COO-1210-93

ACQUIRED IMMUNOLOGIC TOLERANCE IN CHIMERAS
AND HISTOCOMPATIBILITY FACTORS IN CATTLE
AND THEIR RELATIONSHIP TO THOSE IN HUMANS

Final Report

MASTER

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ABSTRACT

During the course of this project we have studied 35 pairs of chimeric cattle twins. It is now clear that fractionated doses of whole-body ^{60}Co irradiation can cause marked shifts in the proportions of the two erythrocyte populations that make up the chimeric mixture. However, it has not been possible to eliminate one of the two cell types and thus abrogate the acquired immunologic tolerance. The results of our extensive skin-grafting experiments are remarkable because they show that a chimeric twin may mount a sufficient immune response to reject its cotwin's skin while remaining completely tolerant to erythropoietic elements of its cotwin. These findings are compatible with our results showing that the proportions of mixed lymphocytes and mixed erythrocytes do not correlate. Our present hypothesis is that some kind of a blocking factor controls the proportions of the two cell types by regulating the amount of immune response that the mixed tissues can make against each other.

In conjunction with these studies, we have acquired sufficient data to define a major histocompatibility locus in cattle using alloimmune anti-lymphocyte typing sera as well as the mixed lymphocyte culture technic. Many of the antisera that we have produced are cytotoxic for human lymphocytes and are potential sources of histocompatibility typing reagents.

This project has also yielded a considerable number of new immunogenetic parameters for cattle, monkeys and birds. Such parameters are useful for basic and applied studies in immunology.

LIST OF PUBLICATIONS DURING TOTAL TERM OF
CONTRACT - COO-1210

NOTE: * Reprint or preprint included with this report.

Reprint or preprint previously submitted

+ Reprints not available

- #COO-1210-1: Original Formal Research Proposal (April 4, 1962).
- #COO-1210-2: First Progress and Renewal Report (March 1, 1963).
- #COO-1210-3: Possible Somatic Recombination in Twin Cattle with Erythrocyte Mosaicism. Proc. XI Intl. Cong. Gen. I: 193, 1963 with Janis Friedman, Audrey Fregin and Joan Caulton.
- #COO-1210-4: Effects of Irradiation on Erythrocyte Chimerism in Dizygotic Cattle Twins. Paper presented to Protection and Recovery Conference, Oak Ridge, Tenn., Nov., 1963, with Audrey Fregin, Joan Caulton, R.G. Cragle and E. W. Swanson.
- #COO-1210-5: Special Summary of Research Program for Committee Review (May 1, 1963).
- #COO-1210-6: Chimerism and Tolerance in Cattle Twins. Paper presented to the 48th Ross Conference on Aplastic Anemia, Sept., 1963.
- #COO-1210-7: Second Progress and Renewal Report (March 15, 1964).
- #COO-1210-8: Possible Somatic Cell Mating in Twin Cattle with Erythrocyte Mosaicism. Proc. Natl. Acad. Sci. 51: 1036, 1964, with Janis Friedman and Audrey Fregin.

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- #COO-1210-21: Studies of Allotypes in Cattle. II. Isoprecipitins Produced Against Globulin. Presented by title to the 10th Congr. European Society of Animal Blood Group Reserach - Paris, July, 1966, by J. Rapacz and W. H. Stone.
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I. MAIN RESEARCH ACCOMPLISHMENTS

A. Background

Most cattle dizygotic twins contain two antigenically distinct kinds of blood cells derived from a reciprocal exchange of tissues during gestation. Such twins exhibit erythrocyte chimerism which represents a state of acquired immunologic tolerance, since the immune system of each twin is unresponsive to the erythropoietic tissues of the other twin. Also, chimeric twins show a high degree of tolerance for each other's skin and kidney grafts. Thus, chimerism is an important phenomenon in studying problems of immunobiology involving transplantation, and tolerance. It is also important in somatic cell genetics involving interactions between genetically distinct tissues.

The early years of this project were devoted to experimental treatment (mainly irradiation) of chimeric twin cattle. Subsequently we observed, summarized, and analyzed the results of these treatments. Then, new experiments suggested by the outcome of our work were undertaken. For example, we expanded our scope to include a study of chimerism (artificially produce) in birds; and later initiated studies on leukocyte antigens of cattle and blood types and allotypes in Rhesus monkeys. In addition to these specific accomplishments, there have been many "spin-off" benefits from this project. We have detected several new immunogenetic parameters in cattle and monkeys and have been able to use them to study other phenomena - for example, maternal-fetal interactions.

