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Maternal-Fetal Histoincompatibility in Rats: An Escape from Adversity[1]

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Summary

A central question in reproductive immunology concerns the mechanisms whereby embryos and fetuses customarily escape adverse consequences of maternal-fetal antigen incompatibilities, with respect to both paternally derived histoincompatibility (*H*) and embryonic differentiation antigens. The question has most often been focused upon the immunogenically potent antigens of major systems, such as *H-2* in mice and *HL-A* in humans, and has become more critical as increasingly sensitive serological assays have provided evidence for initial *H* antigen expression in preimplantation embryos.

Data from breeding experiments with rats, in which a comparable and most probably homologous *H* system (*Ag-B*) occurs, have suggested that the consequences of maternal-fetal antigen interactions may vary, *i.e.*, be adverse or beneficial, depending upon which *H* antigens are involved. In segregating populations from a number of inbred strain crosses, *Ag-B* incompatibility appears to protect the embryo from the adverse consequences of maternal immune reactivity toward other genetically defined antigens. Segregating backcross progeny that are *Ag-B* compatible with maternal tissues (but that differ for unknown numbers of other *H* antigens) exhibited preweaning runting and abnormal lymphoid tissue development and, under stressful environmental conditions, frequently succumbed prior to 30 days of age. A higher incidence and earlier time of preweaning deaths occurred with increasing parity. Similar patterns of mortality were not seen among backcross progeny of reciprocal matings, *i.e.*, F_1 hybrid females mated to inbred males. These and other factors suggest an immune basis for the phenomenon. More extreme samples involve matings in which the parental strains are *Ag-B* compatible but differ for other antigens. Progeny either were not obtained or, in cases in which a few births occurred, failed to survive to weaning.

Introduction

A major unresolved question of mammalian reproductive biology has been the nature of the protective mechanisms whereby a fetus generally escapes immune attack by maternal lymphoid cells, even though the latter may be competent to react against paternally derived histocompatibility antigens. Although on occasion immunologically oriented anomalies do occur, mammalian reproduction is mainly devoid of these problems.

The enigma of fetal protection has been highlighted in recent years by many indications that alterations of the maternal immune system in response to fetal antigens do occur normally. Fetally induced maternal tolerance or enhancement (3, 7), as well as antibody elicitation and lymphocyte sensitization, have all been demonstrated in one or more species (1, 8).

The question is particularly intriguing because the polymorphism of major histocompatibility systems (*H-2*, *Ag-B*, and *HL-A* of mice, rats, and humans, respectively) is so considerable (2, 9) that most pregnancies within randomly breeding populations do involve highly immunogenic incompatibilities.

Recent studies of rats (12, 13) suggested that antigenic incompatibility of fetus to mother may be either advantageous or deleterious, depending upon the antigen locus involved. Moreover, incompatibility at the major histocompatibility locus, *Ag-B*, was not only advantageous, but appeared necessary for optimum development and survivability of progeny. The data have reversed the usual question, "Why is the antigenically incompatible fetus not rejected as a homograft?" to "How does antigenic incompatibility prevent fetal adversity?"

In this report, a survey of the up-dated rat data is presented and discussed in terms of its possible relevance to the topic of this meeting, *i.e.*, fetal and embryonic antigens in cancer.

Materials and Methods

Rats. The inbred strains used for the backcross matings were, with few exceptions, from colonies raised at the Wistar Institute. The homozygous strains and their *Ag-B* genotypes (14, 15) were Lewis, (L)-$Ag\text{-}B^1$; Wistar Furth, (WF)-$Ag\text{-}B^2$; Brown Norway, (BN)-$Ag\text{-}B^3$; "D" agouti, (DA)-$Ag\text{-}B^4$; August-28807 (AUG)-$Ag\text{-}B^5$; the congenic strains (14), BN.B2-($Ag\text{-}B^2$) and BN.B4-($Ag\text{-}B^4$); and, finally, derivatives of an HO strain obtained from Dr. J. Howard (Oxford) and of the A2(A1) strain of Dr. N. F. Anderson (Edinburgh) (10), which were $Ag\text{-}B^5$ and $Ag\text{-}B^2$, respectively.

