STUDIES OF THE RAT KAPPA CHAIN ALLOTYPES

by

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INTRODUCTION

Literature Review

An allotypic variant on rat immunoglobulin molecules was first reported by Barabus and Kelus in 1967 (2). These workers injected Wistar BB serum into "black and white hooded" recipient rats. Following 12 weekly injections an antiserum resulted that precipitated with donor serum in a 1% agar gel. Evidence from immunoelectrophoresis and Sephadex G-200 filtration suggested that the allotypic specificity was in the IgG immunoglobulins as it could not be detected in the macroglobulin fraction from G200 gel filtration.

Further evidence for the existence of an Ig allotype in the rat was provided by Wistar (67). DA anti-Salmonella flagella antibody-antigen complexes were injected into Lewis rats. Antiallotype antibodies were detected both by a microprecipitation assay using ^{125}I labeled IgG and by a type of Coombs assay using antibody coated sheep erythrocytes. The antiallotype activity could be inhibited by both IgG and IgM antibodies as well as by purified light chains. Since it had been shown by Hood et al. that the light chains of the rat are 95% kappa type, it appeared that this allotypic specificity designated RI-1 was a genetic marker for the kappa chain (28).

Gutman and Weissman continued this line of work raising antisera to two possibly allelic allotypic specificities (23). They surveyed a number of inbred strains which were positive for one marker or the

other but never both. These two "alleles" were designated RI-1^a and RI-1^b. These investigators also showed that the RI-1 genes were not linked to AgB (the major histocompatibility complex of the rat), or to the hooded, agouti or albino coat color loci.

Light chain allotypes were also studied by Armerding, who detected two alleles that he named W-1 and SD-1 (1). These allotypes were shown to be on the light chain and were present on IgM and two subclasses of IgG immunoglobulins. It appears that the SD-1 marker is the same as that designated RI-1^a by Wistar and the RL-1^a allotype of Gutman and Weissman. The strain distribution of the RI-1^b allele described by Gutman and Weissman corresponds to that of the W-1 gene product of Armerding.

The most extensive survey of inbred strains for the kappa allotypes was reported by Beckers et al. (5). These investigators introduced a new nomenclature for the genes in order to be consistent with designations proposed previously for alpha chains: IK(1a) corresponds to RI-1^b and W-1, while IK(1b) corresponds to RI-1^a and SD-1. Throughout the remainder of this thesis the nomenclature of Beckers et al. has been used. The results of the strain surveys reported by all of the groups are summarized in Table 1.

In 1971, Rokhlin, Vengerova and Nezlin demonstrated that the gene loci controlling the kappa allotypic determinants segregate in a Mendelian fashion and are allelic in nature (55). Further studies on the k chain allotypes by these investigators indicate that:

1) the kappa allotypes are the result of multiple amino acid differences between alleles as shown by peptide mapping of tryptic and cyanogen bromide fragments (65);

Table 1. Distribution of the kappa allotypes in inbred strains of rats (5, 20, 54)

IK (1a) RL2; W-1; RI-1^b)

ACP	BN	LA	RHA
Agus	BP	LEP	Selfed
AS	BS	Lewis	Sherman
AS_2	Buffalo	Long-Evans	*Sprague-Dawley
Atrichus	Cap/Cub	Lou/C/Ws1	+SD F11
August	CAR	Lou/Ws1	WA
AVN	CAS	Lou/M/Ws1	Wistar AG
AXC	E3	Lou/C/IH/Ws1	Wistar Fu
BDX/Cub	F45	M520	Wistar R
BDE	HO	Peth	Zimmerman
Birmingham AA	HS	PVG/C	
Birmingham BB	Hypodactyle	RA	

IK (1b) RL-1; SD-1; RI-1^a)

ACI	Gunn
ACJ	MSU
Albany	OFA
BD	Okamoto
BDV	OM
COP	RLA
Copenhagen	SHR
DA	*Sprague-Dawley
Fisher	NIH Sprague-Dawley F42
F344	Yoshida

*3/14 retired breeders were assayed homozygous Kla/Kla.

+SD-F11 from Tulane University Sprague-Dawley stock selectively bred for responsiveness to Group A streptococcal carbohydrate.

2) these amino acid differences in sequence could be localized to the constant region of the kappa chain (46) and

3) examination of F1 allotypic heterozygotes and wild rats revealed a prevalance of the Klb over the Kla allotype in the serum (54).

This prevalence suggested to the authors that the Klb gene was a "more active gene" and had greater "adaptive value" than the Kla allele. The possibility of new, as yet unreported, allotypic specificities being present in the wild animals was not explored.

Another study of wild populations of Rattus norvegicus was done by Gutman in 1977 (20). Using inhibition of passive hemagglutination and a solid phase radioimmunoassay to detect and quantitate the serological identity of the proteins from wild rats, Gutman found the prevalence of Klb in wild rats from Australia. His survey showed 62 of 64 wild rats trapped at three different distant locations, homozygous for the Klb allele. However, this was not seen in the wild rat populations studied in Japan where 25 wild animals were trapped at three different locations. In this survey only 3 of 25 were shown to be homozygous for the Klb allele whereas 11 of 25 were homozygous Kla and 11 heterozygous Kla/Klb (20).

To date the Kla and Klb alleles are the only allotypic specificities found on the kappa chain. Direct evidence for a new specificity could have come from these studies of wild populations. A new specificity would be indicated by the finding of an individual animal which is negative for both alleles or an animal which is determined to be negative for one allele and positive for the other, but this positive quantitation does not account for all the kappa chains

detected by other means. Neither situation was encountered among 249 wild animals studied by Nezlin et al. or Gutman. Both groups reported that every serum gave an unambiguous positive reaction for one another or both of the known allotypes. The possibility that new specificities do exist in the wild still remains; however, they must be present either at low frequencies or in forms not detected by the antisera used thus far. Both possibilities could be addressed by examination of a large number of wild rats from a wide variety of populations.

Evidence for new allotypic subspecificities on rat kappa chains has been demonstrated by Gutman and Moriwaka through the study of closely related rodents (22). These studies were able to detect at least four subspecificities of the Kla allotype through examination of Rattus rattus subspecies and other Asian rats by passive hemagglutination and allotype specific radioimmunoassays. In addition, unpublished observations by Gutman have detected subspecificities in the Klb allotype among Australian rats. These studies make it evident that we are only at the beginning of our understanding of these genetic markers in rats. More extensive analysis, including sequence analysis will clarify the evolution of kappa chains and the structural basis of their diversity.

One attempt at allotype suppression in the rat was contained in a study by Rokhlin and Nezlin (54). Neonatal F1 heterozygotes were injected with "pure rabbit anti RI-2 (Kla) antibodies" 24 hours after birth and followed for four months. A suppression in the Kla allotype was observed over 3 months as compared to a normal group;

however, no attempt to further quantitate this effect was made, nor was any data given on either the specificity of their rabbit anti-rat allotype antiserum or the behavior of the nonsuppressed allotype.

A study of the ratio of the two allelic variants of kappa chains was conducted by Nezlin and Rokhlin in hybrids rats and hybrids immune to dansyl and azobenzoate haptens (53). This work reconfirmed the prevalence of the Klb allotype in normal Fl hybrids whether or not the mother was homozygous Kla or Klb. In addition, these experiments showed that the ratio of the allotypes in antibody specific to the hapten changed after repeated immunization with the haptens. Two Fl hybrid crosses were used, each crossing a Klb/Klb maternal strain with a different Kla/Kla paternal strain. Antibody to azobenzoate isolated from one hybrid group, designated MW-Fl, had a 1:1 ratio of the allotypes after primary immunization. Upon secondary challenge the percentage of Klb increased to 70% in specific antibody fractions. This shift to Klb prevalence was maintained after the third immunization with azobenzoate. The response of the second Fl hybrids, designated MA-Fl, to the azobenzoate hapten failed to show the prevalence of the Klb allele in all specific antibody fractions. Instead, the percentage of the Klb molecules on the average was the same as those molecules positive for the Kla allele; however, this second experimental group demonstrated a wide variation among individuals. The ratio of Klb to Kla molecules in the serum of individuals ranged from 1:4 to 4:1.

The opposite picture was found in the response of these same Fl hybrids to the dansyl hapten DNS (1-dimethylaminoaphthalene-5-sulfonyl)

(53). In this case the other hybrid group, MA-F1 showed the large prevalence of the Klb allotype in specific antibody whereas the MW-F1 hybrids exhibited wide individual fluctuation and a mean ratio of 1:1.

A study of the amino acid sequence of the constant region of rat kappa chains by Gutman et al. showed that these two serologically detectable forms differed considerably (21). Of the 81 residues of the constant region examined, 11 amino acid substitutions, as well as one size difference were found, confirming the conclusion of Vengerova and Rokhlin that these proteins differed at multiple sites. Since one of these genes could not have been derived from the other by a single point mutation, Gutman and colleagues referred to Kla and Klb as "complex allotypes" and suggested that these genes existed in tandem, or as very closely linked genes, rather than as true alleles. This hypothesis had first been proposed by Bodmer in discussing the polymorphism of mammalian histocompatibility complexes (6). He suggested that serologically detected allelic differences may actually be the products of different closely linked genes and that the genetic polymorphism of regulator genes control which allotype is expressed. This means that every individual of a species has in its genome the information for all "alleles" of an allotype and that the mechanism for controlling the expression of this information is that which is inherited in a Mendelian fashion. From this hypothesis, one would predict that occasionally an individual would be found that expressed the "wrong" or unexpected allotype based on its (genotype) pedigree.