B. Results

1. Effect of irradiation on chimerism: The data show that whole body ^{60}Co irradiation causes a substantial change in the proportions of the two cell types in chimeric mixtures. Less than 50% of the twins that received a single dose of irradiation showed a significant change (10% or greater). However, over 80% of the twins that received fractionated doses (either 50r/week or 25r/week) showed very marked changes. Finally, less than 20% of the non-irradiated controls showed a significant change. It is apparent from these results that fractionated irradiation markedly increases the frequency and magnitude of the changes in the proportions of the two cell types in chimeric twins.

We do not understand the mechanism that maintains the equilibrium between the two hematopoietic tissues in these twins, nor the mechanism that causes the shifts. The effects of irradiation are quite variable. For example, the direction of shift is not correlated with the majority or minority cell types nor with the genotype of the twin. Also, the shift may be rapid or gradual and the magnitude is variable also, ranging from 10% to 90%. In some twins, a complete reversal in the cell proportions were noted after fractionated doses of irradiation. In others the proportions of the two cell types reached so close to 100%

it was virtually impossible to detect a second cell type for certain. Therefore, it was not possible to say if these two twins were no longer chimeric for the erythrocytes.

Changes in the proportions of cell types generally occur in the same direction but not necessarily the same magnitude in both twins of a pair treated alike. The significance of this is unknown; but clearly, the physiologic mechanisms controlling the shifts must be similar, and it appears that the direction of shift is somehow pre-determined. These results argue strongly that both twins of a pair are remarkably similar in the response of their hematopoietic tissues to irradiation.

Our present hypothesis to explain these results is that chimeric twins do indeed have immunologic competence to react against their co-twin's tissues, but some kind of a blocking factor modulates this reaction. We will test this hypothesis using the mixed lymphocyte culture technic.

2. Studies on skin grafts: The fates of reciprocal skin grafts were studied extensively in 35 pairs of twins. Several sets were grafted a second, third, or fourth time to observe the heightened immunologic response. In summary, the results showed that: (1) autografts persisted indefinitely; allografts were completely rejected within 12 to 14 days, and the fate of co-twin grafts varied considerably. (2) Irradiation whether single doses or fractionated doses did not affect the fate of co-twin skin grafts. (3) Similarly, other treatments had no effect. These treatments were Neutron irradiation, ^{90}Sr (ingested and deposited in bone tissues), chemical treatments with 5-fluorouracil, 6-mercaptopurine and chloramphenicol, induced anemia, passive immunization with blood typing reagents, and kidney transplantation. (4) The co-twin graft rejection was not sharply delineated like a typical allograft, but was slow and smoldering. Thus, three graft reaction parameters were studied (a) Onset of rejection reaction, (b) Duration of reaction, and (c) Completion of reaction. About 16% (10/62) of the grafts showed the onset of rejection by 100 days, but none was completely rejected at that time. By 200 days about 47% (28/60) had begun rejection and about 26% (16/60) had been completely rejected. At 300 days, over 60% (34/56) had gone through the initial stages of rejecting (Onset) and 50% (28/56) had completely rejected (Completion) their co-twin grafts. After this time, the difference between the percentage of grafts undergoing rejection and the percentage already rejected steadily decreased until at about 800 days (over two years) when all of the rejections were complete. On the average the duration of reaction period was 80 days. This was a measure of the time elapsed from the onset to the completion of rejection. The percent rejection (70%) remained relatively constant for nearly three additional years. Out of 41 twins who ultimately rejected their co-twin's grafts, only one rejected beyond 27 months and only three rejected beyond 18 month post-grafting. (5) Significantly, members of a twin pair often showed asymmetric graft responses. In 12 sets, one twin retained its co-twin's grafts while the other twin rejected them. During the five years of observation, we found no twin pairs in which both members retained their co-twin's grafts. During this same period, we found 10 additional pairs in which both members ultimately rejected their

co-twin's grafts. However, the members of a twin pair often responded quite differently with respect to the three rejection parameters. Significantly, this asymmetry was not associated ($P > .05$) with differences in the blood types of the two erythrocyte populations or in the proportion of lymphocyte types (XX vs. XY) in the chimeric mixture. (6) Repeated grafts confirmed that the rejection of co-twin grafts was the result of histocompatibility differences and that tolerance to histocompatibility antigens of skin in chimeric twins varies considerably, but is hardly complete. As expected, the times for complete rejection decreases with succeeding grafts. For example, the duration of the rejection reaction and the time for the completion of rejection were reduced in 4⁰ responses to about one-fourth the values typical for 1⁰ responses. (7) In general, the survival of a co-twin skin graft was not affected by subsequent skin grafts. Furthermore, the rejection response to skin was not indicative of responses to kidney grafts. Some kidney grafts have survived three to four years longer than skin grafts. These data further confirm that the rejection of co-twin skin grafts do not affect the survival of co-twin kidney grafts or vice versa.

