trodes. This fact is considered as indicating that no effect due to electrolysis is present in our records.

4) The effects of impurities in the ANS preparation used (Eastman Organic) were ruled out by experiments demonstrating no difference in the optical signals when recrystallized samples were used. Artifacts arising from possible impurities in the rhodamine B and pyronin B preparations (Allied Chemicals) were not ruled out.

Attempts to interpret the experimental results described above are severely hampered by the fact that the physicochemical factors which influence the quantum yield of fluorescence in rhodamine B and pyronin B are not well known. The slow and progressive change in fluorescence seen in Figs. 1 and 2 suggests that an electrophoretic effect (accumulation or depletion of the dye and/or salts in and near the membrane) may be present. Various ions in the axon interior are expected to affect the rate (and the direction) of migration of the large dye molecules during voltage-clamp. Furthermore, changes in concentration of these ions can affect the conformation of the macromolecules (proteins and phospholipids) which in turn may influence the quantum yield of fluorescence. (Note that fluorescence of pyronin B in aqueous solution can be strongly quenched by addition of various macromolecules to the solution.)

In the case of ANS, it appears reasonable to assume that a finite portion of the fluorescent light from the axon in the resting state derives from the dye molecules located in the hydrophobic layer (*9*) in the axon membrane. Since the increase in the membrane conductance is considered to be associated with an increase in the water content of the major diffusion barrier in the axon membrane, a decrease in fluorescence during nerve excitation is expected (*5*). In order to explain the increase in fluorescence associated with a hyperpolarizing voltage pulse, one might postulate a change in the thickness of the hydrophobic layer during hyperpolarization. Further experimental and theoretical studies are needed to understand the physicochemical nature of the findings described in this report.

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**References and Notes**

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## Susceptibility to an Avian Leukosis-Sarcoma Virus: Close Association with an Erythrocyte Isoantigen

Abstract. *A dominant gene for susceptibility to early steps of cellular infection by subgroup B avian leukosis-sarcoma viruses is associated with the presence of an erythrocyte isoantigen. This gene may control both an isoantigen and a cell membrane receptor for an oncogenic virus.*

Host cell susceptibility to the early events of infection by subgroups A and B avian leukosis-sarcoma viruses is controlled in each case by a dominant allele at one of two independent, autosomal loci (*1*). Dominance of susceptibility indicates that the presence of a specific cell-membrane receptor substance is required for virus infection (*2*). It has been suggested that this receptor may also be antigenic and thus be detectable by immunologic methods (*1*). We now present evidence for a close association between the occurrence of an erythrocyte isoantigen and susceptibility to subgroup B leukosis-sarcoma viruses.

Individual chickens of inbred RPRL line 100, known to be segregating at the loci controlling susceptibility to viral subgroups A and B, are assigned genotypes each generation by test mating with a known double-recessive line (RPRL line 7, subline 2) (*1*). Progeny embryos are tested for susceptibility by inoculation of Rous sarcoma virus (RSV) of subgroups A [BH-RSV (RAV-1)] and B [BH-RSV(RAV-2)] on the chorioallantoic membrane (CAM) (*1, 3*).

Erythrocytes from a group of these birds were tested for agglutination with a large panel of independently prepared isoantiserums specific for antigens controlled by 11 blood group systems (*4*)—A, B, C, D, E, H, I, J, K, L, and P—and with an isoantiserum containing a new fraction of agglutinins, designated R1 (*5*). Among the birds tested, segregation was disclosed for the previously established I, K, and L systems and for the new R system. The distribution of blood group classes for these four systems among the parents classified for susceptibility (eight or more embryos surveyed for each of the virus subgroups) is presented in Table 1. Inspection of these data shows

Table 1. The distribution of blood group classes (BGC) for four segregating systems among parents independently assigned susceptibility genotypes for the subgroups A and B leukosis-sarcoma viruses. Each bird was mated to a homozygous resistant line and assigned a genotype based on at least eight embryos for each subgroup assayed on the chorioallantoic membrane. The lower case letter (*a* or *b*) represents the locus concerned with the virus subgroup. The *r* and *s* superscripts represent resistance and susceptibility, respectively. The results signify the number of birds assigned to each class.

| BGC* | Susceptibility genotypes (No.) | | | | | |
|---|---|---|---|---|---|---|
| | Subgroup A | | | Subgroup B | | |
| | $a^sa^s$ | $a^sa^r$ | $a^ra^r$ | $b^sb^s$ | $b^sb^r$ | $b^rb^r$ |
| $I^2I^2$ | 2 | 4 | 4 | 4 | 9 | 3 |
| $I^2I^s$ | 0 | 6 | 6 | 12 | 6 | 2 |
| $I^sI^s$ | 0 | 1 | 1 | 3 | 1 | 0 |
| $K^2$— | 2 | 7 | 6 | 16 | 8 | 2 |
| *kk* | 0 | 4 | 5 | 3 | 8 | 3 |
| $L^1L^1$ | 1 | 5 | 5 | 9 | 4 | 0 |
| $L^1L^2$ | 1 | 5 | 4 | 8 | 8 | 4 |
| $L^2L^2$ | 0 | 1 | 2 | 2 | 4 | 1 |
| $R^1$— | 2 | 10 | 9 | 19 | 16 | 0 |
| *rr* | 0 | 1 | 2 | 0 | 0 | 5 |

* The upper-case letter represents the blood group locus (or system) and the numbered superscript the specificity of the corresponding cell surface antigen. For the K and R systems only K2 and R1 reagents, respectively, were available; thus, two genotypes in each system are indistinguishable, for example, R1-positive birds are either $R^1R^1$ or $R^1r$ and are designated $R^1$—.

that there is no obvious association between blood group classes of the I, K, or L systems and the genotypes for subgroup A or B virus susceptibility. On the other hand, there is a distinct association between the R blood group classes and subgroup B virus susceptibility; all 35 birds possessing the $b^s$ susceptibility allele (19 $b^sb^s$ and 16 $b^sb^r$) belong to the R1-positive class ($R^1$—) while the five birds of the resistant genotype $b^rb^r$ were R1-negative ($rr$).

