Tests on Immune Sera Produced Against Calf Thymus Chromosome.* (27098)

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Because antigenic expression is usually independent of the background genome (including the alleles of the genes in question), it has been suggested that erythrocyte antigens may be more or less immediate products of their causative genes(1,2). To gain some insight into the mechanism of production of these gene products, it seemed worthwhile to search for specific serological reactions between the erythrocyte antigens and antibodies produced against chromosomal material. Means for studying these problems are provided by the wealth of antigenic factors on bovine erythrocytes(3), by standard serological technics of hemolysis, hemolysis-inhibition and absorption, and by the availability of calf thymus lymphocytes from which relatively pure chromosomal material can be isolated.

Two major lines of evidence suggest that nuclear material can elicit antibody production. The first is the identification in the serum of patients with systemic lupus erythematosus (and other diseases) of a number of factors capable of reacting with different constituents of the cell nucleus(4,5). The reaction appears typical of antigen-antibody reactions since passive cutaneous anaphylaxis can be produced by injecting guinea pigs with "L.E." serum or purified "L.E." factor along with calf thymus or salmon sperm DNA(6). The second line of evidence is the production of antibodies in rabbits against a DNA-protein extract of the bacterium *Brucellus abortus*(7) and related species(8). Of the several bands of precipitation obtained in the gel diffusion reaction between the antigen used for injection (or DNA from calf thymus or salmon sperm) and the resulting antiserum, one or more were Feulgen positive. This band could be altered by pre-treatment of the antigen with deoxyribonuclease, but not by pre-treatment with trypsin or chymotrypsin.

Chromosome preparations. Thymus glands were obtained from unselected, 3- and 4-month-old calves, at slaughter, and stored at –20°C until used. Blood for typing was obtained from each calf. The procedure for isolating chromosomes and the properties of these preparations are detailed elsewhere(9). An additional straining through muslin removed clumps arising from the final centrifugation.

Immunizations and bleedings. Each of 6 adult rabbits received injections on alternate days consisting of 1 ml of about a 15% suspension of the chromosome preparation from the thymus of a single calf, No. 11. The first 4 injections were intravenous, but after 2 of the rabbits died from anaphylactic shock, intraperitoneal injections were used. The rabbits were bled on the 9th and 10th day following last injection. Two of these rabbits were given booster injections 3 weeks later, and bled on the fourth day thereafter. Blood was collected in dry tubes from the heart or ear vein. It was allowed to clot, the serum decanted, centrifuged, and heated to 56°C for 30 min. to destroy complement activity. The sera were stored at –20°C without preservative until used.

Testing procedures. Red blood cell samples used for testing the immune sera were obtained from cows of various genotypes by bleeding from the jugular vein into tubes containing a small amount of isotonic sodium citrate and stored at 4°C until used. The technics for testing hemolysis and hemolysis-inhibition are described elsewhere(10). Hemolysis was judged by eye and graded from "0" (no lysis) to "4" (complete lysis). The titer of a hemolytic serum is defined as the reciprocal of the highest dilution of the se-

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rum which gave more than a "3" reaction after 4 hours incubation. Inhibition was considered *strong* if the inhibitant reduced an otherwise "4" reaction to "0", and *weak* if the inhibitant reduced the reaction to about "1". Absorptions(10) were performed by combining one vol of the serum with one vol of the absorbing material (red cells or chromosome preparation) and incubating for one min. at room temp (24 ± 2°C), then for 15 min. at room temp with a second volume of the absorbant, and finally for 30 min. at room temp and 30 min. at 4°C with a third volume of absorbant.

Hemolytic activity of anti-chromosome sera. The immune sera of all 4 rabbits showed reactions ranging in titer from 16 to 128 with 25 unselected cattle cells, while the normal sera were non-reactive. To determine if these reactions were specific, various aliquots of the immune sera were absorbed with the red cells or chromosome preparations from cows whose red cells contained antigenic factors partly common to calf No. 11 whose chromosomes were used to produce the immune sera. For example, the 13 detectable antigenic factors on the red cells of calf No. 11 were B_1, G, O_1, Y_2, E'_1, D', C_1, E, X_2, L', F, J, and H'. The red cells of another cow used for one of the absorptions had all of these factors *except* G, Y_2, E'_1, D' and F. Thus, antibodies with specificity for any of these 5 factors, if present, should remain in the immune serum after absorption. However, when the absorbed sera were tested against a panel of cattle cells with known antigenic factors no hemolysis was noted. Since absorption with any single sample of cells or chromosome preparation removed all hemolysins for all other cells, it was concluded that the anti-chromosome sera contained only species-specific antibodies.