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Breeding Experiments. The initial finding of *Ag-B* heterozygote advantage was based upon retrospective analyses of segregating populations from reciprocal backcross matings (12, 13). Information concerning preweaning mortality, sex ratio at birth, litter size, etc., was not available; subsequent experiments have included these parameters. Mating cages are checked daily; litters are sexed and counted at or within 1 day of birth, observed daily for the appearance of abnormal development, and weighed on Days 10, 21, 30, and 60 after birth. Coat colors or patterns are noted at the 10th day and are rechecked at weaning. *Ag-B* phenotype was determined by hemagglutination tests performed between 3 and 4 weeks of age. The polyvinylpyrrolidone agglutination test and the preparation of the specific antibody reagents in use have been described (15). The *Ag-C* phenotype was detected initially by suitably absorbed rabbit anti-rat sera and, later, by alloimmune anti-C1 antibody.

Results and Discussion

The initial data from which these maternal-fetal studies have developed were obtained from attempts to determine genetic linkage of the *Ag-B* locus to loci determining various coat colors or patterns that were segregating within the inbred strains. Reciprocal backcross matings were used. Chart 1 illustrates the genetic interrelationships of the reciprocal backcross matings, as well as the direction of potential immune reactivity between mother and fetus.

Although no linkage was found, the data compiled over several years (from backcrosses of several inbred strain pairs) did reveal a consistent excess of *Ag-B* heterozygotes. The excess, although not extreme, was consistent and statistically significant. Following a suggestion by Clarke and Kirby (4) that antigen incompatibility might be beneficial to fetuses, the data were grouped according to the genotype of the mother: F_1 hybrid or inbred strain. On subdividing the data (Table 1, Group A) it became clear that the excess *Ag-B* heterozygosity noted in the total population was entirely a characteristic of the backcross matings in which the mother was of an inbred strain and therefore competent to react against paternally derived antigens of the fetuses. In contrast, among progeny of F_1 hybrid mothers, the *Ag-B* segregation approximated the equality expected.

It appeared that fetuses possessing a major histoincompatibility with respect to maternal lymphoid tissues enjoyed a selective advantage. Subsequent breeding data have confirmed the repeatability and predictability of the phenomenon (Table 1, Group B). The BN and DA strains and the F_1 BN/DA hybrids have been used for the more detailed studies. In addition, 10 other *Ag-B*-incompatible pairs consisting of 6 inbred parental strains and 7 F_1 hybrid combinations have been studied to a lesser extent. *Ag-B* heterozygote advantage among progeny of inbred strain mothers occurred in all but 2 strain combinations, WF and BN, and A1 and BN. In both of these crosses, the *Ag-B* incompatibility is B2 and anti-B3. Whether the failure to exhibit heterozygote excess is due to this fact or to an absence of other relevant antigen incompatibilities remains to be determined. The availability of the "negative" strain combinations should be useful in attempts to determine the basis for the observed *Ag-B* heterozygote advantage in other strain combinations or, conversely, the homozygote disadvantage. The summarized data alone provide no clues as to the reasons for the different segregation patterns, although they do show a sex difference. The excess of *Ag-B* heterozygotes is more obvious among the male progeny (Tables 1 to 3).

Careful observations of events during successive breeding of BN and DA backcrosses over several years have revealed that the *Ag-B*-compatible deficiency results from an active reduction of this group and is not simply the reflection of increased numbers of *Ag-B* heterozygotes due to a nonspecific selective advantage of the latter.

Birth records indicate that the reduction of expected numbers of *Ag-B*-compatible offspring may occur prenatally or postnatally depending upon the genetics of the

Chart 1. Maternal-fetal antigenic interrelationships of reciprocal backcross matings. Progeny of either mating should be similarly heterogeneous for all genes distinguishing the parental strains. However, when an F_1 hybrid is the female parent, fetuses do not possess potentially immunogenic histocompatibility antigens (with the exception of specific male antigens), because the F_1 hybrid mother is a composite of both parental genomes. On the contrary, when a female of either parental strain is the mother, the fetuses do possess paternally derived histocompatibility antigens which are potentially immunogenic for maternal tissues. These include antigens of both the major *Ag-B* histocompatibility system and all other antigens distinguishing the 2 strains being studied, in this case, BN and DA.

