

A "HYBRID" ANTIGEN IN MONKEYS

(*Theropithecus gelada* × *Papio hamadryas*)

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Immunogenetic researches have revealed the principles of inheritance of the antigens of the erythrocytes of man [14, 17] and animals [9-11] and the blood serum antigens of animals [2-5, 15]. It has been found that the inheritance of antigens does not obey the law of complete recessiveness. Hybrids contain antigens characteristic of the parent species, and when hybrids are crossed this antigenic complex is dissociated in accordance with the hypothesis of unifactorial inheritance. Certain writers have found a particular form of hybrid antigenic specificity by means of hemagglutination reactions in erythrocytes [7, 8, 12, 13] and by precipitation in the serum of hybrid birds [1]. Recently, however, certain workers have been unable to detect a hybrid antigen in the tissues of embryos of hybrid sea urchins [6] and in the erythrocytes of hybrid birds [16].

An electrophoretic analysis of the serum of hybrid birds (*Streptopelia risoria* × *Streptopelia humilis*; *Streptopelia risoria* × *Columba livia*) and mammals (*Bos taurus* × *Bos bison*) carried out by Scheinberg [15] showed that the serum of the hybrid contains practically no components other than the serum proteins of the parent species.

Work has recently been published which does not confirm the previous findings in respect to the presence of specific hybrid antigens in hybrids. For this reason we have made a special investigation of hybrid monkeys, using the highly sensitive immunological reaction of anaphylaxis.

EXPERIMENTAL METHOD

We studied blood antigens (of erythrocytes and serum) of the intergeneric F₁ hybrid ♂ *Theropithecus gelada* × *Papio hamadryas* ♀. The age of the hybrid was 1 year, and of the parents: ♀ - 5 years, ♂ - 14 years. The hybrid was obtained at the Institute of Experimental Pathology and Therapy. In its external form it occupied an intermediate position between the parent species.

We used the anaphylaxis with desensitization reaction in guinea pigs. Animals weighing 300-350 g were sensitized by subcutaneous injection of antigens from erythrocytes (0.8 ml of a 25% suspension) and blood serum (0.15 ml) of the hybrid.

On the 21st day after the sensitizing injection all the animals were desensitized to the paternal (*T. gelada*) antigens by means of several subcutaneous and intraperitoneal injections of erythrocytes or serum. Next day total desensitization was verified (intravenously), and if desensitization was not complete a further test was made. In some cases several such tests had to be made before complete desensitization was obtained and the guinea pig failed to react to the intravenous injection of paternal erythrocytes or serum. When desensitization to the paternal antigens was complete, the guinea pigs were desensitized to the maternal (*P. hamadryas*) antigens by intravenous injections of the mother's erythrocytes or serum. A test for total desensitization was made after 2 h, and if desensitization was incomplete a second test was made. When complete desensitization had been obtained after 2 h a reacting dose of antigen from the erythrocytes or serum of the hybrid was given. The erythrocytes were washed repeatedly in physiological saline to remove serum and plasma. For the sensitizing injection a 25% suspension of washed erythrocytes was used. For the desensitizing and reacting injections, the erythrocytes were hemolyzed in distilled water, the cell membranes

TABLE 1. Anaphylaxis with Desensitization Reaction in Guinea Pigs Sensitized with Erythrocytes of a Hybrid Monkey and Desensitized with the Parents' Erythrocytes, in Response to Injection of the Hybrid's Erythrocytes

Guinea pig No.	Desensitiza- tion (hemolyzed paternal erythro- cytes, 1.0-1.5 ml subcutaneously and 1 ml intraperi- toneally per animal)	Test for total desensiti- zation (1 ml of hemolyzed paternal erythrocytes intravenously)		First reacting injection (1 ml of hemo- lyzed mater- nal erythro- cytes intra- venously)	Test for total de- sensitization (1 ml of hemolyzed maternal erythro- cytes intravenously)		Second reacting injection (1 ml of hemolyzed hybrid's erythro- cytes intra- venously)
		first reaction	subsequent reaction		subsequent reaction		
1	++	-	No injection given	++	-	-	
2	++	-	No injection given	+	-	-	
3	++	-	No injection given	+	-	-	
4	++	-	No injection given	+	-	-	
5	+	-	No injection given	+	-	-	
6	++	-	No injection given	+	-	-	
7	+	-	No injection given	+	-	-	
8	++	-	No injection given	+	-	-	
9	+	-	No injection given	+	-	-	
10	+	++	-	++	-	-	
11	++	+	-	++	-	-	
12	+	++	-	+	-	-	
13	++	+	-	++	-	-	
14	+	++	-	+++	-	-	
15	+	++++	-				

Legend: + tremor, rubbing the nose and ears, disheveled hair, dyspnea, slight fall of temperature; ++ the same but more marked, frequent sneezing; +++ the same but still more marked, convulsions, cough, lateral position, animal survived; ++++ all signs very marked, animal died; — no signs of anaphylactic shock.

