IMMUNOLOGICAL CROSS-REACTIONS BETWEEN HUMAN AND ANIMAL α-FETOPROTEINS

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Cross-reactions between human and animal α-fetoproteins (α-FP) were studied in the agar diffusion test with antisera obtained by immunizing rabbits with purified α-FP by injection into a lymph gland. The antisera used revealed species-specific determinants in homologous α-FP and cross-reacting determinants in heterologous α-FP. It was shown that antibodies against species-specific and cross-reacting α-FP determinants can be isolated.

Among the proteins present in the embryonic sera of animals and man, α-fetoprotein (α-FP) is one of the most interesting. This protein appears in the blood of animals with primary and transplanted hepatomas, and in the blood of patients with hepatocellular carcinoma and teratoblastomas [4]. It is important to have methods of identifying α-FP among the other embryonic serum proteins of animals belonging to different species. One such method is the determination of immunological similarity between α-FP of different origins. Immunodiffusion methods have revealed common antigenic determinants in the α-FP of mice and rats [2, 8] and in α-FP from man, monkeys, dogs, pigs, cats, sheep, and armadilloes [9, 11, 12]. Cross-reactions between α-FP of man, mice, and rats have not been detected by direct immunochemical methods [9, 11, 12]. The similarity between them has been demonstrated only by immunoautoradiography [3].

In the present investigation cross-reactions were studied between α-FP of man, mice, rats, and cows by the agar-diffusion test using rabbit antisera [7] with a high content of antibodies against human, mouse, and rat α-FP.

EXPERIMENTAL METHOD

The following sera were used to compare to α-FP: human fetal serum (HFS), neonatal mouse serum (NMS), neonatal rat serum (NRS), and bovine fetal serum (BFS).

Antisera against human (anti-α-HFP), mouse (anti-α-MFP), and rat (anti-α-RFP) α-FP were obtained by immunizing rabbits with purified preparations of α-FP [6] injected into a lymph gland by the scheme described previously [7]. The very small quantities of antibodies against impurities were absorbed by adult serum of the corresponding species. The anti-α-FP used, when tested with homologous fetal or neonatal sera, revealed only one antigen identical with the purified α-FP.

Antibodies against cross-reacting and species-specific determinants of mouse α-FP were obtained from the anti-α-MFP by means of NMS and RMS polymerized with glutaraldehyde by the method of Avrameas and Ternynck [10]. The absorbents were prepared from 1 ml NMS and 1 ml RMS. Antibodies were isolated from 1 ml anti-α-MFP. For this purpose, 4 ml of anti-α-MFP in a dilution of 1:4 was treated initially with absorbent from RMS for 60 min at room temperature. The antiserum was removed by centrifugation at 2000 rpm, after which the absorbent was carefully washed free from serum and the fixed antibodies against cross-reacting determinants were removed with 4 ml glycine–HCl buffer, pH 2.8, to give eluate I (EL.I). After treatment with absorbent from NRS, the anti-α-MFP did not react in the diffusion

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TABLE 1. Composition of Mixture of Antigens Used to Detect Cross-Reactions of α-FP of Different Species

Antiserum	Mixture of antigen*			
	NMS	NRS	HFS	BFS
Anti-α-MFB	1/15	1/15	1/5	–
Anti-α-RFP	1/20	1/10	1/10	–
Anti-α-HFP	1/20	1/20	1/5	1/10

*Numbers denote final dilution of components in mixture corresponding to equivalent proportions of α-FP of each species of antiserum preliminarily determined by titration in the agar-diffusion test.