Table 1

Distribution of Ag-B heterozygotes and homozygotes among progeny of reciprocal backcrosses

Group A represents a summary of the initial data obtained from backcrosses of several strain combinations (13). Group B is a summary of backcrosses involving only the BN and DA strains and the F_1 BN/DA hybrid. (Xx and xx designate *Ag-B* heterozygotes and homozygotes, respectively.)

		Mother			
		F_1		Inbred strain	
Group	Surviving progeny	Xx	xx	Xx	xx
A	Females	50	45	85	58
	Males	38	38	79	51
	Total	88	83	164	109
		$\chi^2 = 0.46$		$\chi^2 = 10.80$	
B	Females	79	70	146	104
	Males	57	67	150	98
	Total	136	137	296	202
		$\chi^2 = 0.0018$		$\chi^2 = 17.742$	

Table 2

Association of Ag-B heterozygosity with maternal genotype, infant mortality, and sex of progeny

One of the 4 breeding experiments contributing to the backcross summary in Table 1, Group B. Mortality was high in this particular experiment but appears independent of *Ag-B* distribution among surviving progeny. Data show influence of sex in distorted ratios; males are more subject to the anomaly.

		Ag-B distribution among surviving progeny			
		Females		Males	
Parental crosses	Infant mortality (%)	Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
BN × F_1BN/DA	16	54	51	42	29
DA × F_1BN/DA	66	12	18	17	5
Total		66	69	59	34
F_1BN/DA × BN	65	36	37	31	30
F_1BN/DA × DA	58	12	9	9	10
Total		48	46	40	40

Table 3

Association of maternal-fetal Ag-B compatibility and a runting syndrome in backcross progeny

Last of 4 experiments contributing to the summary in Table 1, Group B. Although an equal proportion of runted individuals died, regardless of *Ag-B* phenotype, the actual number was higher among the *Ag-B* group because more of these exhibited runting.

	Progeny tested[a]				60-day mortality		
	Females		Males		Total	Among	
Classification	*Ag-B*$^+$	*Ag-B*$^-$	*Ag-B*$^+$	*Ag-B*$^-$		*Ag-B*$^+$	*Ag-B*$^-$
Normal	41	35	35	22	5/133[b] (3.4%)	1/75	4/58
Runted	10	15	7	21	24/53 (45.3%)	7/17	17/36
60-day survivors	47	41	39	33			

[a] The designations *Ag-B*$^+$ and *Ag-B*$^-$ signify the presence or absence of the paternally derived *Ag-B* allele and, therefore, indicate incompatibility or compatibility, respectively, with maternal lymphoid tissues.

[b] Number of rats that died by Day 60/total number of rats.

particular mating. For example, BN × F_1BN/DA matings resulted in a deficiency of males and, among surviving males, a marked deficiency of *Ag-B*-compatible offspring (Table 2). This is despite a limited postnatal mortality. Presumably, some of the disadvantaged *Ag-B* homozygous males were eliminated prior to birth. When DA × F_1 BN/DA matings were examined, however, it was clear that most of the selection against *Ag-B*-compatible progeny occurred after birth. This may reflect different non-*Ag-B* immunogenicity in the 2 combinations.

High mortality frequently occurs among offspring of F_1 hybrid mothers (Table 2 and unpublished data), but the surviving population does not exhibit distorted *Ag-B* ratios.

The sex difference that is a feature of the overall studies is evident among the progeny of BN and DA females in Table 2. Males almost always exhibit a greater *Ag-B* heterozygote excess, which, of course, reflects the greater vulnerability of the male *Ag-B* homozygote.

When postnatal, the reduction of the *Ag-B*-compatible class is in some strain combinations the result of a wasting syndrome characterized by anomalies usually associated with the GVH[2] syndrome (5): ruffled fur, hunched position, and, on occasion, dermatitis. The severity of the symptoms and the time of death are related to parity. In early litters, deaths usually occur between 15 and 21 days whereas, in later litters, the onset is progressively earlier (13). In severe cases, runted individuals exhibit abnormal lymphoid tissues showing a lack of germinal centers and acellularity (13). When less severe, the runted individuals are detectable mainly by weight. Among the groups studied, all runted young did not die (Table 3), and those surviving until 60 days appeared to be fairly long-lived. It is possible that seasonal or other environmental factors may determine whether mortality or recovery of retarded young will predominate in different experiments. Table 3 shows the greater incidence of runting with *Ag-B* maternal-fetal compatibility, although the association is not an all-or-none phenomenon.