Several reports have appeared in recent years describing situations in which the presence of unexpected "alleles" have been

found. 1) A rabbit of phenotype ala3/b4b5 has been found to express three alleles of the group a (a1, a2 and a3) and three alleles of the group b (b4, b5 and b6) after hyperimmunization with freeze dried Micrococcus lysodeikticus bacteria. Isolated light chains were shown to contain the b4, b5 and b6 specificities and the heavy chains of the a2 specificity were indistinguishable from normal a2 chains by tryptic peptide "map" determination (61). 2) Data presented by Mudgett et al. suggest that a high percentage (50%) of normal rabbit sera express very low amounts of group a allotypes not anticipated by breeding date (45). 3) Lobb et al. detected an apparent discrepancy by immunofluorescence between the genotype and the expressed phenotype of an individual when cells were grown in culture. This conclusion was drawn from the result that both Gm(a) and Gm(b) molecules were detected on cells from a $^{+}b^{-}$ or a $^{-}b^{+}$ donors (37). 4) Rivat et al. also detected the "wrong" or unexpected C_H allotypes in the cultural supernates from human mixed lymphocyte reactions (52). 5) Under conditions of stress, certain mice express an IgG2a allele unexpectedly. The animals from a congenic partner strain of Balb/c-mice, the ICR CB-17 were specially bred so as not to differ from Balb/c in any known way except to carry immunoglobulin structural genes of the C57BL/Ka allotype. Under certain circumstances (bacterial septicemia, onset of a spontaneous tumor, hyperimmunization) the Balb/c allotype appeared in the serum of some ICR CB-17 mice, thought previously to be allotypically homozygous according the Mendelian inheritance of mouse allotype marker (7). The appearance

of this previously "hidden" allotype was usually transient and associated only with immunoglobulins of the IgG (IgG2a) class. The regulator hypothesis of Bodner is not proved conclusively by any of these observations, but, each supports the view that allotypes are closely linked genes which are controlled by a polymorphic regulator mechanism. This regulatory mechanism exhibits the genetic polymorphism associated with the phenotypic expression of the allotypes. However, under certain infrequent conditions, such as neoplasia, septicemia in vitro cultivation or hyperimmunization which place a stress on the cell the regulatory mechanism proves to be "leaky" allowing the expression of the "wrong" or unexpected allotype.

It has been shown by Hunt and Duvall that in most cases the kappa chain allotypes are expressed exclusively by thymus independent B cells (29). In an attempt to prove that these markers were found solely on B cells, radiation chimaeras in which only thymus dependent cells could carry the allotypic marker were constructed. Inbred rats were thymectomized, irradiated, marrow-reconstituted, and allowed to recover as T cell depleted rats. They then received an intravenous injection of thymocytes from either an F1 hybrid (semi allogeneic transfer) or from an allotype congenic strain (syngeneic transfer). The animals were then immunized and their sera analyzed for the presence of allotype of the thymocyte donor allele. In 24 of 26 rats there was no detectable allotype derived from the thymus cell donor in the specific antibodies produced. Whether the significant quantities of the "wrong" allotype present in two of 26 rats was 1) thymocyte specified, 2) derived from B cell contamination by

parathymic lymph nodes or 3) from the release of the wrong allotype by a leaky regulatory mechanism has not been determined.

Immunoglobulin allotypes have provided the cellular markers which have elucidated some of the mechanisms which control the immune response. One such mechanism is based on a network of idiotype anti-idiotype interactions which regulate antibody levels (31). In addition to an idiotype-based network an allotype based network has been suggested (51). The latter idea is based on the demonstration by Herzenberg et al. that in a particular mouse strain, carrier primed T cells from Ig 1a (one allele of mouse IgG2a H-chains) homozygotes collaborated in a secondary anti-DNP response with Igla allotype but not Iglb allotype B cells from DNP primed Igla/Iglb heterozygotes (26).

Previous work by these investigators has shown that perinatal exposure of Balb/c(1a) x SJL (1b) F1 mice to antibody to the paternal Iglb immunoglobulins generates a population of specific suppressor T cells which completely suppress production of Iglb molecules in the majority of exposed mice over six months of age (30). These specific suppressor T cells were shown to act only on the allotype specific T helper population (26). Thus in this allotype suppression system, three cell sets are involved in a network based on an H chain allotype, the Ig 1b, specific helper cells which help only B cells bearing the Iglb allotype and suppressor T cells which specifically suppress these allotype specific T helper cells.

The existence of regulatory network in the mouse based on allotypes was recently confirmed by Bosma et al. (8). These investigators were able to demonstrate allotype specific T suppressor cells in

normal non-immune mice through the use of adoptive lymphocyte transfers between allotype congenic mouse strains. Donors and recipient mice differed only in allotype and yet a donor plasmacytoma which produced the donor allotype was unable to proliferate and kill the majority of recipient animals of a differing allotype. Further studies demonstrated that normal spleen cells from a congenic donor did not produce immunoglobulin in recipient mice unless 1) the donor and recipient were of the same 1b allotype, or 2) the recipient was x-irradiated. These authors named this phemomenon allotype dependent barriers. These barriers which existed in normal mice are mediated by allotype specific suppressor T cells. It is attractive to speculate that these barriers in normal mice are a self regulating network which prevent the expression of the "wrong" allotype and are in fact, the effector mechanism of a polymorphic regulator of closely linked genes.

One of the most fundamental questions for establishing the generality of an allotype based network is whether a similar phenomenon can be demonstrated in another allotype system. The rat K allotypes provide an inbred model system to test the observation of an allotype based cellular network on inbred model system.

Mason et al. used congenic rat strains which differed only in the K allotypes in an attempt to demonstrate L chain allotype specific T helper cells (41). The source of T cells was the K1b/K1b homozygous strain which had been primed several weeks earlier with dinitro-phenyl-bovine gamma globulin (DNP-BGG). They bred an F1 hybrid (K1b/K1b) from allotype congenic strains for their source of B cells or used a K1a/K1a strain. B cell donors had

been primed previously with DNP-BGG. Various mixtures of B cells, T cells and DNP-BGG were injected intravenously into irradiated 1b/1b homozygotes and the number of K1a anti-DNP plaque forming cells per recipient spleen determined one week later. The results showed that helper T cells in the rat lacked specificity for light chain allotypes. That is, the K1b/K1b T helper cells cooperated fully with the K1a/K1a B cells to respond to DNP. The authors could not demonstrate any allotype-specific helper T cells like those shown for the IgG2a H-chain allotype of the mouse.

Suppression of allotype has proven to be a powerful tool in immunogenetic research. It is clear that allotype suppression has provided information on the regulation of immunoglobulin in mice and rabbits. The study of the suppression of paternal allotype in F1 hybrid heterozygotes has led to the definition of two types of allotype suppression. In heterozygous F1 mice (other than Balb/c x SJL) from mothers immune to the paternal H-chain allotype, a short term suppression is seen with the litter reaching control levels of the paternal H-chain allotype by 17 weeks of age. However, the majority of animals from a particular cross of mouse strains (Balb/c x SJL) exhibit a chronic suppression of the paternal H-chains allotype. In this instance very few animals exhibit any detectable paternal allotype at six months of age (26, 30).

A similar situation is seen with suppression of the b group (K chains) allotypes in the rabbit. Some offspring of mothers immune to the paternal allotype exhibit a short term suppression, recovering to normal level of paternal allotype by 60-80 days (40). While some

animals exhibited depressed levels of the paternal allotype for over 3 years (40). Work on the mechanism of this chronic suppression has elucidated two intervals and perhaps two mechanisms of the suppression. The first phase of the suppression is characterized by the ability to neutralize the suppression by administration of minute quantities of the suppressed allotype. This is usually effective until approximately 2 months of age after which the animals are refractory to this treatment (9). The second phase characterized by suppression of the allotype beyond 2 months of age and often for the life of the animal is non-neutralizeable with allotype (9). It has been postulated to be mediated by an active cellular suppression mechanism; however, work to confirm this hypothesis has been hampered by the lack of inbred rabbits.

Previous studies have characterized the anticarbohydrate antibodies induced by streptococcal vaccines in sharks (10), chickens (36), Japanese quail (Leslie, unpublished observations) and rats (35, 56). These studies helped to confirm the initial observation by Osterland et al. with rabbit anti-streptococcal carbohydrate antibodies, which demonstrated an electrophoretic uniformity and a serum concentration much like myeloma proteins (49).

Further studies have examined the genetic influences on the immune response of rats to streptococcal A carbohydrate (SACHO (58). Through selective brother-sister inbreeding of Sprague-Dawley rats, high and low precipitating antibody-producing strains were established.

Additional genetic and structural studies have been done to demonstrate and evaluate a crossreactive rat idiotype, ID-1, to SACHO (32, 57, 58, 59, 60). The detection of a crossreactive idiotype on rat anti-SACHO antibodies obtained from different selectively bred and from nine inbred rat strains suggests the occurrence of a similar or identical antibody variable region genes in unrelated animals. The amino terminal sequence analysis of rat anti-SACHO H-chains isolated from idiotypically related antibody preparations support this view (32). The inability to detect ID-1 on anti-SACHO antibodies produced by goats, rabbits, chickens, and nurse sharks suggests the species specificity of ID-1.

Several factors make suppression of allotype in the rat a valuable model for research. As in other mammals, very little immunoglobulin is secreted by the neonate; most that is present in the circulation at birth is derived from the mother (24, 44). Studies on the fate of radiolabeled immunoglobulin have shown that maternal antibody is responsible for immune protection of the young rat while it is developing its own immunologic competence (24, 43, 44). This presents an interesting opportunity for the modulation of gene products in the progeny through calculated manipulations in specific maternal antibody levels. The complex multideterminant nature of the target allotype and the precipitating antibodies which are elicited are two facts which favored the plausibility of allotype suppression in the rat. In addition, unrelated studies have demonstrated the capacity of antibody to affect immunoglobulin subclass ratios as well as to suppress rat lymphocytes in vitro (12,13,34). The rat

provides a unique experimental system to study allotype suppression. There are defined allotypes on the alpha, gamma 2b heavy chains as well as the kappa allotypes (3, 4). Thus the rat is the only inbred species with heavy and light chain serologically defined allotypic markers. This not only presents the opportunity of evaluating the generality of the murine allotype studies, but also extending the knowledge of H and L chain marker interactions established in the rabbit.

The significance of allotype suppression as a general phenomenon stems from the fact that it may constitute the only clear example in mammals of an antibody induced modulation of phenotype. Some investigators believe that it may represent a special case of immune inhibition of gene activity exerted at the level of immunocompetent lymphocytes (9). There is no firm evidence for the latter generalization; nonetheless, allotype modulation in the mouse is now often quoted as one operational model for the study of immune regulation.

Statement of the Problem

Allotypy in immunoglobulins was first described by Grubb in the human and Oudin in the rabbit in 1956 (19, 50). Oudin coined the term allotype to describe serum proteins which were present in distinct antigenic forms in some but not all members of a species. These antigenically unique forms segregated in a Mendelian fashion, were not sex linked and were co-dominant in heterozygous individuals.

The suppression of allotype has proven to be a powerful tool in immunogenetic research. Such suppression has provided information on the regulation of immunoglobulin synthesis in mice and rabbits. To date allotype suppression has not been extensively investigated in the rat, although the rat provides the only highly inbred species with defined H and L chain allotypes.