In addition to the parents included in Table 1, there were 22 others having fewer than eight embryos surveyed for each virus subgroup. Combining these data, there was a total of 57 R1-positive parents producing 83.1 percent susceptible embryos among 557 assayed, while from nine R1-negative parents only one susceptible embryo occurred among 75 assayed. Analysis of these and corresponding data for other blood group systems showed that only the R system-subgroup B virus susceptibility relationship was statistically significant.

These data indicate that there is a close association between the $R_1$ antigen and cellular susceptibility to subgroup B leukosis-sarcoma viruses. Independent confirmation of this association in line 100 was sought in two ways. Nineteen additional progeny-tested line 100 birds were typed for the $R_1$ antigen. The cells of 12 homozygous resistant ($b^rb^r$) birds did not possess the $R_1$ antigen, while the cells of six out of seven previously classified as heterozygotes ($b^sb^r$) did have the $R_1$ antigen. The exceptional parent produced only two susceptible embryos among a total of 17 and should perhaps be considered to represent a technical error in genotype classification for susceptibility. Otherwise, the data on this second group of line 100 agree with the association originally observed (Table 1).

A second, more direct method of checking this association was employed as follows. Known heterozygous ($b^sb^r$) line 100 males exhibiting the $R_1$ antigen were mated with homozygous resistant ($b^rb^r$) line 7, subline 2 females lacking the $R_1$ antigen. At 11 days of incubation, the embryos were inoculated on the CAM with a dose of subgroup B RSV known to give over 100 pocks on the membrane of susceptible embryos (*1*). At 19 days of incubation, blood was removed from an allantoic vein, and the CAM's were classified for susceptibility. Differentiation between resistant and susceptible CAM's was clear; susceptible embryos had no less than 30 pocks, while resistant embryos had no more than two. The agglutination tests were read by an observer who had no knowledge of the membrane classification. There were 191 susceptible embryos and they were R1-positive; there were 194 resistant embryos and they were R1-negative. Two embryos had the $R_1$ antigen but were classified as resistant. The expected 1:1 ratio was observed for both traits. The two exceptional embryos could have resulted from recombination or technical error.

This close association has been observed in line 100 and in some related lines; however, in a few other lines, now under study, the $R_1$ antigen may be absent from the cells of individuals transmitting susceptibility to subgroup B viruses. For example, in the inbred line in which the R1 isoagglutinins were produced (DeKalb line 2), there exist R1-positive susceptible, R1-negative susceptible, and R1-negative resistant types.

Either of two hypotheses could explain these data. Under the first hypothesis, the $R_1$ antigen and subgroup B RSV susceptibility in line 100 could be controlled by the same gene. The susceptible R1-negative individuals observed in DeKalb line 2 and some other lines may be accounted for by assuming the existence of at least two $b^s$ alleles—one R1-positive and the others R1-negative. Under the second hypothesis, the susceptibility to subgroup B RSV and the $R_1$ antigen may be controlled by closely linked genes or by a complex locus. Thus, the existence of susceptible individuals not possessing $R_1$ antigen could represent recombination. However, to date we have not found conclusive evidence of recombination within line 100, nor have we located a single adult bird having the $R_1$ antigen, regardless of line, which does not carry susceptibility to subgroup B virus. Only the reciprocal situation appears to exist with certainty, while both would be expected if recombination occurred with detectable frequency.

If subsequent data validates the first hypothesis, this will be the first demonstration of a single gene controlling a viral receptor and an isoantigen. In the mouse, susceptibility to leukemogenic viruses is influenced quantitatively by H-2 alleles (*6*), possibly as a result of differential antibody response by various H-2 genotypes (*7*). Although the specific gene action resulting in allelic differences in susceptibility may differ between loci, selective mechanisms operating in populations of animals infected with ever changing viral phenotypes could play a prominent role in maintaining certain isoantigenic polymorphisms (*7*).

A relation has been shown between glycoproteins that include the M and N human blood group specificities and the receptors for hemagglutination by influenza viruses (*8*). A complete correspondence between the $R_1$ and isoantigen and susceptibility to B subgroup leukosis-sarcoma viruses would afford material that should prove useful in investigating the specific molecular structure of a viral receptor segregating within a population.

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5. Each blood group gene is represented by italics ($R^1$); the corresponding red cell antigen is designated by the system letter with a subscript ($R_1$); and the corresponding antibody is written R1. The R1 agglutinins are present in each of five antiserums resulting from a series of repeat immunizations of a male recipient with the blood cells from a single female donor. Description of the immunogenetic analysis of these antiserums and data from matings designed to establish the genetic relationship of the $R_1$ agglutinogen with prior blood systems are in preparation.
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