FIRST CASE OF A NONALLELIC INTERACTION PRODUCT AS A SPECIES-SPECIFIC RED CELL ANTIGEN¹

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Received September 9, 1968

IMMUNOGENETIC species relationships have been disclosed most notably by IRWIN and associates with doves and pigeons (family Columbidae). By backcrossing fertile species-hybrids to a parental species, progeny were obtained that demonstrated assortment of the genetic factors controlling antigens (e.g. CUMLEY and IRWIN 1942). Antigenic differences between the species have been investigated in considerable detail (e.g. IRWIN and MILLER 1961), but the major antigenic variables were summarized by IRWIN (1959) into several groups. These groups were designated by numbers originally indicating their order of discovery and lately renamed to imply a related antigenic system in several species. Thus, group 1 is immunogenetically related in *Streptopelia chinensis*, *S. senegalensis*, *S. humilis*, *S. orientalis*, and *Columba guinea* and symbolically identified as ch-1, se-1, hu-1, etc.

This neat packaging must be supplemented by certain exceptions—the “hybrid substances” or interaction products. First discovered by IRWIN (1932) in hybrids between pearlneck, *S. chinensis*, and ring neck doves, *S. risoria*, such antigens were missing from both parents, but were regularly produced by the hybrids. The problem became more pertinent to general immunogenetic theory with further such discoveries in ducks, rabbits and fowl (McGIBBON 1944; COHEN, 1956; and BACHARACH, McGIBBON and IRWIN 1960) and the discovery that certain pure homozygous species give agglutination with antisera specific for a “hybrid substance” (see MILLER 1956).

Some studies on the “hybrid substance” disclosed interaction of alleles (see BRYAN and MILLER 1953; MILLER 1956; COHEN 1956). But a “hybrid substance” reactivity in a pure homozygous species is evidence that the substance is not the interaction product of two alleles. Of course, this reasoning could be upset by assuming immunologic crossreaction (or less orthodox genetic situations).

PALM and IRWIN (1962) first found a nonallelic interaction product associated with the species-specific group-4 antigenic character of four species of doves. This new interaction product was still associated with hybrids and backcrosses thereof, since it required the genome of a fifth species, *risoria*, in the background. IRWIN (1966 a, b) noted that the gene or antigen interacting with that for group 4 (or

¹ Journal Paper No. J-6057 of the Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames. Supported in part by Project 1681, and by the Summer Research Participation Program of the National Science Foundation, Fund No. GE 7597.

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one linked to it) was presumably the one controlling the *risoria* alternative for the group 8 species-specific antigens, that is, homozygotes for the groups 4 and 8 lacked the interaction product.

MATERIALS AND METHODS

The dove colony available included many *Streptopelia risoria*, the ring neck dove, eight *S. humilis* (also called *tranquebarica*), the dwarf turtle dove, and backcross hybrids of these two species derived from a single F_1 female (MILLER 1964). Also available were many domestic pigeons, *Columba livia*, several mourning doves, *Zenaidura macroura*, and a few F_1 generic hybrids between *C. livia* and *S. risoria* and between *Z. macroura* and *S. risoria*.

Two antisera R18 and R19 were produced in rabbits by multiple (3 times a week for 4 weeks) intravenous injections of $\frac{1}{2}$ to 1 ml of washed red cells of dwarf turtle doves diluted to about a 10% suspension in 0.92% saline. The antisera and absorbed reagents were stored frozen. Red cell reactivity was determined by macroscopic inspection of saline agglutination tests. Critical combinations that appeared to lack reaction were checked microscopically for traces of agglutination, and sometimes absorptions with appropriate red cells were used to confirm presence or absence of a particular specificity. For further details see MILLER (1956) or references in STIMFLING and IRWIN (1960).

Species-specific antigens hu-1, hu-4, and hu-8 in backcross hybrids of *humilis* with *risoria* have been described by STIMFLING and IRWIN (1960) and further noted by PALM and IRWIN (1962) and MILLER (1964). They appear genetically independent of each other, and except for hu-4, which seems to lack an alternative in *risoria*, they appear controlled by genes co-dominant to ring dove alternatives, (MILLER and BRYAN 1953; STIMFLING and IRWIN 1960). While 3 species, *S. capicola*, *orientalis*, and *senegalensis*, have exhibited individual differences in the presence or absence of detected group 4 specificities, all individuals of *S. chinensis* and *S. humilis* have always possessed 4 and 8 group antigens as expected of a homozygous species character (PALM and IRWIN 1962).

RESULTS

The reactions of the antisera and absorbed reagents with various cell types are indicated in Table 1. Aliquots of antisera R18 and R19 before and after absorption at $\frac{1}{4}$ dilution by an excess of red cells of ring neck doves possessed a titer of 1/4096 to 1/16,384 with test red cells from the dwarf turtle dove, the homologous cell type. Red cells from backcross doves possessing hu-1 and hu-8 also were nearly as strongly reactive, but those from hu-4 birds were nonreactive.