The objectives of this thesis are four-fold:

1) to produce alloantiserum to the rat kappa allotypes by cross immunization of inbred rat strains,

2) to establish allotype suppression in heterozygous F1 hybrid animals using two approaches,

a) perinatal administration to F1 hybrids of anti-allotype antibody to the paternal allotype and

b) immunization of females against the paternal allotype prior to breeding, followed by examination of allotype levels in the F1 heterozygous progeny.

3) to determine the time course of the allotype suppression, and

4) to analyze "suppressed" animals for compensation by the nonsuppressed allotype through increased serum levels. Allelic compensation for the suppressed allotype appears to be a homeostatic mechanism which is often observed in allotype suppressed rabbits. Total immunoglobulin values in these animals are comparable to normal controls despite the abnormal depletion of one allotype.

MATERIALS AND METHODS

Animals

August (AUG), Copenhagen (Cop) and Marshal 520 (M520) rats were obtained from ARS/Sprague-Dawley laboratories. Fisher 344 (F344) rats were obtained from Simonsen laboratories. Outbred Sprague-Dawley retired breeders were obtained from Charles River Labs. All animals were maintained in groups of 2-4 on Purina Chow Pellets (Ralston Purina, St. Louis, Mo.) and water ad libitum.

Buffers

1) Tris buffered saline (TBS)

Stock solution

Trizma HCL	132.2 g
Trizma Base	19.4 g
Sodium Azide	100.0 g
Disodium EDTA	37.2 g
Distilled H_2O	q.s. 1 liter
pH	7.4

This solution is filtered through Whatman #1 paper prior to use.

Working buffer - 0.01 M Tris-Tris-HCl

Stock solution	160 ml
NaCl	132.48 g
Distilled H_2O	q.s. 16 liter
pH	7.4

2) Immunodiffusion buffer

Sodium Barbital	6.98 g
NaCl	6.00 g
Thimersol (Lilly)	100 mg
Distilled H_2O	q.s. 1
pH	7.40

1% agarose in immunodiffusion buffer is immunodiffusion agar.

3) Immunodiffusion buffer with polyethylene glycol (PEG)

2 g of PEC (average molecular weight 3000-3700) is added to 100 ml of 2 above.

1% agarose in PEG immunodiffusion buffer is PEG immunodiffusion agar.

Streptococcal vaccine

Streptococcal vaccine (GASV) was prepared from a lyophilized stock culture of Group A Streptococci, strain J17A4, originally obtained from Dr. Richard Krause of the Rockefeller University. Briefly, 10 liters of an 18 hour Todd-Hewitt broth culture of the streptococci were heat-killed at 60° C for 90 minutes. The cultures were streaked on blood agar plates before heating to establish their purity and after heating to confirm that all streptococci were killed. The

streptococci were collected by centrifugation and the cells washed several times with sterile saline. The streptococcal pellet was suspended in 250 ml of sterile saline adjusted to pH 2, containing 500 mg of pepsin. The mixture was incubated with continuous mixing for 2 hours at 37° C, centrifuged and washed several times with sterile saline. The final product was suspended in sterile saline at a concentration of 10 mg/ml (dry weight) and cultured to check sterility.

Immunization regimens

1) Autologous anti-allotype

Alloantisera containing antibodies to the kappa allotypes were raised by two methods. Both methods are modifications of conventional methods used in the mouse.

a) Inbred strains of rats were first immunized with Group A Streptococcal vaccine (GASV). The specifically purified IgG antibodies (by molecular weight from Sephadex G200 gel filtration) elicited to the Group A Streptococcal carbohydrate (SACHO) were used as the antigen. The following strains presumed to be mismatched at the K allotype locus were reciprocally immunized with 100 µg specifically purified IgG anti-SACHO antibody which had been incubated for two hours with 0.5 mg of GASV and emulsified in an equal volume of complete Freund's adjuvant (CFA):

August (K1a) into Copenhagen (K1b) - anti-K1a

Copenhagen (K1b) into August (K1a) - anti-K1b

M520 (K1a) into F344 (K1b) - anti K1a

F344 (K1b) into M520 (K1a) - anti-K1b

The total volume of immunogen given each animal per injection was 0.2 ml. The following schedule was used:

Week		Week	
1	footpads	5	subcutaneous
2	subcutaneous	6	IP - bleed & test
3	intraperitoneal (IP)	7	subcutaneous - induced ascites
4	subcutaneous	8	IP

After 8 weeks of the schedule animals were immunized every two weeks. Animals were bled beginning prior to the 6th week of immunization and their sera tested for the presence of precipitating antibodies by double diffusion against the antigen in PEG agar.

An ascites was induced at week six using a modification of the method of Tung et al. (63). Briefly, the antigen or sterile saline is emulsified in CFA in a 1:9 ratio (Ag:CFA). One ml of this emulsion is injected intraperitoneally. This is continued on a weekly basis with animals developing a substantial ascites normally after the 2nd or 3rd injection. With the onset of a noticeable ascites animals were checked daily and drained of ascites as necessary. Production of the ascites was more pronounced in females with individuals producing as much as 75 mls per tap and yielding a total volume of over 200 mls of fluid. Using this method and weekly bleedings a considerable pool of alloantiserum was obtained.

b) The second method utilized in the production of alloantisera to the kappa allotypes involved a modification of the

procedure of Warner et al. (66). Strains serving as the source of immunogen were first immunized repeatedly (3 to 4 times over two weeks) with 1-3 x 10^7 spleen cells from the strain to be immunized. This same protocol has been used by DeWitt to produce anti-AgB alloantisera (anti-rat MHC) (14). Three to four days following the last injection of spleen cells the animals were bled out and their serum pooled. This pool was successively fractionated with 15% w/v sodium sulfate followed by two salt fractionations of 14% w/v sodium sulfate. After extensive dialysis the gamma globulin rich fraction was applied to upward flow Sephadex G200 columns and the peak corresponding to a rabbit IgG marker concentrated and recycled over G200. This recycled IgG peak served as the immunogen for the strain which had donated the spleen cells. The same immunization schedule was followed as before, except that no GASV was used in the emulsion.

Immunization protocol for Group A Streptococcal vaccine (GASV)

Animals immunized with GASV are treated in the following manner: Animals are bled from the tail vein (Prebleed) and immunized on 3 consecutive days with 1 mg/injection of GASV. This is done weekly for three consecutive weeks (wk 1, 2 & 3), with the animals being bled at the onset of each weekly immunization schedule. The animals are then rested 4 to 8 weeks. The secondary series follows an identical time schedule as the first series with animals being bled at the beginning of the 3 day immunization series; however, they are given 0.2 mg per injection of GASV during the secondary series (wk 4, 5 & 6). Four days after the last injection of week 6 the animals are bled out.

Passive hemagglutination for the detection of the allotypes

Passive hemagglutination of antibody sensitized sheep erythrocytes (SRBC) as described by Gutman was used with minor modifications to evaluate individual serum and ascites samples for antiallotypic antibody (23).

Antibody to SRBC was obtained by intraperitoneal injection of 0.1 ml of a 25% suspension of SRBC (Prepared Media Labs, Tualatin, Ore.) into normal animals homozygous Klb/Klb and Kla/Kla. The animals were bled six days later and the serum was heat inactivated by a 30 minute incubation at 56° C. Micro-hemagglutination was carried out in V-bottom titer plates (Cooke Laboratory Products, Alexandria, Va.) with 25 µl of serially diluted serum (in Tris buffered saline) and 25 µl of a 1% suspension of washed SRBC.

SRBC'ε were sensitized with the dilution of anti-SRBC serum from allotypic homozygous donors at a dilution corresponding to one or two wells past the end point of agglutination. After 1-2 hours of end over end mixing at 4° C the cells were washed once with TBS and resuspended at a 1% suspension.

All sera to be assayed by passive hemagglutination were first heat inactivated (30 minutes at 56° C) and absorbed overnight at 4° C with 10% v/v packed SRBC to remove natural hemagglutinins.

Serum to be tested for antiallotype activity was serially diluted in 25 µl of 1% bovine serum albumin (Fraction V, Pentex, Inc., Kankakee, Ill.) in Tris buffered saline (1% BSA-TBS) and 25 µl of a 1% suspension of sensitized SRBC added. Log_2 titers were read 2

hours later. Controls always included unsensitized SRBC with alloantiserum, sensitized SRBC with no alloantiserum and sensitized SRBC with antiallotypic antiserum of known activity for each preparation of sensitized SRBC. Sensitized SRBC were prepared fresh and discarded after one day.

Serum and ascites pools for each allele were made on the basis of passive hemagglutination titers.

Gel filtration

Gel filtration was performed in 2.6 cm x 100 cm columns packed with Sephadex G200 or G150 matrix (Pharmacia, Upsaala, Sweden) and previously calibrated with known molecular weight markers.

Protein concentration was determined by reading the absorbance at 280 nm. An extinction coefficient of $E_{280\ nm}^{1\%,\ 1cm} = 14.0$ was assumed for IgG (1). The peaks were pooled, concentrated by negative pressure dialysis and recycled over the G200 column.

Preparation of subunits of rat IgG

1) Rat $F(ab')_2$ fragments

$F(ab')_2$ fragments were prepared from representative rat strains homozygous for the Kla or Klb allele using the method of Stankus and Leslie (57). Briefly, IgG isolated from normal serum by successive salt fractionations and G200 gel filtration, as previously described, was digested with 1% (w/w) pepsin (Worthington Biochem. Corp., Freehold, N.J.) in 0.1 M acetate buffer at pH 4.0 at 37° C for 12 hours. After digestion the fragments were filtered through

a Sephadex G150 column calibrated with rabbit IgG and $F(ab')_2$ fragments. The effluent peak corresponding in molecular weight to rabbit $F(ab')_2$ fragments was pooled, concentrated and analyzed by immunoelectrophoresis.

2) H and L chain separation

H and L chains were prepared from the same starting material as was used for the preparation of $F(ab')_2$ fragments at a concentration of 10-20 mg/ml in 0.5 M Tris, pH 8.0. Reduction was done in 0.1 M 2-mercaptoethanol (Sigma, St. Louis, Mo.) for 1 hour at room temperature, followed by alkylation with 0.1 M iodoacetamide for 1 hour at 4° C (67). The resultant mixture was dialyzed overnight against 5.5 M guanidine hydrochloride (GHCl) and placed on a column of Sephadex G200 in 5.0 M guanidine hydrochloride. The absorbance of the effluent fractions was read at 280 nm. Peaks corresponding with rabbit IgG H and L chain markers were pooled, concentrated and recycled over the GHCl-G200. Recycled peaks were concentrated and dialyzed against Tris buffered saline. Antigenic analysis of the recycled peaks by double diffusions revealed no detectable H chain contamination of the L chain peak; however slight L chain contamination was detected in the H chain preparation.