TABLE 2. Anaphylaxis with Desensitization Reaction in Guinea Pigs Sensitized with Serum of a Hybrid Monkey and Desensitized with the Parents' Serum, in Response to Injection of the Hybrid's Serum

Guinea pig No.	Desensitiza- tion (paternal serum, 0.3-0.5 ml subcutan- eously and 0.5 ml intrapertoneally per animal)	Test for total desensiti- zation (0.3 ml of paternal serum intravenously)		First reacting injection (0.3 ml ma- ternal serum intravenously)	Test for total de- sensitization (0.3 ml of maternal serum intravenously)	Second reacting injection (0.4 ml of hybrid's serum intra- venously)
		first reaction	subsequent reaction		subsequent reaction	
1	+	++++				
2	++	+++	-	+	-	-
3	+	+++	-	+	-	-
4	++	-	No injection given	+	-	-
5	++	++++	No injection given			
6	++	-	No injection given	+	-	-
7	+	++++	No injection given			
8	++	-	No injection given	+	-	-
9	+	+++	-	+	-	-
10	++	-	No injection given	+	-	-
11	+	++++				
12	+	+++	-	+	-	-
13	++	-	No injection given	+	-	-
14	++	-	No injection given	+	-	-

Legend: + tremor, rubbing the nose and ears, disheveled hair, dyspnea, slight fall of temperature; ++ the same but more marked; frequent sneezing; +++ the same but still more marked, convulsions, cough, lateral position, animal survived; ++++ all signs very marked, animal died; — no signs of anaphylactic shock.

were removed by centrifugation, and the solution was made isotonic by the addition of a hypertonic solution. The final concentration of antigens corresponded to the concentration of antigens in a 25% suspension of erythrocytes.

EXPERIMENTAL RESULTS

In the first series of experiments 15 guinea pigs, sensitized with antigens of the hybrid's erythrocytes, were desensitized to the antigens of the paternal (*T. gelada*) erythrocytes. After the first desensitizing injection all the guinea pigs developed signs of anaphylactic shock; in 8 of the 15 animals its degree was assessed as ++ and in the other 7 as + (Table 1). After complete desensitization to the antigens of the paternal erythrocytes, the guinea pigs received the first reacting injection of antigen from the maternal erythrocytes (*P. hamadryas*). In response to injection of the maternal antigens, all the guinea pigs developed signs of anaphylactic shock: in one guinea pig it was assessed as +++, in 4 as ++, and in 9 as +. After complete desensitization to antigens of the maternal erythrocytes, the guinea pigs received the second reacting injection of hybrid's erythrocytes; no signs of anaphylactic shock were observed.

The results of the first series of experiments showed that the hybrid's erythrocytes contained no specific hybrid antigens and that the antigenic composition of the hybrid's erythrocytes was of the nature of a selection of antigens of the parent species.

In the second series of experiments 14 guinea pigs, sensitized with serum antigens of the hybrid, were desensitized to the paternal serum antigens (*T. gelada*). After injection of the first desensitizing dose, all the guinea pigs showed signs of anaphylactic shock (Table 2). In many guinea pigs desensitization did not take place even after two subcutaneous and one intraperitoneal injection of paternal serum, as a result of which, when tested for total desensitization, 4 animals died from anaphylactic shock. After complete desensitization to the paternal serum antigens, the guinea pigs received the first reacting injection of antigen from the maternal serum (*P. hamadryas*). In response to the injection of maternal serum antigens, all the guinea pigs developed signs of anaphylactic shock. After complete desensitization to maternal serum antigens the guinea pigs were given the second reacting injection, consisting of hybrid's serum: no signs of anaphylactic shock were observed (Table 2).

Hence, the results of the second series of experiments showed that the hybrid's serum, like its erythrocytes, contained no hybrid antigens other than those of its parent species.

It follows from these experiments that the guinea pigs which were desensitized to the antigens of one parent reacted weakly to the injection of antigens from the other parent (Tables 1 and 2). These results demonstrate that most of the antigens of the erythrocytes and serum of the hybrid monkey are those common to the parent species. Antigens specific for each species evidently account for only a small part of the total antigenic complex of the hybrid. The results of our experiments are in full agreement with results obtained with hybrid birds [9].

The fact that the injection of the parents' antigens completely removed the sensitization of the guinea pigs to the hybrid's antigens indicates that, firstly, the selection of the hybrid's antigens is determined by the genotypes of its parents and, secondly, there are no specific individual antigens, for the selection of antigens of any individual is determined solely by the difference in the maternal and paternal genotypes. If organisms are hereditarily identical (highly inbred animals and uniovular twins), we know that the antigenic composition of their tissues also is identical.

The study of a hybrid F₁ monkey (*♂ Theropithecus gelada* × *Papio hamadryas* ♀) by means of the anaphylaxis with desensitization reaction has shown that the antigenic composition of the erythrocytes and serum of the hybrid is determined by the antigens of its parents, and contains no specific "hybrid" or individual antigens.

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