R19, after further absorption by cells possessing hu-1 and hu-8 plus cells exhibiting a new factor hu-y (MILLER and WEBER, unpublished), was exhausted of reactivity with any of the cell types and combinations thereof. However, R18 further absorbed by hu-1, hu-4, hu-8, hu-y, hu-1-4, and hu-1-8 type cells still reacted to about three quadrupling dilutions (i.e., 1/16 to 1/64), with the red cells of *humilis* and with those hybrids possessing hu-4 and 8 together. Absorptions with hu-4-8 cells eliminated the reaction as expected. In all, 125 backcross hybrids have been tested with this reagent. Only 8, plus the F_1 whose cells possessed both hu-4 and hu-8 showed reactions. No hu-4-8 bird failed to react. Red cells of eight dwarf turtle doves (all tested) also reacted. Red cells from domestic pigeons, mourning doves, and the two generic hybrid types mentioned were non-reactive.

TABLE 1

Range of titer of unabsorbed and absorbed immune sera obtained from rabbits immunized with dwarf turtle dove (*S. humilis*) erythrocytes

Test cells	Antisera absorbed by pooled <i>S. risoria</i> red cells				
	Unabsorbed antisera				Further absorbed by hu-1, 4, 8, 1-4, 1-8 selected cells
	R18	R19	R18	R19	R18
<i>S. humilis</i>	7	6	7	6	3
Backcross birds					
hu-1	—*	—	—	5	0†
hu-4	—	—	0	0	0
hu-8	—	—	6	6	0
hu-4, 8	—	—	6	6	3
<i>S. risoria</i>	6	5	0	0	0
<i>C. livia</i>	6	5	2	2	0
<i>Zenaidura macroura</i>	6	5	3	1	0
F ₁ <i>risoria/humilis</i>	—	—	—	7	3
F ₁ <i>C. livia/S. risoria</i>	—	—	—	—	0
F ₁ <i>Z. macroura/S. risoria</i>	—	—	—	—	0

* a dash = not tested.

† a zero = no reaction.

Titer in this table is indicated in numbers of quadrupling dilutions i.e. 1 = dilution of test fluid with three parts saline to make a 1/4 dilution, etc. Thus, 6 would indicate a reaction at 1 part test fluid in 4096 parts saline.

DISCUSSION

Interaction products in doves and pigeons were known to occur in the species cross heterozygotes, e.g., for hu-8 and ri-8 (BRYAN 1953), although genes closely linked were not entirely excluded. BRYAN (1953) and MILLER (1956) noted that both d-4 and d-11 (now ch-4 and ch-8) of *S. chinensis*, genetically independent of each other, nevertheless, cross-reacted with specificities against the single antigen C, now C^s of *Columba guinea* (in contrast to *livia*) which has its own interaction product. Therefore, the nonallelic loci controlling groups 4 and 8 might interact with each other as PALM and IRWIN (1962) and IRWIN (1966 a, b) discovered for hu-4 and ri-8 and we found for hu-4-8. One can even postulate that the C locus might be "ancestral" (IRWIN and MILLER 1961) and in the genus *Streptopelia* has duplicated with slight divergencies of specificity to groups 4 and 8. This is reminiscent of the situation noted by IRWIN and CUMLEY (1945).

The observation that red cells of other species could cross react with a reagent for a "hybrid substance" was long known (see IRWIN and CUMLEY 1945; MILLER 1956), but the nature of the genetic components was not known (BRYAN 1953; PALM and IRWIN 1962). The probable nature of the "hybrid-substance" reaction of cells of a regular species now is obvious as interacting loci. It was disclosed only by hybridization and assortment into identifiable genetic components. However, the possible serological crossreaction of reagents for interaction products controlled by alleles with the product of a third allele in a homozygous species is not a mutually exclusive situation with that of nonallelic interaction. Both may be

operating. Note in this context that the bison specific character [R'-S'] is cross-reactive with R' and S' occurring separately in cattle (MILLER 1966).

The suggestion by Fox (1949) that nonallelic interaction products are perhaps more common than suspected because of the constancy of the genetic background is supported by our data. Indeed, the immunogenetic analysis is beginning to fit quite comfortably with classic genetic phenomena now that we know of antigens controlled by recessive genes (STORMONT 1951; MILLER 1965), genetic inhibition of antigenic products (RENDEL, NEIMANN-SORENSEN, and IRWIN 1954; LEVINE *et al.* 1955), and now nonallelic interaction.

SUMMARY

A reagent was made from rabbit antisera against red cells of the dwarf turtle dove by absorptions including hu-4 and hu-8 antigenic cell types from backcross birds of the dwarf turtle dove. This reagent reacted only with group 4-8 cells of the presumed homozygous species or in heterozygous backcross hybrids with both 4 and 8 together. Since the genes controlling hu-4 and hu-8 are independent, this represents the first nonallelic immunogenetic product detected directly as a species character.

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