Preparation of immunoadsorbants

August IgG (K1a/K1a), F344 IgG (K1b/K1b), human Bence-Jones lambda, chicken anti-human lambda, and a human IgG_2K myeloma protein immunoadsorbants were prepared by the procedure of Cuatrecasas (11). Sepharose 4B or 4BCL was washed with distilled H_2O (~100 v/v

Sepharose). The washed, packed Sepharose (1.5-2 ml Sepharose per 4 mg of protein to be conjugated) was resuspended in an equal volume of distilled water. A 10% w/v solution of CNBr in distilled water was prepared and 10 ml of this solution was added per 10 ml of packed Sepharose. The reaction was kept on ice and the pH maintained at 11.0 with 4N NaOH until stable. The activated Sepharose was washed with 1-2 liters cold (4° C) distilled water and subsequently with 1-2 liters cold 0.5 M $NaHCO_3$, pH 9.0. The washed, packed, activated Sepharose was resuspended in an equal volume of 0.5 M $NaHCO_3$, pH 9.0, the protein (previously dialyzed into the bicarbonate buffer) added, and the mixture gently mixed overnight at 4° C. The resultant immunoadsorbant was washed extensively with Tris buffered saline, 3 M NaSCN and finally with Tris buffered saline a second time. Samples were applied directly to the immunoadsorbants in small glass columns and the unbound material washed until the effluents had readings at 280 nm of less than 0.010. The columns were then eluted with 3 M NaSCN until the absorption at 280 nm decreased to less than 0.010 and finally extensively washed with Tris buffered saline to remove the NaSCN.

Rabbit antiserum to rat lambda chain

Rabbit antiserum to rat lambda chains was prepared as follows: rat lambda containing immunoglobulins were isolated from normal rat serum by virtue of their crossreactivity with human lambda (48, 60). A chicken anti-human Bence-Jones lambda protein immunoadsorbant was used to purity rat lambda-containing immunoglobulins (60). Material

eluted from this column with 3M NaSCN was dialyzed, concentrated and used as the immunogen.

Two rabbits were given 100 µg each of the immunogen in the footpads, followed by a subcutaneous injection of an additional 100 µg at several sites on the back three weeks later. Bleedings were taken weekly from the lateral ear vein. Bleedings were pooled and purified by elution from an immunoadsorbant of purified human Bence-Jones lambda protein. The eluate from the lambda immunoadsorbant column was then placed over a human IgG_2K myeloma immunoadsorbant to remove any contaminating anti-K, anti-L or IgG H chain antibodies. The resultant antiserum exhibited the following characteristics by gel double diffusion analysis.

1) Single precipitin lines of partial identity were observed when the rabbit anti-rat lambda and a commercial goat anti-human lambda (Meloy Laboratories) were reacted with human lambda isolated from immune gamma globulin by the chicken anti-human immunoadsorbant.

2) Single lines of partial identity were observed when the rabbit anti-rat lambda was reacted with human lambda from immune gamma globulin and lambda-containing rat IgG (by molecular weight from gel filtration).

3) The rabbit anti-rat lambda antiserum also detected a reaction of partial identity between a human myeloma protein defined as IgD-lambda and the rat lambda-containing immunoglobulin preparation.

4) The rabbit anti-rat lambda reacts with two precipitin bands when reacted against dilutions of normal rat serum from the

inbred strains available. Every strain gives a precipitin reaction. Single reaction lines are obtained against normal "IgM" and "IgG" (by molecular weight-gel filtration) from all inbred strains tested.

5) A strong reaction is obtained when the rabbit anti-rat lambda antiserum is reacted against isolated L chain preparations.

6) No reaction at a wide range of dilutions was seen when the rabbit anti-rat lambda was set against specifically purified rat anti-SACHO antibodies or anti-SACHO $F(ab')_2$ fragments in gel diffusion. These antibodies have previously been shown to be devoid of lambda type chains by gel diffusion (60).

Alloassays for the detection and quantitation of the K allotypes

Radioimmunoassays (RIA) were developed for the detection and quantitation of the two alleles of the rat kappa allotypes.

First, pools of Kla and Klb alloantisera were made on the basis of reactivity in double diffusion and by the relative titers of individual serum and ascites samples by passive hemagglutination of allotype sensitized SRBC. The anti-Kla and anti-Klb pools were then specifically purified by passage over Kla/Kla IgG or Klb/Klb IgG immunoadsorbants. The 3 M NaSCN eluates from these columns were dialyzed extensively against Tris buffered saline and concentrated.

These specifically purified antiallotypic antibody preparations were then each conjugated to N-hydroxysuccinimide-activated Sepharose 4B-CL beads (Pharmacia, Upsaala, Sweden) which were described by Gottlieb et al. (18). The conjugated beads were brought up in a volume of Tris buffered saline with 2% w/v BSA (TBS-BSA) equivalent

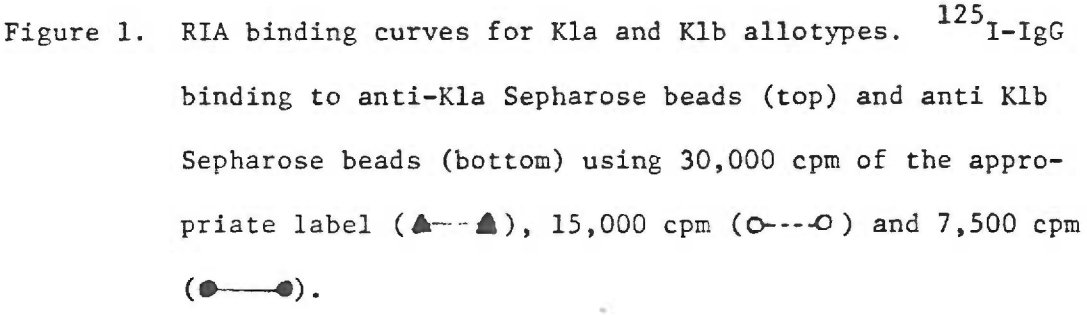
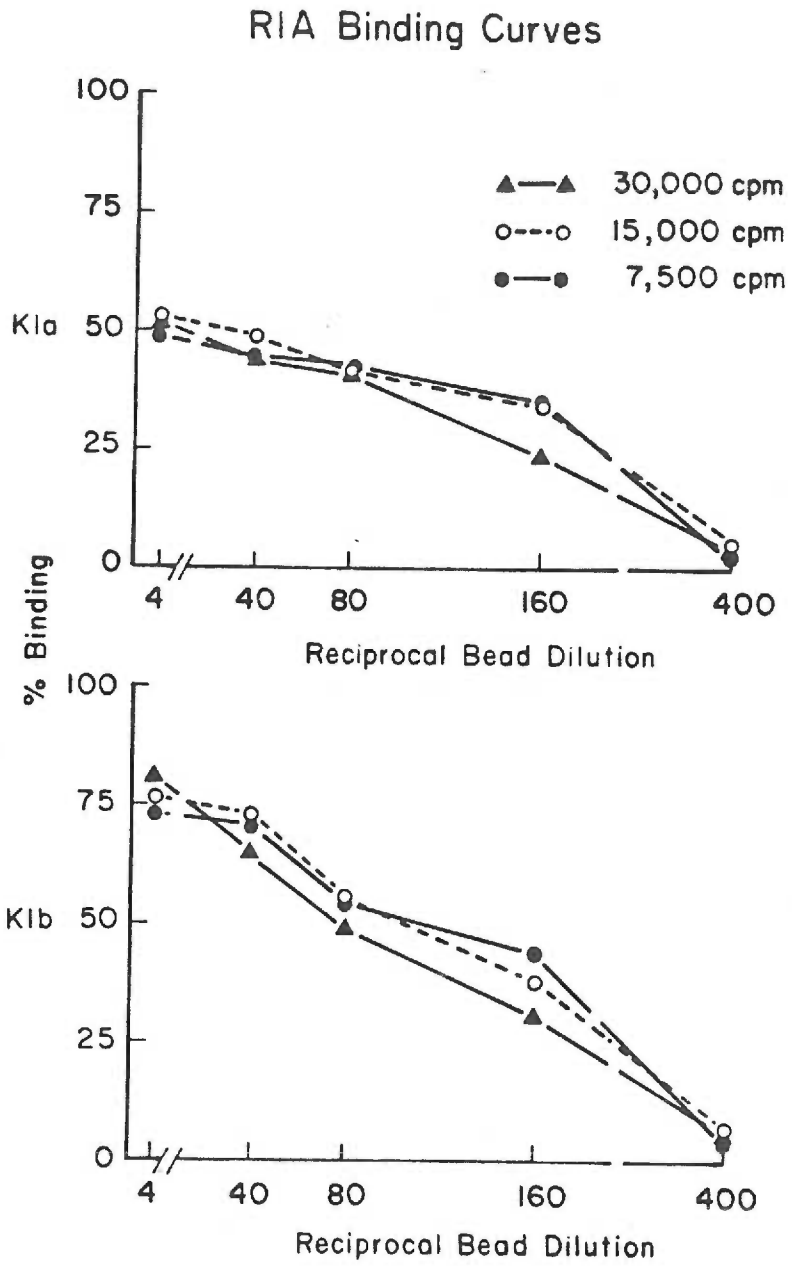


Figure 1. RIA binding curves for Kla and Klb allotypes. ^{125}I-IgG binding to anti-Kla Sepharose beads (top) and anti Klb Sepharose beads (bottom) using 30,000 cpm of the appropriate label (▲---▲), 15,000 cpm (○---○) and 7,500 cpm (●——●).


RIA Binding Curves
100
75
Kla 50
25
0
% Binding
30,000 cpm
15,000 cpm
7,500 cpm
4
40
80
160
400
Reciprocal Bead Dilution
100
75
Klb 50
25
0
4
40
80
160
400
Reciprocal Bead Dilution

to 3 times their packed volume.