Inhibiting activity of absorbed anti-chromosome sera. The absence of specific hemolysins that are thought to possess some sort of structural complementation toward the antigenic factors of the red cells prompted tests to detect factors structurally similar to the red cell antigens. If present, the immune sera might be expected specifically to inhibit antibodies reactive with some of the 13 blood factors defined by the genotype of calf No. 11, but not inhibit other antibodies. To test this possibility, the immune sera were used as inhibitants in tests with approximately 20 standard blood typing reagents (antibodies) including reagents reactive with the 13 blood factors of calf No. 11 as well as others. One drop of undiluted anti-chromosome serum and 2 drops of blood typing reagent were incubated for 30 min. Then, one drop of a 3% suspension of red cells was added that would normally be lysed by these reagents. The absence of a hemolytic reaction would indicate that the antibodies had been inhibited and were not available to react with the cells.

When the anti-chromosome sera were absorbed with *red cells* before testing for inhibition of the reagents, inhibition was practically never observed. (One absorbed serum showed weak inhibition for antibodies specific for 2 of the 13 blood factors defined by the genotype of calf No. 11, while a serum absorbed with the cells of another cow showed weak inhibition for one factor present and one not present on the cells of calf No. 11.) In contrast, when the immune sera were absorbed with *chromosome material* from another calf, and then tested for inhibition, the inhibition was usually strong. However, inhibition was noted for factors absent as well as present on the red cells of calf No. 11. For instance, one antichromosome serum absorbed with a chromosome preparation from another calf showed strong inhibition activity for the 13 blood factors of calf No. 11 and also for 10 of 12 blood factors not present on the cells of calf No. 11.

It seems unlikely that the chromosome preparations used in the absorptions introduced the inhibiting capacity into the absorbed sera, because rabbit normal sera produced little or no inhibition when similarly absorbed by the same chromosome preparations or by red blood cells. In addition, another group of rabbits that had been injected with a chromosome preparation mixed with adjuvants produced sera with no hemolytic activity, but moderate and strong inhibition was demonstrable without absorption.

Other tests with the anti-chromosome sera. Absorptions of cattle blood typing reagents

F, V, J and Z, with chromosome preparations resulted in some decrease in titer. However, the absorptions were non-specific, since the decrease was independent of whether or not the chromosomes had been obtained from a calf carrying these antigenic factors.

Some of the anti-chromosome sera were tested for agglutinins against the red blood cells of rabbits, doves, rats and humans. No difference in reactivity was observed for these cells between the immune sera and the normal sera.

Discussion. Immune sera produced in rabbits against chromosome preparations from calf thymus have been shown to possess species-specific hemolytic activity toward cattle erythrocytes. It was not possible to demonstrate type-specificity since attempts to selectively absorb out some of the hemolysins with erythrocytes or chromosome preparations resulted in a complete loss of hemolytic activity. If the hemolysins were directed against the cattle DNA of the chromosome preparations rather than against the protein portion, then any activity directed toward cattle red cells, which do not possess a nucleus and presumably lack DNA, would indicate that substances on the surface of the red cells had configurations similar to DNA. The presence of anti-DNA antibodies would have been strongly suggested had we been able to show that the anti-chromosome serum specifically hemolyzed red cells from only those animals that shared erythrocyte antigens with the animal used as the source of the chromosomes. However, the inability to show this type-specificity argues that the hemolysins were elicited by proteins common to the chromosome preparations and to the red blood cells and possessed by all members of the species. It is possible that material adsorbed to the chromosomes in the course of preparation is responsible for these reactions.

After absorption of the hemolysins with chromosome preparations, the immune sera displayed inhibiting activity toward antibodies reactive with cattle cells. This inhibition activity gave no clear pattern with respect to the antigenic constitution of the calf whose chromosomes were used for the injections, therefore, no conclusions can be drawn with respect to type-specificity. The reason for the loss of inhibiting activity of antichromosome sera after absorption with red blood cells, but not after absorption with chromosome preparations is not known.

Summary. Rabbit antisera prepared by injection of cattle chromosomal material contained species-specific, but not type-specific hemolysins for cattle erythrocytes. These sera also showed an unexplained inhibiting activity toward standard antisera reactive with cattle erythrocytes.

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