3. Kidney transplantation: Among 12 chimeric co-twin kidney grafts, 8 survived and 5 continued to function normally for over 2 years. Analyses of transplanted kidney function as compared to the hosts own kidney revealed that the transplanted kidney was functioning at almost normal levels. In fact, in two cows, we removed the hosts' kidneys and noted that the transplanted kidney alone could perform the clearance function necessary to sustain the animals. As noted above, the fate of skin grafts and kidney grafts appeared to be independent.

4. General physiologic effects of irradiation: As a by-product of these studies we have accumulated a considerable amount of unique data on irradiation effects in cattle. Some twins were treated with neutron radiation, others with ⁹⁰Sr (orally). Over 1,500 individual animal gamma exposures were made. The results indicate that cattle can tolerate far more irradiation when the dose is small and repeated than believed possible.

5. Comparison of erythrocyte and lymphocyte chimerism: Another aspect of our studies on chimerism has involved a comparison of the karyotype mixture with the red cell mixture. The proportions of XY:XX cells were significantly different between members of 3 of the 5 twin pairs. In contrast, the proportions of the two types of erythrocytes were significantly different in only one pair. Irradiation caused consistent and unidirectional shifts in the erythrocyte proportions of all 4 irradiated pairs, but the control pair did not shift. In contrast, the leukocyte proportions varied considerably from one determination to another so that consistent changes were not obvious. However, regressions of erythrocyte on leukocyte proportions within each twin gave $r \geq +0.5$ in only 2 of the 10 twins indicating that chimerism for the erythrocytes and for the leukocytes is not often positively correlated. Male twins did not necessarily have a majority of XY cells; nor did the female twins have a majority of XX cells indicating that the host's germinal tissue is not always favored.

Three pairs gave asymmetric graft responses to each other's skin, whereas one pair rejected and the 5th pair accepted each other's grafts. This behavior was not correlated in any obvious way with the leukocyte or erythrocyte proportions and was independent of the irradiation. If peripheral leukocytes carry the same histocompatibility antigens as skin, it is difficult to explain the rejection of co-twin allografts. Perhaps blocking factor plays a role. The differential effect of irradiation on erythrocytes but not on leukocytes might be explained by the relative differences in radioresistance and longevity of the different stem cells.

6. Somatic cell mating in cattle twins and in parabiotic birds: The twins in this project were continually assayed for evidence of hybrid erythrocyte types which may have resulted from somatic cell mating. Hybrid cells are usually first detected by noting an excess (over 100%) of lysis with reagents detecting allelic antigens. Thus, we surveyed all of our twins to determine if excess lysis were occurring. Only two cases were found among the 35 sets of twins studied over a 5 year period. Thus, it is apparent that somatic cell mating is either extremely rare or if not the resulting hybrid cells are not detectable.

Chimeras were induced in doves (Streptopelia) by making parabionts of embryonating eggs that carried genes for erythrocyte antigens, which were readily identified. The parabiotic pairs were chosen so that new antigenic specificities would appear if somatic cell mating took place. However, no evidence of somatic cell mating was noted. Erythrocytic chimerism was no longer detectable in some birds after varying periods of time. In a few others tolerance was presumably lost, since their plasma contained antibodies against cellular antigens that either were present, or had been present, in the bird's circulation.

7. Studies on cattle histocompatibility antigens of lymphocytes: Beginning in the 1971-1972 grant period of this project, we began to concentrate on this area of research. We have been following several leads, some of which appear to be extremely promising. However, this work is still in progress and is by no means complete.

(1) Alloimmune cytotoxic sera for cattle lymphocytes: One of the advantages of using cattle for these studies has been the unlimited quantities of lymphocytes, absorbing cells, and sera available. Thus, a definitive serologic analysis of these type-specific antigens has been possible.

We have studied 10 alloimmune sera produced by injecting cows with lymphocytes from their respective calves. These sera contained antibodies that detected SD antigens of lymphocytes. In addition, serum from multiparous cows showed cytotoxicity and some were fractionated into specific reagents. A survey of our antisera showed that they were detecting polymorphisms of the lymphocytes. Some monospecific typing sera were isolated and analyzed serologically and genetically. We have preliminary data indicating that other monospecific or oligospecific reagents can indeed be obtained from these sera. These results will definitely establish an SD (serologically defined) major histocompatibility locus for cattle for the first time.

(2) Mixed lymphocyte culture tests: We have succeeded in perfecting this technic for cattle and have analyzed several hundred sire families (half-sibs). The data have not yet been thoroughly analyzed, but they appear to unequivocally establish an LD (lymphocyte defined) locus in cattle.