Normal F344 (K1b/K1b) IgG (by salt fractionation and gel filtration) and normal August (K1a/K1a) IgG (by salt fractionation and gel filtration) were radiolabeled by the procedure of McConahey and Dixon (42). Binding curves for the two assays were determined by varying both the bead dilution and the counts per minute (cpm) of label used. Figure 1 shows the binding curves obtained for representative bead preparations of each allotype. From these curves working dilutions of beads and label were resolved. The radioimmunoassay was done by placing 100 µl of the optimal bead dilution, 50 µl of 2% TBS-BSA diluent, 25 µl of the optimal label dilution and 25 µl of the unknown sample dilution in a 400 µl microfuge tube (total volume 200 µl). The tubes are vortexed and mixed continuously end-over-end for 12-18 hours at 4° C. The tubes are then centrifuged in a Beckman Microfuge (Beckman Instruments, Mountain View, Ca.) at 15,000 rpm (∿10,000 x g) for 5 minutes at room temperature. 100 µl of the supernatant is withdrawn and counted in a gamma counter in addition to the remaining pellet. Percent binding is determined by:

$$\%B = 100 \times \frac{\text{cpm pellet} - \text{cpm supernatant}}{\text{cpm pellet} + \text{cpm supernatant}}$$

Inhibition of radiolabel binding to the conjugated beads was used to quantitate the unknown serum. A standard curve was plotted (% inhibition versus amount inhibitor) by using known amounts of K1b positive immunoglobulin or K1a positive immunoglobulin to inhibit the assays. Using a program developed by Daniel Gray (Veterans Hospital, Portland, Ore.) and the Wang series 700 calculator (Wang Laboratories,

Merrill, Mass.), the standard curves of each assay were plotted, a quadratic regression run, 95% confidence limits determined, and unknowns calculated for each assay. A quadratic regression was run on the standard curves rather than the more theoretically correct logit curve because it fit the data best. Using this regression, assays were repeated when greater than 1% of the error was accounted to running the regression (coefficient of correlation of ≥ 0.99).

Percent inhibition is calculated from the following formula:

$$\%I = 100 - \frac{B_t - B_c}{B_{max} - B_c} \times 100$$

Where: B_t = % binding of the unknown test sample

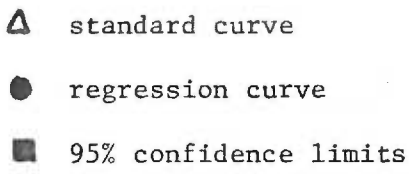
B_c = % binding maximum inhibition

B_{max} = maximum uninhibited % binding

Figures 2 and 3 are representative standard curves for both alleles. Each shows the standard curve, regression and 95% confidence limits. Unknown test sera were assayed at several dilutions in order that the most linear and accurate portion of the curve was used to determine allotype values. A typical assay of unknowns was run with uninhibited tubes to determine maximum binding; replicate tubes with 25 µl whole serum for 0% binding; replicate tubes with normal rat serum homozygous for one allele at a dilution to give 50% inhibition and to serve as an internal standard; and finally the dilutions used to prepare the standard curve.

Results are expressed as mg/ml of allotype and each determination represents the mean of at least 2 radioimmunoassays.

Figure. Standard curve for Kla RIA. RIA is as described in Materials and Methods.



Abcissa is a $\log_{10}$ scale of allotype concentration.

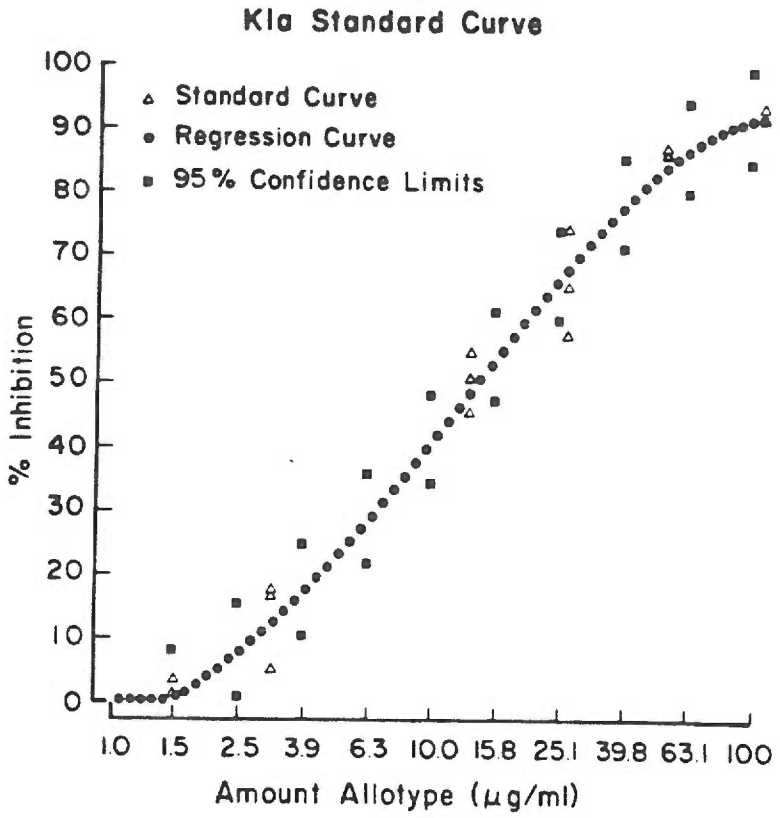
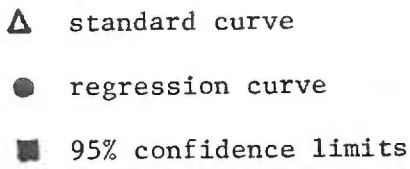
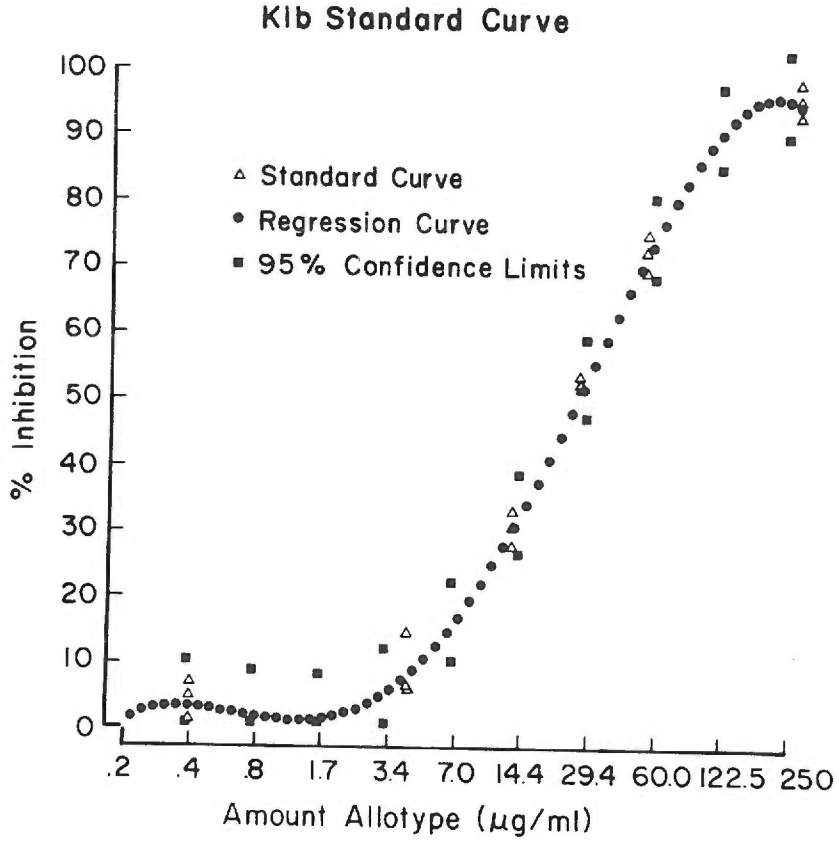

Kla Standard Curve
Standard Curve
Regression Curve
95% Confidence Limits
% Inhibition
100
90
80
70
60
50
40
30
20
10
0
1.0
1.5
2.5
3.9
6.3
10.0
15.8
25.1
39.8
63.1
100
Amount Allotype (μg/ml)

Figure 3. Standard curve for Klb RIA. RIA is as described in Materials and Methods.



Abcissa is a $\log_{10}$ scale of allotype concentration.


Klb Standard Curve
△ Standard Curve
• Regression Curve
▪ 95% Confidence Limits
% Inhibition
100
90
80
70
60
50
40
30
20
10
0
.2
.4
.8
1.7
3.4
7.0
14.4
29.4
60.0
122.5
250
Amount Allotype (µg/ml)

Total serum protein

Total serum protein was determined by refractometry (Bausch & Lomb) and quantitated using a standard BSA curve.

Immunoelectrophoresis (IE)

Immunoelectrophoretic analysis was performed in IE agar. IE agar was prepared by adding 5.4 g sodium barbital, 2.3 g Na acetate, 58.2 ml of 0.1 N HCl and 10 ml of a 1% thimersol solution to 1 liter of distilled water. The final pH was 8.6 and the ionicity 0.05. Addition of 1% (w/v) agarose was made to the above for IE agar. The electrophoretic runs were made in a Gelman electrophoresis chamber at a constant current of 13 mamp per tray (27 x 5.5 cm) for 75 min.

Cellulose acetate electrophoresis (CA).

Cellulose acetate electrophoresis was performed with a Beckman Microzone electrophoresis cell. Serum samples were supported in a cellulose acetate medium, and electrophoretic runs were made at 250 V for 20 min in barbital buffer (Beckman, Buffer B-2), pH 8.6 and ionic strength 0.075. The cellulose acetate strips were stained for 10 min with Ponceau S, rinsed in 5% (v/v) acetic acid, dehydrated in absolute methanol, and cleared in an 18% (v/v) acetic acid/methanol solution.

The CA strips were scanned using a Quick scan (Helena Labs) integrating gel scanner and the percentage of serum proteins in each peak calculated.

Quantitation of serum immunoglobulin class levels by radial immunodiffusion (RID)

RID plates were prepared by mixing either 0.6 ml or 1.5 ml of rabbit anti-rat IgM, goat anti-rat IgG2a, goat anti-rat IgG2b, goat anti-rat IgG2c or sheep anti-rat IgG1 with a 1% (w/v) solution of Seakem agarose (MCI Biomedical) in immunodiffusion buffer at 50° C, to yield a final antiserum dilution of 1:10. Six mls of this mixture was applied to a 7.5 cm x 5 cm slide and 15 ml, to a 10 cm x 8 cm lantern slide. Wells 2.3 mm in diameter were cut in the solidified agarose just prior to use. Rat sera were added to the wells and the reactions were allowed to proceed for 5-24 hrs at 4° C in a moist chamber. Dilutions of rat serum whose immunoglobulin class levels were known were included on each plate. After incubation the diameters of the precipitin rings were measured to the nearest 0.1 mm with a Bausch and Lomb measuring magnifier and darkfield lighting.