To test these observations, that *Ag-B*-compatible offspring were being subjected to what appeared to be an immune reaction of the GVH type, matings of *Ag-B*-compatible, but otherwise antigenically dissimilar, inbred strains were set up and observed for the appearance of the wasting syndrome among the progeny. The preliminary results were completely unexpected. Of 8 *Ag-B*-compatible matings involving 3 strain combinations, only 1 produced

[2] The abbreviation used is: GVH, graft-*versus*-host.

live offspring, all of which were runted and failed to survive until weaning. Fertility tests (*i.e.*, remating of individuals with either syngeneic or *Ag-B*-incompatible mates) of approximately 25% of the parents from the nonproductive matings were productive. Although there are only a small number of tests within each category of strain combination, and the reasons for failure of reproduction, despite fertility of those parents tested, must be elaborated, it is very clear that in rat matings in which strong *Ag-B* incompatibility is lacking, reproductive performance is severely curtailed.

Several factors have supported the impression that immune interaction rather than conventional heterosis is the underlying cause of the phenomenon: (*a*) the failure to observe similar selective pressures (even when mortality is severe) among progeny of the F_1 hybrid females which are unable to interact immunologically with their fetuses; (*b*) the occurrence in the depressed *Ag-B*-compatible class of a wasting syndrome similar to that occurring with GVH reactions; (*c*) the increased severity of the runting syndrome with the parity of the immunocompetent inbred strain mother; (*d*) the failure to observe similar patterns of mortality among syngeneic strains; and, finally, (*e*) the disappearance of heterozygote excess in later stages of congenic strain development (Table 4) as the progeny become antigenically more like their mother except for the *Ag-B* allele being transferred.

If the supposition of immune interactions is correct, then the deleterious attack upon *Ag-B*-compatible offspring must be directed toward other antigens of the fetus, either paternally derived histocompatibility antigens or stage-specific differentiation antigens, or both, to which the mother can respond immunologically. The population of *Ag-B*-incompatible progeny should be equally heterogeneous for these other antigens; the fact that they are less affected (Table 3) than their *Ag*-incompatible sibs appears, therefore, to be due to some protective effect of *Ag-B* incompatibility *per se*.

The population statistics appear to result from 2 selective pressures: (*a*) a positive one favoring *Ag-B*-incompatible progeny (which may or may not be immunological); and (*b*) a negative one for those progeny which, because of *Ag-B* compatibility with their mothers, are more subject to what appears to be an immune attack to other, non-*Ag-B* paternally derived antigens.

In an attempt to detect the chromosomal location of relevant antigens, attention was focused upon the distribution of other phenotypic markers appearing in the segregating backcross populations. These have included the *Ag-C* blood group for which 2 alleles, C^1 and C^2 [formerly the *C-D* blood antigens of Owen (11)] are known; the *albino*, *agouti*, and *hooding* genes for coat color or pattern; and *fuzzy*, a new hypotrichosis mutation (J. Palm, to be published). In mice, the chromosomes bearing the *albino* and *agouti* loci are each characterized by 2 or more histocompatibility loci, and in the rat at least 1 serologically detected antigen is linked with the *albino* locus. This and other apparent homologies of mouse and rat chromosomes (6, 15) have made the segregation ratios of these loci of special interest in the research for a deficient heterozygote class among *Ag-B*-compatible progeny.

Problems have been encountered in analyzing the data because the phenotypic markers are not equally represented in the various strain combinations used in the backcrosses. To detect a deficiency, among the *Ag-B*-compatible groups, of heterozygotes for any other locus requires that only those groups varying for the particular marker and exhibiting an *Ag-B* homozygote deficiency be used. The numbers of rats in most such groups are as yet insufficient for conclusive statements. However, a suggestion that this relationship may exist has appeared for the *albino* locus.

In 3 strain combinations where there were deficient numbers of *Ag-B*-compatible progeny, there occurred also an excess of *albino* offspring or, in other words, a deficiency of the heterozygote *Cc* class which might possess paternally derived antigens linked to the wild-type allele for color. Further analyses are in progress and will be the subject of a separate report.

