for Fig. 2. All the determinants of the V_2 (100 per cent) have matching determinants in the virus V_1. The antigenic kinship is asymmetric

Let us then compare virus V_1 with virus V_2. Consider the subset of V_1 antigenic determinants each of which can be found in the virus V_2 (even though any matching determinant in V_2 has already been taken into account for any other determinant). In the earlier example of [B]-sense kinship there was no determinant of T_1 (Fig. 1) because there was no T_1 on V_2. But all the rest of the determinants are separately matchable. Thus, there are five matchable determinants, i.e. 50 per cent. There is, in fact, a [D]-sense kinship between V_1 and V_2 viruses but only in one direction (Fig. 2). However, in the opposite direction the kinship is 100 per cent because each V_2 determinant can be found on virus V_1 (Fig. 3). This definition is asymmetric and will help us later to interpret the phenomenon of asymmetry in 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 a determinant is possessed by one of the two viruses, or the postulate [6] does not hold.

III. The Relationship Between the Kinship Definitions and Experimental Data

The main task of the proposed mathematical model was to describe the mechanism of serological cross reactivity in terms of the above-described postulates and to analyze which of the above kinds of kinships best describes the experimental data (8). The mathematical structure simplified by basic equation [1] (see Appendix) permitted us to come to the following conclusions.

In the case when the virus V_2 is kin to the virus V_1 according to the [C]-sense kinship (i.e. the universally constant percentage p from the postulate [5] is taken into account) the experimental value d of the kinship ([A]-sense

kinship) is approximately equal to the value of kinship according to the [D]-sense kinship (the percentage of the determinant types in V_1 which are represented by at least one determinant in V_2). In the case of symmetric cross reactivity the viruses V_1 and V_2 can be considered to be related by [C]-sense kinship. There is no direct quantitative connection between the experimental values of the cross reactivity ([A]-sense kinship) and the percentage of the determinants which are common in both viruses ([B]-sense kinship). Therefore, [D]-sense kinship is the most suitable means of describing in general terms the kinship between the V_1 and V_2 viruses measured by serological cross reactivity ([A]-sense kinship).

IV. Explanation of the Phenomenon of Asymmetric Cross Reactivity

The phenomenon of asymmetric cross reactivity has been met with pretty often but is not understood or explained. The usual formula for expressing cross reactivity is that suggested by ARCHETTI and HORSFALL (5), namely, $r=\sqrt{r_1 \cdot r_2}$ where r_1 is the ratio obtained by dividing the heterologous titre of V_2 virus by the homologous titre of V_1, and r_2 is the ratio obtained when the heterologous titre of V_1 virus is divided by the homologous titre of V_2 virus. Such a formula evens out any asymmetric cross reactivity.

In the present studies (8) asymmetric cross reactivity was often found. An anti-V_1 serum inhibited V_2 virus activities while the anti-V_2 serum either did not inhibit them at all (one-sided asymmetry), or the titre of heterologous inhibition by anti-V_2 serum was significantly lower than that shown by anti-V_1 serum (two-sided asymmetry).

In the context of the above definitions of antigenic kinship the explanation of the phenomenon of the asymmetry of the cross reactivity is that it is due to [D]-sense kinship. As can be seen (see Appendix), both the two-sided and, especially, one-sided asymmetric cross reactivity (for which no explanation has been proposed till now) can be explained on the basis of the [D]-sense kinship definition either by taking different values of p, or by different selections of the determinant types.

Discussion

The aim of these studies was to develop a hypothesis for our serological results by using a general combinatorial mathematical model. Of the six postulates of the general hypothesis the first reflects a well established fact (6). The second postulate has a solid experimental basis (8, 10, 14, 15). The third and the fourth are not definitely established facts and form the core of the hypothesis. The fifth postulate is a simple inference from the accepted mechanisms of inhibition of the viral HA and Nase activities. The sixth postulate is a simplification made for the sake of convenience of the mathematical model.

A newly formulated hypothesis can be tested internally and also by comparing it with any available alternative. Such an alternative runs as follows:

The experimentally found antigenic relationships between avian PMV serotypes are not due to the selection of specific combinantions of unchanging determinants but to gradual changes in the determinants themselves. Such changed determinants induce correspondingly changed antibodies but they still retain some kinship to the original type of antibodies induced by the original determinants. This would be shown as cross reactivity between both the "old" and "new" determinants which is a model of the cross reactivity between different serotypes. Thus, the latter is an expression of a total antigenicity which is due to all the determinants which have undergone the changes.

The difference between the combinatorial model and the alternative hypothesis is that the former is based on the "all or none" principle, implying that any changes of antigenic determinants occur by leaps and a new determinant displays no cross reactivity with the original determinant. As the suggested model can describe all the phenomena connected with the experimental data this supports the view that antigenic determinants change by leaps.