(3) A study of cross-reactions between cattle and human antilymphocyte sera: Antisera obtained from six cows immunized with lymphocytes from their calves, and absorbed with human erythrocytes still exhibited strong cytotoxic activities against human lymphocytes. Each antiserum showed differential cytotoxicity patterns with a panel of over 60 human cells. These patterns were not correlated with the known HL-A antigens. We have also produced antisera in cattle immunized with human lymphocytes and kidney tissues. The resulting antisera showed differential reactivities with a panel of human test cells suggesting that type-specific antibodies had been produced.

8. Studies on cattle and monkey allotypes (inherited serum alloantigens):

(1) Cattle immunoglobulin allotypes: A new alloimmune sera which detect light chain allotypes, called B1 and B2 were discovered. Curiously, these sera formed 2 or 3 precipitin bands with reactive animals. In double diffusion using polyethylene glycol in agarose, one band required the first component of complement (C1) to form. Evidence suggests that the other bands were due to determinants on different molecules. Gene frequencies for these markers in several cattle breeds have been calculated. From a study of families the evidence is clear that the chromosome region controlling these new specificities is transmitted like autosomal dominant genes. There was no evidence against Hardy-Weinberg equilibrium.

(2) Rhesus monkey allotypes: The allotypic marker, called F53 is an autosomal dominant trait and is carried by α_1 -macroglobulin molecules. Unfortunately, we have not been able to produce an antiserum against the allelic product. New immunizations with purified IgG have yielded three strong antisera. We are currently studying the allotypic markers detected by these sera. Our F53 antiserum did not parallel the reactions of the allotypic sera described in rhesus by Balner's group at the Primate Lab, Rijswijk, the Netherlands.

9. Studies on blood groups of Rhesus monkeys: We studied over 2,000 monkeys (Macaca mulatta) at the University of Wisconsin Regional Primate Research Center. By alloimmunization and absorption, we produced 25 specific blood typing reagents (agglutinins). The blood factor that each reagent detects behaves as a Mendelian dominant character. Genetic studies indicate that these factors are distributed in at least 10 independent blood group systems. We discovered that transplacental immunization of the mother by fetal red cells regularly occurs in this species. Sera obtained postpartum from 253 female rhesus monkeys were tested for agglutinins reactive with the erythrocytes of their mates or infants. Approximately 20% of parturients, but less than 3% of 40 males and 31 virgin females exhibited antibodies. Antibodies were detected in 26% of the mothers who bore blood group incompatible infants in contrast to only 9% of the mothers with compatible offspring. Subsequent pregnancies seemed to increase the incidence of antibodies but not significantly. Thus, blood group incompatibility may lead to transplacental

alloimmunization of the mother. Unlike the situation in man, hemolytic disease was not observed, even when the erythrocytes of the rhesus infants were coated with maternal antibodies.

10. Miscellaneous studies supported in part by this project:

(1) Phylogenetic studies of cattle breeds and bird species: Methods have now been worked out whereby the immunogenetic parameters can provide an excellent unbiased estimate of the evolutionary history of different cattle breeds. Using the blood groups, allotypes and enzyme polymorphisms we have studied various breeds from different parts of the world. For example, data on Spanish and Portuguese cattle yielded a phylogenetic tree that showed that both of these breeds are very closely related to American Longhorn cattle. We have demonstrated the practical use of these studies by showing that they provide a basis for the rational development of future breeding and preservation programs that should permit the best use of the germ plasm resources of the species.

Over the years we have continued our long term analysis of the antigenic specificities in pigeons and doves that are produced by gene interaction (hybrid substance). The ability of genes to effect one antigenic product, plus another by interaction with an allele or with a gene on another chromosome has provided a model for gene action in support of the Overdominance theory of heterosis.

(2) Maintenance of cattle and Rhesus monkey blood typing capabilities and concomittant research and services: The production, analysis, and use of cattle and monkey blood typing reagents for our studies, and for various collaborative studies have always consumed significant amounts of our time and energies. We have maintained stocks of over 80 cattle and 25 monkey reagents and hundreds of immune sera, and frozen red cell samples. We have exchanged reagents with laboratories at U. Cal., Davis; Ohio State U.; Texas A & M; Canadian Dept. of Agriculture at Guelph; Primate Laboratory, University of Oregon; and N.Y.U.. We have supplied reagents and advice to laboratories in Barcelona, Zaragoza and Cordoba, Spain, Milan, Italy, and in LaPlata, Argentina. We have updated our computer programs and data retrieval systems so that we can generate extensive information for family studies, gene frequencies, linkage, etc.

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END OF REPORT