Anti-rat antisera

The goat anti-rat L, goat anti-IgG2a, rabbit anti-IgG2b, goat anti-IgG2c, rabbit anti-rat IgM, sheep anti-rat IgG1 and goat anti-whole rat serum, were the kind gifts of Dr. Gerrie A. Leslie, University of Oregon Health Sciences Center.

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immunoglobulin. None of the other antiallotype antisera contained precipitating antibody; therefore, common methods of quantitation, based on precipitating antibody, i.e. reverse radial immunodiffusion, could not be used to measure amounts of antiallotype antibody in each antiserum. Antiallotype antibody in the antisera was detected and a semi-quantitative comparison of the three antisera to each allotype made by PHA. A comparison of the anti-Klb antisera by PHA gave the following agglutination titers: Aug anti-Klb (MHC) 1:2048, Aug anti-Klb (GASV), 1:256, M520 anti-Klb (GASV) 1:128. Given that these data were obtained using the same preparation of sensitized SRBC, it appeared that the Aug anti-Klb (MHC) antiserum had the greatest amount of antiallotype antibody and was used throughout the remainder of these studies. Asimilar comparison of the anti-Kla antisera showed little difference in antiallotypic activity as judged by PHA. The Cop anti-Kla (MHC), the Cop anti-Kla (GASV) and the F344 anti-Kla (GASV) had PHA titers of 1:64 . The Cop anti-Kla antisera produced from both immunization methods were pooled and used in these studies.

Specificity of the alloantisera

A question crucial to these studies was the specificity of the antisera to the kappa allotypes. Inhibition of the radioimmunoassay (RIA) with various samples was used to assess the specificity of each antiserum. Using the RIA it was possible to accurately detect as little as 50 ng of the Klb allotype and 80 ng of the Kla allotype.

A survey of several strains of rats revealed that only strains reported to be homozygous for an allele would inhibit the RIA directed

against that particular allele (Table 2). Three of the strains homozygous for Kla failed to give complete inhibition of the RIA. It is uncertain if this is significant and real or represents the fluctuation in replicate tubes from the strain survey RIA. The survey did reveal a difference from previous reports on the allotype of outbred Sprague-Dawley (SD) rats. Previous investigators have uniformly reported this strain as homozygous Klb (1, 5, 54). My RIA, conducted on 14 retired breeders obtained from Charles River Laboratories, showed 3/14 animals were homozygous Klb while the remainder were homozygous Kla (Table 3). Selectively inbred strains of SD rats were established based on responsiveness to SACHO from outbred SD stock obtained from Tulane University. It is of interest to note that examination of serum pools from four filial generations of high and low responders showed these animals were uniformly homozygous Kla.

Localization of the Kla and Klb allotypes on immunoglobulins was accomplished by inhibiting the RIA with fragments of immunoglobulin molecules (Table 4). IgM and IgG from appropriate rat strains were obtained by salt fractionation with Na_2SO_4 and Sephadex G-200 gel filtration. Antigenic analysis of these fractions by Ouchterlony double diffusion demonstrated that the IgM fraction contained a slight, but detectable, amount of IgG; whereas the IgG fraction contained no detectable contaminating IgM. Other serum proteins are present in both fractions as judged by immunoelectrophoresis using a goat anti-whole rat serum antiserum. When the IgM and IgG rich-fractions from appropriate strains were assayed in the Kla and Klb RIA, both fractions

Table 2. Distribution of Kla and Klb allotypic specificities in various strains of rats

Serum inhibitor (25 µl)	Number tested	Percent inhibition of RIA	
		Kla	Klb
August (Aug)	Pool>10	100	0
Copenhagen (Cop)	Pool>10	0	100
Fisher-344 (F344)	Pool>10	0	100
Lou/C/Wsl	1	95	0
Lou/Mn	Pool>10	100	0
Spontaneous hypertensive rat (SHR)	Pool 8	0	100
Marshall 520 (M520)	Pool 10	100	0
Brown Norway (BN)	1	100	0
Buffalo	Pool 3	100	0
Wistar Furth (W/Fu)	Pool 10	92	0
Sprague-Dawley low responder SACHO F7[Δ]	4	100	0
Sprague-Dawley high responder SACHO F9[Δ]	Pool 10	100	0
Sprague-Dawley high responder SACHO F11[Δ]	Pool 10	95	0
Sprague-Dawley high responder SACHO F12[Δ]	14	100	0
Sprague-Dawley inbred NIH-F42[+]	Pool 15	0	100
Sprague-Dawley outbred (SD)[*]	14	11/14	3/14

[+]Inbred Sprague-Dawley F42 obtained from NIH, Bethesda, Md.

[*]Outbred Sprague-Dawley obtained from Charles River Laboratories, North Wilmington, Mass. 3/14 animals gave 100% inhibition of the Klb RIA; the other gave 100 % inhibition of the Kla RIA.

[Δ]Selectively inbred Sprague-Dawleys for responsiveness to Group A streptococcal carbohydrate from outbred stock obtained from Tulane University.

Table 3. Distribution of K1a and K1b allotypic specificities in 14 outbred Sprague-Dawley rats. In each case 25 µl of undiluted serum was used to inhibit the RIA.

	Percent inhibition of RIA	
Animal number	K1a	K1b
SD 1	0	100
2	100	0
3	100	0
4	100	0
5	100	0
6	0	100
7	100	0
8	100	0
9	100	0
10	100	0
11	0	100
12	100	0
13	100	0
14	100	0

Table 4. Localization of Kla and Klb allotypic specificities on kappa light chains by inhibition of the radioimmunoassays

		Percent inhibition of RIA	
Inhibitor (25 µl)	Concentration	Kla	Klb
No inhibitor	-	0	0
Kla serum	undilute	100	0
Kla IgM	1 mg/ml	100	0
Kla IgG	1 mg/ml	100	0
Kla IgG $(F(ab')_2$	1 mg/ml	100	0
Kla IgG H chains	*	5-7	0
Kla IgG L chains	*	75	0
Klb serum	undilute	0	100
Klb IgM	1 mg/ml	0	100
Klb IgG	1 mg/ml	0	100
Klb IgG $F(ab')_2$	1 mg/ml	0	100
Klb IgG H chains	*	0	8-10
Klb IgG L chains	*	0	77
(Kla x Klb) F1 hybrid serum	undilute	100	100

*Equivalent amounts of H and L preparations added to each assay based on absorbance at 280 nm of approximately 0.450.

inhibited completely the appropriate RIA (Table 4). $F(ab')_2$ fragments were prepared from representative strains (Aug & F344) by pepsin digestion of specifically purified anti-SACHO IgG. Non-immune IgG fractions as described above, were also used for preparing $F(ab')_2$ fragments. The digest was applied to a Sephadex G-150 column and the peak corresponding to a rabbit IgG $F(ab')_2$ marker, was collected and concentrated. Analysis of this peak by immunoelectrophoresis using an anti-whole rat serum reagent showed a single arc in the IgG region. Inhibition of the K1a and K1b allotype RIA with $F(ab')_2$ fragments prepared from Aug (K1a) and F344 (K1b) strains was as complete as an equivalent amount of IgG (Table 4). H and L chains were prepared from the IgG of strains of each allotype and analyzed by Ouchterlony double diffusion. Light chain preparations contained no detectable H contamination, but reacted strongly with a goat anti rat L reagent; however, H chain preparations had a slight but detectable contamination with L chains. The inhibition of the RIA with L chains prepared from strains of each allotype localized the specificity of each antiserum to the L chain. When equivalent amounts of the H and L chain preparations (as determined by absorbance at 280 nm) were assayed in each allotype RIA greater than 75% inhibition was seen with L chain preparations of the appropriate strain; whereas H chain preparations of the appropriate strain caused 8% or less inhibition of the RIA. The inhibition obtained with the H chain preparations is most likely caused by the small amounts of contaminating L chains detected by gel diffusion in the H chain sample.

Rat immunoglobulin fractions were depleted of the lambda type of light chain based on its crossreactivity with human lambda chain (48, 60). Using the rabbit anti rat lambda reagent, no lambda chain was detected by immunoelectrophoresis or Ouchterlony double diffusion; however, these lambda depleted samples were still able to inhibit completely the appropriate RIA. A study of specifically purified anti-SACHO antibodies from SD rats has shown them to contain only K type light chains (60). These same specifically purified anti-SACHO antibodies inhibited fully the appropriate allotype assay.

In order to prove that the anti-Kla and anti-Klb antisera did not share antibody to common determinants, one allotype's radiolabel was incubated with antiserum to the alternate allotype. No direct binding of the inappropriate radiolabel could be detected in either radioimmunoassay over a wide range of concentration.

Reproducibility of the radioimmunoassays for allotype

The validity of each RIA was rigidly controlled by assessing any statistically significant deviation in 1) the standard curve assayed in triplicate with each RIA and 2) an internal control which closely resembled test samples.

Standards for each assay consisted of dilutions of the same IgG preparation used for radiolabeling. Before unknowns were analyzed the standard curve was plotted, a quadratic regression calculated and 95% confidence limits determined. Assays with standards outside the 95% confidence limits of previous curves were repeated. Figures 2 and 3 show representative standards, regression curves and 95%

confidence limits obtained for each allotype RIA.

Unknown serum samples were assayed at various dilutions in order to bring the level of inhibition to the most sensitive linear portion of the standard curve. The concentration of allotype in an internal control was determined for each assay. This internal control consisted of a dilution of normal serum from an inbred homozygous Kla or Klb. Using the internal control, assays which generated acceptable standard curves, but erroneous unknown values could be detected. Assay to assay fluctuations in this internal control were seen; however, any assay which produced values significantly different ($p>0.01$) from the mean of previous assays was repeated.

Allotype suppression in heterozygous F1 hybrid rats

1) Neonatal administration of antiallotype antiserum

The ability to affect the expression of the paternal allotype (Klb) was examined following passive injection of antiallotypic antiserum to neonatal animals within 12 hours of birth. The Klb allotype was selected as the target of suppression because the Aug anti-Klb (MHC) antiserum against it contained precipitating antibodies, unlike the anti-Kla antisera and had a high antiallotypic titer by PHA. The same strains (Aug x Cop) were used for the F1 cross that produced the anti-Klb antiserum to minimize heterologous effects. Undiluted anti-Klb serum was given to the neonates in this study. The PHA titer of this antiserum pool was 1:128.

An August x Cop litter was divided into experimental and control groups approximately 12 hrs after birth. Eight experimental animals

received an 0.05 ml intraperitoneal injection of neat August anti-Klb antiserum. The four control littermates received 0.05 ml of normal August serum. Both groups were injected every 48 hours for a total of seven injections. Following the first injection the volume was increased to 0.1 ml per injection.