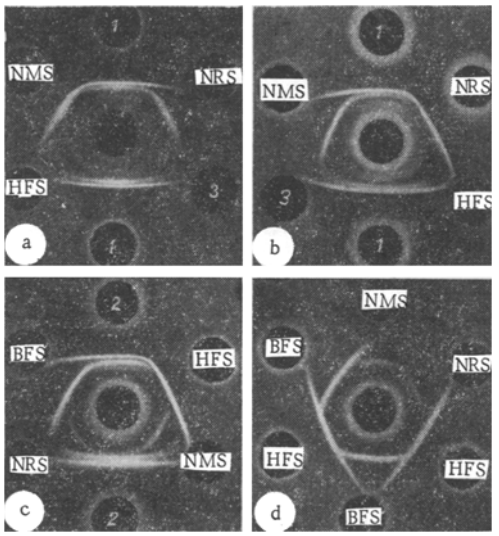

Fig. 1. Cross-sections of human, mouse, rat, and bovine α-FP. NMS, NRS, HFS, BFS) neonatal mouse and rat sera, human and bovine fetal, respectively. 1) Mixture of NMR, NRS, and HFS; 2) mixture of NMS, NRS, HFS, and BFS; 3) physiological saline. Antisera against α-FP in central wells: a) mouse; b) rat; c, d) human.

test with NRS, indicating complete absorption of the cross-reacting antibodies. The same antiserum was then treated with absorbent from NMS and 4 ml of eluate of antibodies against species-specific determinants of α-MFP was obtained (EL. II). The eluates were neutralized with Na_2CO_3 and diffusion tested for the presence of antibodies against NRS and NMS. The agar diffusion test was carried out in the micromodification of Gusev and Tsvetkov [5].

EXPERIMENTAL RESULTS

The anti-α-FP tested in the agar diffusion test with NMS, NRS, HFS, and BFS reacted not only with homologous, but also with heterologous antigens. To compare human, mouse, rat, and bovine α-FP from each of the antisera obtained, a mixture of NMS, NRS, HFS, and BFS was prepared in such a way that the α-FP of each species was present in the mixture in optimal dilution for the particular antiserum. The compositions of the mixture of sera are given in Table 1 and their reactions in agar with the various antisera are given in Fig. 1.

The diffusion test as performed in this way revealed three types of interaction between the heterologous antigens and the same antiserum simultaneously: reactions of identity, of partial identity, and of nonidentity. As Fig. 1a shows, each antigen of the mixture formed its own precipitation line with the anti-α-MFP, as shown by the reaction of complete identity of each of the α-FPs composing the mixture and the homologous α-FP in the separate well. Partial identity of the mouse, rat, and human α-FP follows from the fact that the precipitation lines formed by α-RFP and α-HFP with anti-α-MFP merged into the precipitation line formed by α-MFP and the homologous antiserum. The "spur" thus formed in this test curved toward the well with antiserum and merged with the precipitation line formed by α-MFP in the mixture. A similar picture of partial identity was obtained by comparing the diffusion reaction of human and rat α-FP with anti-α-MFP. Finally, the reaction of nonidentity is illustrated in Fig. 1 by the precipitation lines formed by the α-MFP in the mixture and the anti-α-MFP, which rest against the wells containing NRS and HFS.

It is thus clear from Fig. 1a that anti-α-MFP contains at least three types of antibodies: antibodies against the species-specific determinant of α-MFP (AT_1), antibodies against the determinant common to both α-RFP and α-MFP (AT_2), and antibodies against the determinant common to α-MFP, α-RFP, and α-HFP (AT_3). A mixture of the three α-FPs was made up so that each component reacted with antibodies of its own species: α-MFP with AT_1, α-RFP with AT_2, and α-HFP with AT_3. Whereas each of these α-FPs reacted separately with anti-α-MFP, the precipitate was formed somewhat differently: α-MFP was precipitated by AT_1, AT_2, and AT_3, α-RFP by AT_2 and AT_3, and α-HFP by AT_3. That is why the three precipitation lines of the α-FP mixture merged into the precipitation line formed by α-MFP and anti-α-MFP, and the precipitation line formed by α-RFP and anti-α-MFP.

