Vaccinia Virus Hemagglutination Receptor in Chickens Determined by K Isoantigen Locus (36947)

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(Introduced by S. E. Mergenhagen)

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Hemagglutination by vaccinia and some other viruses, as well as by purified phospholipids and a variety of Wasserman antigens is an inherited trait in the chicken (1). Failure to agglutinate is a recessive autosomal trait not previously associated with any other known gene (2). We have now found that alleles determining the K system of erythrocyte (RBC) isoantigens appear to control quantitatively the hemagglutination titer for vaccinia virus. Specific *K* genotypes of chicken RBC may permit a very sensitive assay for viruses that have a phospholipid hemagglutinin. This observation may also provide an explanation for some of the polymorphism of RBC surface antigens based on the selective value of cell surface properties other than antigen–antibody reactions, in this case phospholipid absorption.

RBC of mature individual birds from several lines polymorphic for the 12 isoagglutinating blood group systems A, B, C, D, E, H, I, J, K, L, P and R were tested for isoantigens of each system using a large panel of isoantisera (3–4).

Red cells of these birds were tested for hemagglutination by mumps, rubella, and vaccinia viruses using standard techniques (5). All virus hemagglutinins were produced in the Laboratory of Viral Immunology, Division of Biologic Standards. We thank Dr. L. Barker for the vaccinia hemagglutinin and Dr. F. Ennis for the mumps hemagglutinin, each grown on chicken chorioallantoic membrane. The rubella hemagglutinin was grown on BHK/21 cells (6). Retests on the same bleedings and on repeat bleedings of individuals over a 3-mo period each gave a mean difference of less than one twofold dilution for each of the three viral antigens.

The range of hemagglutination titers for vaccinia was 0 to 10,240 for adult cells while pooled cells from 1-day-old chicks did not agglutinate. There was no difference in titer distribution between the sexes nor in regard to 11 of the 12 RBC antigen systems.

The genotypes at the *K* locus were significantly related to titer of vaccinia agglutination (Table I). Testing the normal approximation of the binomial, the mean of the log of the titers of the four homozygous *KK* types differed from the mean of the three *Kk* types ($p < .001$) and both *KK* and *Kk* differed from *kk* homozygotes ($p < .001$). The mean titer for vaccinia of cells possessing the three codominant isoantigens when homozygous ranked in the order $K_1 > K_2 > K_3$ suggesting that the RBC receptor sites may have differed in avidity for vaccinia as well as antigenic specificity. The recessive allele *k* when heterozygous reduced the mean titer of its associated allele (K^1, K^2 or K^3) against vaccinia by about fourfold and RBC of the *kk* genotype, which does not have a known isoantigen, did not agglutinate. In addition to reduction of hemagglutination titer when compared with homozygotes (K^1K^1, K^2K^2 and K^3K^3), the RBC heterozygous for the *k* allele showed marked variation among individuals within the same genotype.

In addition to the two K^1K^1 birds with titers of 1024, one K^1 positive bird gave repeated titers of 10,240. It was the offspring of a $K^1k \times K^1k$ cross but was not proven by breeding to be K^1K^1. Homozygous K^1K^1 birds should be of practical value by provid-

TABLE I. The Relationship of *K* Genotype as Determined by Direct Typing and Progeny Testing to Vaccinia Agglutination Titer in Adult Chicken Erythrocytes.

	No. of chickens; titer:									
Genotype	<10	10	20	40	80	160	320	640	1280	2560
K^1K^1									2	
K^1K^2						1		1	5	1
K^2K^2						3	4	1	2	
K^2K^3					2	1	1		1	
Total *KK*					2	5	5	2	10	1
K^1k	2		1	1	3	1	3			
K^2k	1	6	2	11	2	9	9			
K^3k	6									
Total *Kk*	9	6	3	12	5	10	12			
kk	59									

ing a more uniform source of RBC in agglutination tests for a variety of pox viruses and Wasserman antigens.

All chicken RBC were also tested with mumps and rubella viruses. Whether from chicks or adults they showed equivalent hemagglutination for mumps virus. The hemagglutination with rubella virus showed no association with any of the 12 isoantigen systems tested nor did it show any simple genetic pattern of familial segregation. Rubella titer was examined by sex, age, and laying status (Tables II). Males gave significantly higher titers than females in each of four lines tested. Two different tests of different individual birds classified by laying status or by age in terms of generations within the flock suggested that titer increases with age in both sexes and that laying birds may have lower titers than nonlaying birds (Table II). The data are insufficient for analysis of the heritability of the titer variations. Fifty individual 1-day-old chicks from a different flock each gave a titer of 32.

Hemagglutination due to vaccinia differs from that caused by other viruses because agglutination is due to a phospholipid particle separate and distinct from the virus. It can be removed from agglutinated RBC with homologous antiserum (but not by washing) without inactivating the RBC sites. Hemagglutinin separates from infective virus in organic solvents and can be inactivated by lecithinase and cobra venom (7–9).

These properties suggest that the receptor site controlled by the *K* locus reacts to the general chemical structure of phospholipid in a weak and reversible way. The various codominant *K* alleles might then be associated with the presence of several antigenically different types of receptor site of varying avidity for phospholipid antigens while the *k* allele results in the presence of an inactive site or no site at all.

There was no microscopic evidence of a mixed population of agglutinating and nonagglutinating cells in *k* heterozygotes. This suggests that both alleles are active in all cells of heterozygotes, as expected from the codominance of the *K* alleles. However, the increased titer range among different cells heterozygous for *k* suggests that there may be second order heterogeneity in number of sites produced on the cell by different alleles controlling structures of similar antigenic specificity.

The posthatching developmental pattern of vaccinia hemagglutination differs from that of mumps and rubella. The absence of vaccinia hemagglutination in cells from 1-day-old chicks indicates that the response to vac-

TABLE II. Relationship Between Rubella Hemagglutination Titer and Sex, Age, and Laying Status of Chickens.[a]

	Titer					
	<8	8	16	32	64	128
Total males			27	38	11	5
Total females	42	45	29	14	6	
Test A (Jul.)						
S generation males					3	5
V generation males				8	7	
R generation females	2	1		9	3	
S generation females	9	2	2	3	3	
V generation females	13	3	1	1		
Test B (Oct.)						
males			27	30	1	
R generation females						
laying		8	3			
nonlaying		2	6	16	5	
T generation females						
laying	18	23	4			
nonlaying	2	4		1		

[a] R, S, T, V, indicate the order of consecutive generations.

cinia hemagglutinin is a property which matures during development, either as a result of ubiquitous environmental stimuli or as the result of a switch in the active genes involved in the synthesis of red cell membranes, similar to changes in hemoglobin in frog metamorphosis and human development (10–12). In either case, the ability to develop susceptibility to vaccinia hemagglutination is related to the *K* locus genotype.

Cellular susceptibility to subgroup B avian leukosis-sarcoma viruses is closely associated with an inherited RBC antigen (4) determined by an allele of the R system, genetically distinct from the K system. The association of RBC receptors for viruses and antibodies may provide an explanation for much of the polymorphic molecular variation of cell surfaces. The presence of easily identifiable isoantigens should provide a useful tool for the study of specificity relationships between cell membranes and viruses.

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