Data from this litter (Litter 1) are summarized in Table 5 and Figure 4. It is clear that the development of Klb is affected by the neonatal administration of anti-Klb antibody; however, this treatment results in a relatively short term effect. The animals appear to be restoring their Klb levels after day 40 but control levels are not reached until about day 53.

Kla levels in certain control and experimental Litter 1 animals show a pronounced and reproducible increase on day 36 (Figure 5, Table 5). This rapid rise and fall in Kla levels is, as yet, unexplained; however, this peak may be the result of a sampling bias since only three of eight experimental and two of four control animals could be measured.

Table 6 presents the total serum kappa chains, as determined by summation of Kla and Klb RIA values, for the means of the experimental and control groups in Litter 1. The Kla to Klb ratio is also included in parentheses for each day examined. The experimental group exhibits from 1.4 to 2.8 mg/ml less serum kappa chains than the control group through 46 days of age. The difference between experimental and control groups through 46 days of age is elucidated further by the Kla:Klb ratio. The Kla:Klb ratio shows the Kla allotype was 5.5 to 22

Table 5. The effects of passive neonatal administration of anti-Klb antiserum on Aug x Cop hybrid Litter 1. Experimental groups received anti-Klb, control littermates received normal Aug serum.

		Allotype Levels - mg/ml				
	Animal	Days of age				
Kla	Number	32	36	42	46	53
Experimental	1-1	2.0	-	-	-	6.8
	1-2	-	5.2	4.8	5.9	6.4
	1-3	3.2	10.3	2.4	6.8	6.1
	1-4	4.0	-	4.7	4.1	4.9
	1-5	5.2	11.1	3.8	3.9	7.5
	1-6	3.5	-	-	-	10.2
	1-7	5.0	-	-	6.0	8.5
	1-8	3.4	-	6.9	5.5	9.5
Control	2-1	-	9.1	2.3	2.7	4.1
	2-2	-	-	4.4	-	-
	2-3	4.1	10.3	2.9	6.0	7.2
	2-4	2.5	-	2.6	5.0	4.4
Exper		3.76	8.90	4.52	5.40	7.50
SEM		0.45	2.26	0.82	0.51	0.68
Control		3.70	9.70	3.10	4.60	5.23
SEM		0.57	0.85	0.54	1.20	1.21
	Animal					
Klb	Number	32	36	42	46	53
Experimental	1-1	1.8	-	-	-	4.3
	1-2	-	0.4	0.9	1.3	2.5
	1-3	-	-	0.9	3.1	3.5
	1-4	0.4	-	0.9	1.3	1.3
	1-5	0.4	0.4	0.7	0.9	1.8
	1-6	0.5	-	-	-	6.5
	1-7	0.3	-	0.9	1.1	1.7
Control	2-1	-	4.1	4.4	3.4	2.9
	2-2	2.7	2.9	4.6	Died	-
	2-3	1.1	2.2	2.4	2.9	3.4
	2-4	2.6	-	3.7	2.6	3.2
Exper		0.63	0.40	0.82	1.40	2.88
SEM		0.26	0	0.04	0.40	0.68
Control		2.13	3.10	3.80	2.96	3.20
SEM		0.64	0.68	0.57	0.28	0.18

Figure 4. Kla and Klb allotype levels (mg/ml) for experimental and control groups Litter 1. Experimental group received passive anti Klb as described in Materials and Methods; control group received normal Aug serum as described. Quantitation was by RIA as described in Materials and Methods.

● Litter 1 control group

○ Litter 1 experimental group

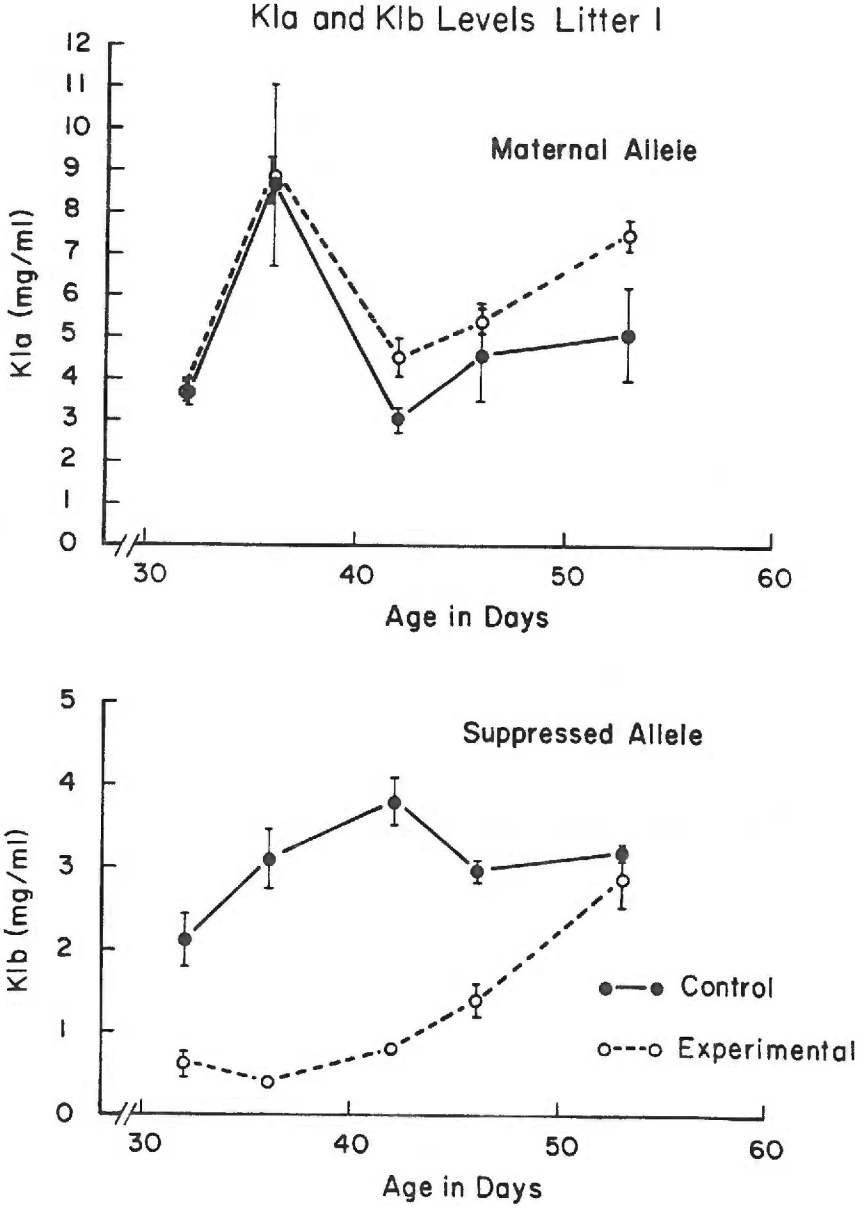

Kla and Klb Levels Litter I
Maternal Allele
Kla (mg/ml)
0
1
2
3
4
5
6
7
8
9
10
11
12
30
40
50
60
Age in Days
Suppressed Allele
Klb (mg/ml)
0
1
2
3
4
5
Control
Experimental
30
40
50
60
Age in Days

Table 6. Total serum kappa chains (Kla + Klb) and ratio of Kla:Klb for the means of control and experimental groups Aug x Cop Litter 1. The amount of kappa chains in mg/ml is listed with the Kla:Klb ratio in parentheses

	mg/ml (Kla:Klb)				
	Days of age				
Group	32	36	42	46	53
Experimental	4.39	9.3	5.34	6.8	10.38
(Kla:Klb)	(5.96)	(22.3)	(5.5)	(3.85)	(2.6)
Control	5.83	12.1	6.9	7.56	8.43
(Kla:Klb)	(1.74)	(3.12)	(0.82)	(1.55)	(1.63)

times more prevalent than the Klb allotype in the experimental group through 46 days of age. The control group, in contrast, ranged between a Kla prevalence three times greater than Klb values, to a slight Klb prevalence on day 42. The data for day 53 show a higher kappa chain level for the experimental group than the control group. The Kla prevalence in the experimental groups also appears to be diminishing by day 53.

2) Maternally induced allotype suppression

The data above on a hybrid litter that neonatally received anti-Klb show a recovery of the Klb allele product to control values by about day 53. In an attempt to suppress the expression of the Klb allotype more completely, females were immunized against the paternal Klb allotype prior to breeding and the allotype levels of the subsequent F1 progeny followed. The females in these experiments were selected from August females on the basis of their serum anti-Klb activity.

The ontogeny of the K allotype in normal August (Kla) x Copenhagen (Klb) F1 hybrids is summarized in Tables 7 and 8 and Figure 5. Litters 10 and 11 are normal control litters. Due to the depletion of the anti-Kla antiserum necessary for the RIA, Kla values were only determined for Litter 10. It can be seen from Figures 4 and 5 that the Kla levels began higher than Klb values in control and experimental animals. This is probably due to maternal immunoglobulin (Kla) uptake by the neonate in utero and while suckling (24, 43, 44).

Table 7. The ontogeny of the Kla and Klb allotypes in control Aug x Cop hybrid Litter 10. The RIA used to quantitate allotypes are as described in Materials and Methods.

Born - 5/15/78

	Allotype Levels - mg/ml Days of Age					
Kla	46	56	61	79	93	103
10-1	5.1	5.2	5.5	6.7	5.8	7.5
10-2	4.1	4.4	4.1	5.7	5.9	5.8
10-3	3.0	3.0	6.0	6.4	7.4	5.4
10-4	3.1	3.0	6.5	5.2	7.7	6.8
10-5	2.9	5.2	6.2	5.8	5.7	6.3
10-6	3.9	3.3	8.0	5.3	8.4	6.6
10-7	-	-	-	7.9	6.1	7.4
10-8	-	-	-	4.7	5.9	-
10-9	-	-	-	7.8	9.2	-
10-10	-	-	-	6.4	9.3	8.6
Mean	3.68	4.02	6.05	6.20	7.14	6.80
SEM	0.38	0.47	0.57	0.36	0.48	0.39

Klb	46	56	61	79	93	103
10-1	9.8	11.1	12.6	14.4	13.6	12.6
10-2	9.7	8.3	12.2	13.4	17.1	15.6
10-3	10.4	10.9	10.6	10.4	10.5	12.9
10-4	8.7	10.1	13.8	10.6	14.3	16.1
10-5	7.8	9.3	9.8	10.8	8.6	15.7
10-6	8.9	8.9	14.4	14.1	12.7	9.3
10-7	-	-	-	15.4	13.3	12.3
10-8	-	-	-	10.8	15.1	Died
10-9	-	-	-	12.2	16.3	Died
10-10	-	-	-	14.7	18.4	14.1
Mean	9.20	9.76	12.2	12.28	12.80	13.7
SEM	0.38	0.458	0.72	0.766	1.33	1.17

Table 8. The ontogeny of the K1a and K1b allotypes in control Aug x Cop hybrid Litter 11. The RIA used to quantitate allotypes are as described in Materials and Methods.