These phenomena include the following: a) asymmetric cross reactivity, and b) "identical" PMVs (with no difference in HI and [or] NI titres between them) differ in their cross reactivity with the other avian PMVs which is expressed either (both) by spectrum or (and) by quantitative patterns of the cross reactivity (8). We have not found any mention of the latter phenomenon in the available literature. However, asymmetric cross reactivity has often been observed, mainly with influenza viruses but also with PMVs (9, 11, 16, 17, 18). The only explanation given was based on "avidity", but our experimental results include eight avian PMV serotypes and we have been able to exclude low avidity of one of the members of a pair of viruses as an explanation for such asymmetry. Our model includes different definitions of antigenic kinship and gives clear explanations for both the phenomena. The model also fits in with the suggestion made in the previous communication (8) that there are "serotype-specific" and "common-to-all-avian-PMVs" parts of the HN glycoprotein-coding gene, on one hand, and "variable" and "conserved" parts of the same gene, on the other.

As HI and NI are tests based on the inhibition of functional activities (haemagglutinating and enzymatic, respectively) and as antigenic and functionally active sites of viral HN glycoprotein may be topologically distinct, we wonder whether the same results would be obtained using antibody binding tests e.g. ELISA, and radioimmunoassay. The study of cross reactivity between avian PMVs should therefore be extended to test further the validity of the model.

Appendix

Suppose there is cross reactivity between V_1 and V_2 viruses. Accordingly, let anti-V_1 antiserum 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 titres.

Consider a concentration of anti-V_1 antibodies that fully inhibits V_1. This means that p per cent of the V_1-belonging determinants have matching antibodies. If in order to bind one determinant a certain efficient number k of antibodies is 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 antibodies. We designate the patterns of the viruses as follows: for the virus V_1 the pattern is: $T_1 : t_{11}$, $T_2 : t_{12}$, $T_3 : t_{13}$, . . ., $Tn : t_{1n}$, 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: $T_1 : t_{21}$, $T_2 : t_{22}$, $T_3 : t_{23}$, . . ., $T_n : t_{2n}$. The antibody pattern in anti-V_1 antiserum is kpt_{11} of the first type, kpt_{12} of the second 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 first type, . . ., 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. Henceforth, we can regard ku as an unseparable value because only relative concentrations are of interest and k is eliminated as a result of division of concentrations:

$\frac{ku_1}{ku_2} = u_1/u_2$. 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 first type, $kupt_{12}$ antibodies of the second type and, . . ., $kupt_{1n}$ antibodies of the n-th type. When reacting with the virus V_2, the anti-V_1 antibodies of the first type fully inhibit upt_{11} determinants of the first type but no 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 first type are bound. Totally, the number of the bound determinants is

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

This must be equal to the percentage p of all the virus V_2 determinants, namely,

$$\sum_{i=1}^{n} min\ (t_{2i},\ upt_{1i}) = p \sum_{i=1}^{n} t_{2i}. \qquad [1]$$

Knowing the exact pattern of both the 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 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 $\left(\frac{1}{u}\right)$ is the indicator of [A]-sense kinship between the viruses.

Example 1: Let the virus V_1 determinants pattern be *5, 0, 5* and the virus V_2 determinants pattern be *0, 0, 10*. Then the equation [1] becomes
$[\min(0{,}5 \cdot \mathrm{up}) + \min(0{,}0 \cdot \mathrm{up}) + \min(10{,}5 \cdot \mathrm{up})] \geq 10\,\mathrm{p}$.
Hence, here $min\ (10,\ 5\ up) \geq 10\,p$.

I.e., either $10\,p \leq 5\ up \leq 10$ or $5\ up \geq 10 \geq 10\,p$. 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 recorded experimentally as the difference between homologous and heterologous inhibition titers expressed in log_2 (8), would in this case be equal to $log_2 2 = 1$. The reciprocal of this value $u = 2$, i.e. 50 per cent, is the precise kinship between the viruses V_1 *and* V_2, using the [D]-sense kinship definition.

Within the limits of the above definitions of antigenic kinship we consider the following explanation of the phenomenon of asymmetric cross reactivity.

Example 2: Consider again the virus V_1 having the determinants pattern *(5, 0, 5)* and the virus V_2 having the determinants pattern *(0, 0, 10)*. The indication of the cross reactivity between the anti-V_1 antiserum and the virus V_2 expressed as a reciprocal value of the fraction between the homologous and heterologous inhibition titres is equal to 2, i.e. the [A]-sense antigenic kinship as well as that in the [B]-sense and [D]-sense is equal to 50 per cent.

Example 3: Let us compute the inverse: namely, the corresponding values of the cross reactivity between the anti-V_2 antiserum and the virus V_1:

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

This equation is satisfied if and only if either

$$10\,p \leq 5 \leq 10\,up$$

or

$$10\,p \leq 10\,up \leq 5.$$

In the former case we have:

$$p \leq 0.5,\ u \geq \frac{1}{2p}.$$

In the other case we have:

$$1 \leq u \leq \frac{1}{2p}$$

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

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