Common antigenic determinants in the molecules of rat, mouse, and human α-FP, and also species-specific determinants of α-RFP were also found with anti-α-RFP (Fig. 1b).

Cross-reactions of human, mouse, rat, and bovine α-FP were studied with the aid of anti-α-HFP. In this case also (Fig. 1c) common determinants were found in the molecules of all four antigens. However,

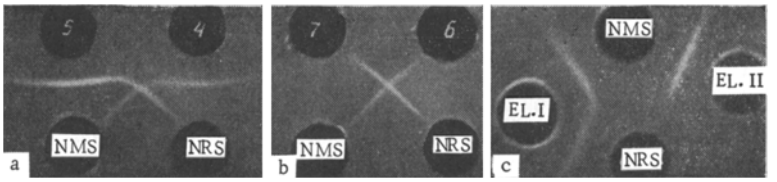

Fig. 2. Cross-exhaustion of antibodies against antigenic determinants common to both α-MFP and α-RFP. EL.I) Eluate of antibodies against antigenic determinant common to both α-RFP and α-MFP; EL.II) eluate of antibodies against species-specific determinant of α-MFP; 4, 5) antisera against α-MFP and α-RFP, respectively; 6) antiserum against α-MFP absorbed by NRS (1 volume anti-α-MFP + 1 volume NRS in dilution 1:60); 7) antiserum against α-RFP absorbed by NMS (1 volume anti-α-RFP + 1 volume NMS in dilution 1:60).

it is interesting to note that common antigenic determinants were found particularly clearly with anti-α-HFP in human, mouse, and rat α-FP, as shown by the complete merging of the precipitation lines formed by mouse and rat α-FP with anti-α-HFP (Fig. 1, c and d). This same antiserum detected both the antigenic determinants common to mouse, rat, and human α-FPs in the bovine α-FP, and also other determinants common to bovine and human α-FPs only. This is shown by the presence of a "spur" formed by the precipitation lines of NMS and NRS with this same antiserum (Fig. 1d).

Differences between the antigenic determinants were particularly clearly revealed when the agar-diffusion test was carried out by the method of double diffusion in two directions [1]. Comparison of the two test systems for rat and mouse α-FP (Fig. 2a) revealed a reaction of partial identity. Each α-FP reacted with both homologous and heterologous antisera. Precipitation with the heterologous antiserum took place because of the presence of antibodies against common determinants in the molecules of rat and mouse α-FP. On the addition of an equivalent quantity of heterologous antigen to both anti-α-RFP and anti-α-MFP, antibodies against the common determinants present in both antisera were exhausted and a reaction of the classical "cross" type (Fig. 2b) was obtained.

The exhaustion experiments show that antibodies against common and species-specific determinants can be separated. The results of diffusion test using eluates of antibodies against common (EL.I) and species-specific determinants (EL.II) prepared from anti-α-MFP are shown in Fig. 2c. It is clear that EL.I reacted with α-MFP and α-RFP by a reaction of complete identity, while EL.II contained antibodies only against the species-specific determinants of α-MFP.

EL.I contained antibodies only against antigenic determinants common to both α-MFP and α-RFP, because this eluate did not precipitate α-HFP. Probably polymerization of NRS in the α-RFP molecule leaves few free determinants common to human, rat, and mouse α-FP.

Rabbit antisera against α-MFP and α-RFP used in this investigation to establish the immunological kinship between mouse, rat, and human α-FPs did not precipitate any of the other heterologous α-FPs studied: cat, dog, guinea pig, bovine, or sheep.* However, it has been shown [12] that exhausted rabbit antisera against goat fetal serum and sheep antiserum against golden hamster fetal serum reacted with rat fetal serum. In order to obtain immune sera for the study of cross-reactions it is evidently better to immunize animals belonging to a different order.

The discovery of direct cross reactions between the α-FPs of man and such animals as mice and rats is particularly important, because carcinoma of the liver is usually studied under experimental conditions in these animals.

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