The data presented bear on several immunobiological

Table 4

Ag-B antigen distribution of progeny from late (8th generation, plus) backcrosses of 2 developing congenic strains

In the later generations of congenic strain development, the progeny are increasingly of the BN genome, except for the chromosomal region including the *Ag-B* locus. The B2 and B4 hybrids are rats of this type that still possess the $Ag\text{-}B^2$ or $Ag\text{-}B^4$ antigen of the WF and DA strains, respectively.

Mating female × male	*Ag-B* phenotype[a]					
	Females		Males		All progeny	
	$Ag\text{-}B^+$	$Ag\text{-}B^-$	$Ag\text{-}B^+$	$Ag\text{-}B^-$	$Ag\text{-}B^+$	$Ag\text{-}B^-$
B2 hybrid × BN	20	19	15	18	35	37
BN × B2 hybrid	11	10	6	15	17	25
B4 hybrid × BN	13	13	10	10	23	23
BN × B4 hybrid	16	16	9	22	24	38

[a] The designations $Ag\text{-}B^+$ and $Ag\text{-}B^-$ signify the presence or absence of the paternally derived *Ag-B* allele and, therefore, indicate incompatibility or compatibility, respectively, with maternal lymphoid tissues.

topics of current interest: polymorphism of major histocompatibility loci, reproductive immunobiology, ontogeny of the immune system, and the biological significance of histocompatibility loci. Studies are under way which may resolve some of the questions in each category, particularly those relating to mechanisms. A discussion of each of these topics is beyond the scope of this survey. However, several questions are of relevance to this symposium, and these will be considered briefly.

First, what are the consequences of the maternal exposure to fetal antigens that appears implicit in the data, especially in the absence of a major histoincompatibility? It may be wondered, for example, whether such exposure of the mother, while permitting sensitization to certain histocompatibility antigens, might also cause tolerance to antigens of the fetal-oncogenic category, thereby rendering the female less able in the future to restrict the growth of carcinomas bearing such antigens, should they occur. Second, what are the consequences in adult life with respect to neoplasia, immune deficiency diseases, and autoimmunity to those progeny surviving the runting syndrome and the abnormal immune development associated with it?

Perhaps the most important question to be considered is that of the relevance of a rat phenomenon to other mammalian species, including man. The *HL-A* haplotypes are numerous, and, in the sense of this report, most matings of unrelated individuals are of the presumptively protective *HL-A*-incompatible type. On first thought, the rat model might seem of little relevance. Nevertheless, little is known of the relative immunogenicity of various haplotype combinations. Variable degrees of maternal immunocompetence in combination with a weakly immunogenic *HL-A* incompatibility might result in processes similar to those described here for rats. The similarity of the placentas of mice, rats, and humans, all of which are the hemochorial type (8) (*i.e.*, having the fewest cell layers separating mother and fetus) may be an important factor in determining the consequences of insufficient major-locus incompatibility. It may not be coincidence that in many of the families in which *HL-A*-associated disease abnormalities of the immune type have been studied, there has been a degree of *HL-A* haplotype compatibility of progeny to mother (2, 9).

Finally, do these observations of presumably optimally housed, well-fed, laboratory rats reflect a normally occurring event among wild populations, possibly representing one mechanism influencing the degree of *Ag-B* allelic polymorphism in the population? Several features of the rat studies are attractive for thinking in this direction. (*a*) The selective pressures, both positive and negative, are not so absolute as to be self-defeating (total elimination of *Ag-B*-compatible progeny would gradually eliminate the polymorphism). Although a higher incidence of mortality of affected young would be expected to occur in feral colonies, given the same kind of immune selective pressures, the system would be self-limited within colonies once the gene frequencies at the relevant non-*Ag-B* loci became stabilized; (*b*) the mechanism described here would tend to eliminate new mutations at non-*Ag-B* loci and thereby, to the extent that histocompatibility antigens (or loci linked to them) reflect functional proteins in the cell, would reduce the acceptability of suboptimal biological changes; finally (*c*) the system appears to be self-perpetuating: as a result of the subtle sex-related selective pressures, sufficient *Ag-B* homozygous females are left to breed with the excess numbers of *Ag-B* heterozygous males at each generation. The repetition of these events in each generation should maintain an *Ag-B* heterozygote excess among the reproductive population, a major requirement for maintenance of a balanced polymorphism.

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