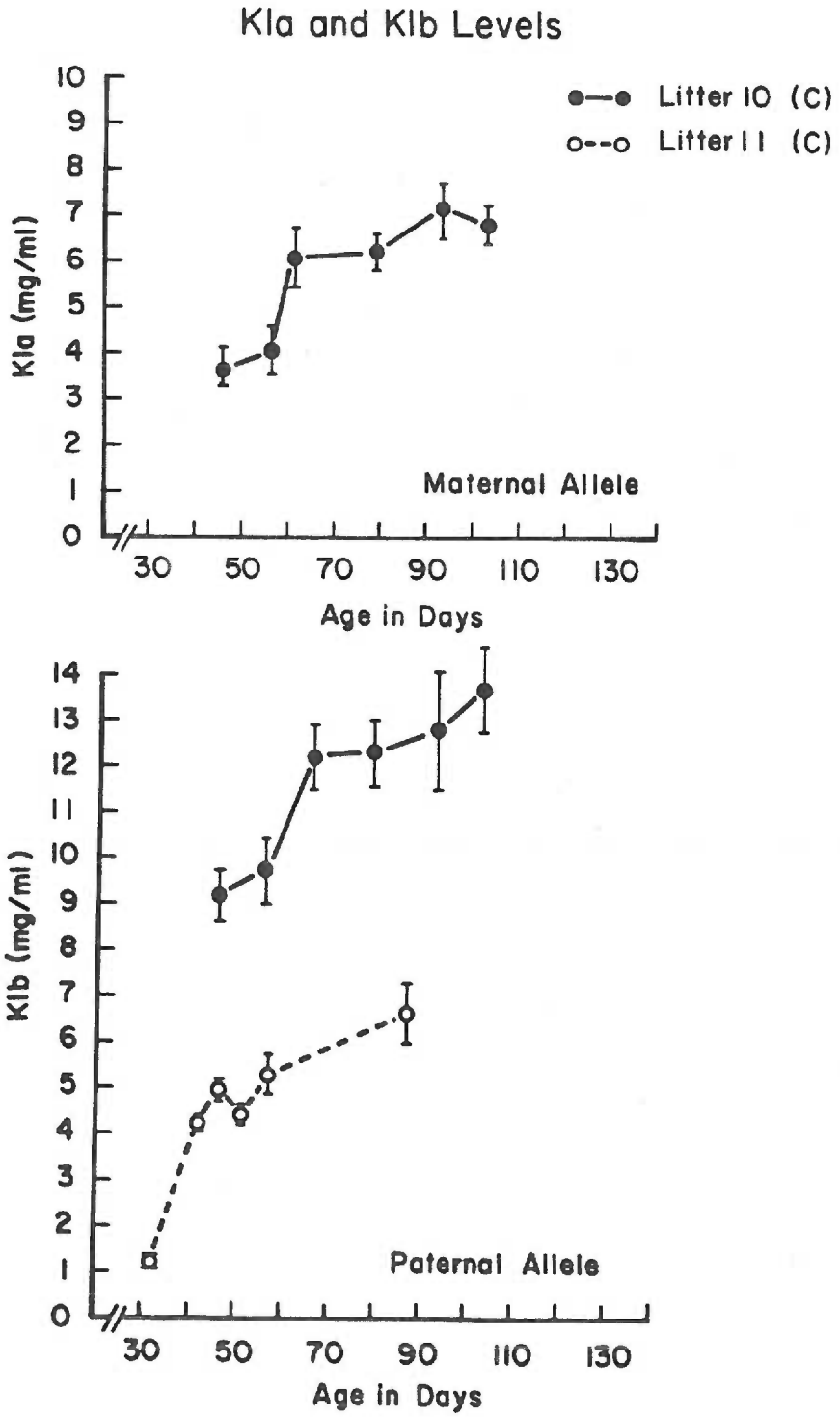
born - 7/17/78

	Allotype Levels mg/ml					
	Days of Age					
K1b	32	42	46	51	57	87
11-1	1.6	4.2	5.0	4.3	4.6	4.6
11-2	1.2	4.1	5.7	4.1	5.4	6.2
11-3	1.3	4.4	4.9	4.9	Died	Died
11-4	0.9	4.5	5.2	4.5	5.1	6.2
11-5	1.1	3.8	4.6	4.3	4.2	5.3
11-6	1.3	3.9	4.4	3.4	4.8	5.7
11-7	2.1	4.9	5.6	4.1	6.5	6.3
11-8	1.0	3.9	5.6	4.1	6.5	6.3
11-9	1.3	4.3	4.3	4.6	7.5	7.4
11-10	0.8	4.3	4.4	5.0	7.5	10.1
Mean	1.26	4.23	4.97	4.39	5.80	6.66
SEM	0.13	0.11	0.19	0.17	0.44	0.59

Figure 5. Kinetics of K1a and K1b allotypes in normal (Aug x Cop) F1 hybrids, Litters 10 and 11. RIA are as described in Materials and Methods.

● Control Litter 10 (C)

○ Control Litter 11 (C)


Kla and Klb Levels
Litter 10 (C)
Litter 11 (C)
Kla (mg/ml)
Maternal Allele
Age in Days
Klb (mg/ml)
Paternal Allele
Age in Days

Early development of the Klb allele can be most readily seen in Litter 11 (Figure 6). If the Litter 11 curve is extrapolated to its intersection with the abcissa, one would predict that significant Klb synthesis in the control neonate does not begin until approximately 26 days of age; however, based on radial immunodiffusion IgM synthesis in other strains begins prior to 7 days of age (G.A. Leslie, personal communication). By day 32 the normal mean Klb value for Litter 11 is 1.2 mg/ml ± 0.13, but by day 42 the mean Klb value is 4.23 mg/ml ± 0.11. A plateau is reached in the Klb levels by approximately 60 days with very little fluctuation seen beyond this age (Figure 7).

Another feature of the normal Aug x Cop hybrids in Litter 10 is the prevalence of the Klb allele at all dates examined (Table 7). The percentage of kappa chains positive for the Klb allele ranges from 71% on day 46 to 64% on day 93. This is in agreement with the data of Rokhlin and Nezlin (53), who observed that approximately 60% of kappa chains from different adult F1 hybrids were positive for the Klb allele before immunization. The ratio of Kla to Klb did change in F1 hybrids studied by Rokhlin and Nezlin following immunization; however, the degree of change in this ratio was dependent on the antigen and the F1 hybrid (53).

Three experimental litters were produced by mothers immunized to the paternal (Klb) allotype. August (Kla/Kla) females, extensively immunized with Cop (Klb/Klb) IgG were bred with normal Copenhagen males. The Klb immune August females were immunized with another 100 µg of Cop IgG one week prior to breeding with the exception of

Figure 6. Development of the Klb allotype between 30 and 65 days of age for control F1 hybrid Litter 11 and maternally suppressed hybrid Litters 4 and 5. RIA are as described in Materials and Methods.

—— Control Litter 11 (C)

—·—· Experimental Litter 4 (S)

- - - Experimental Litter 5 (S)

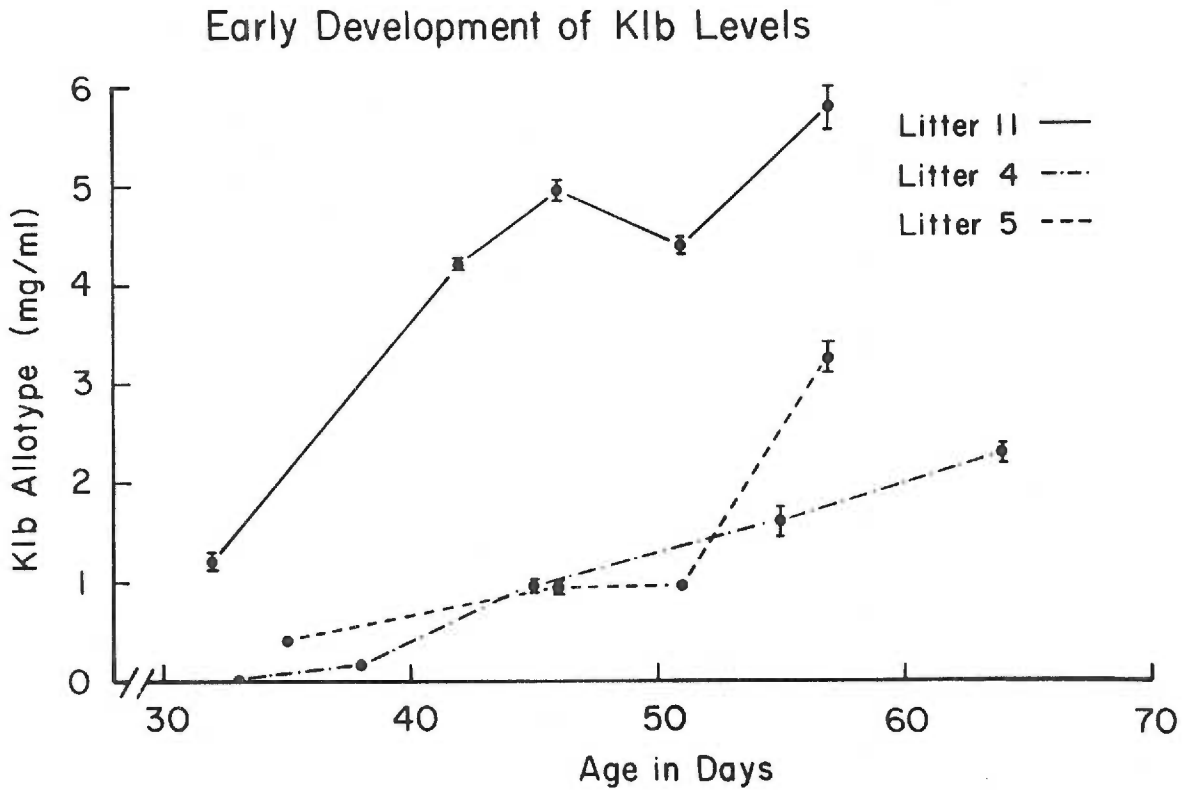
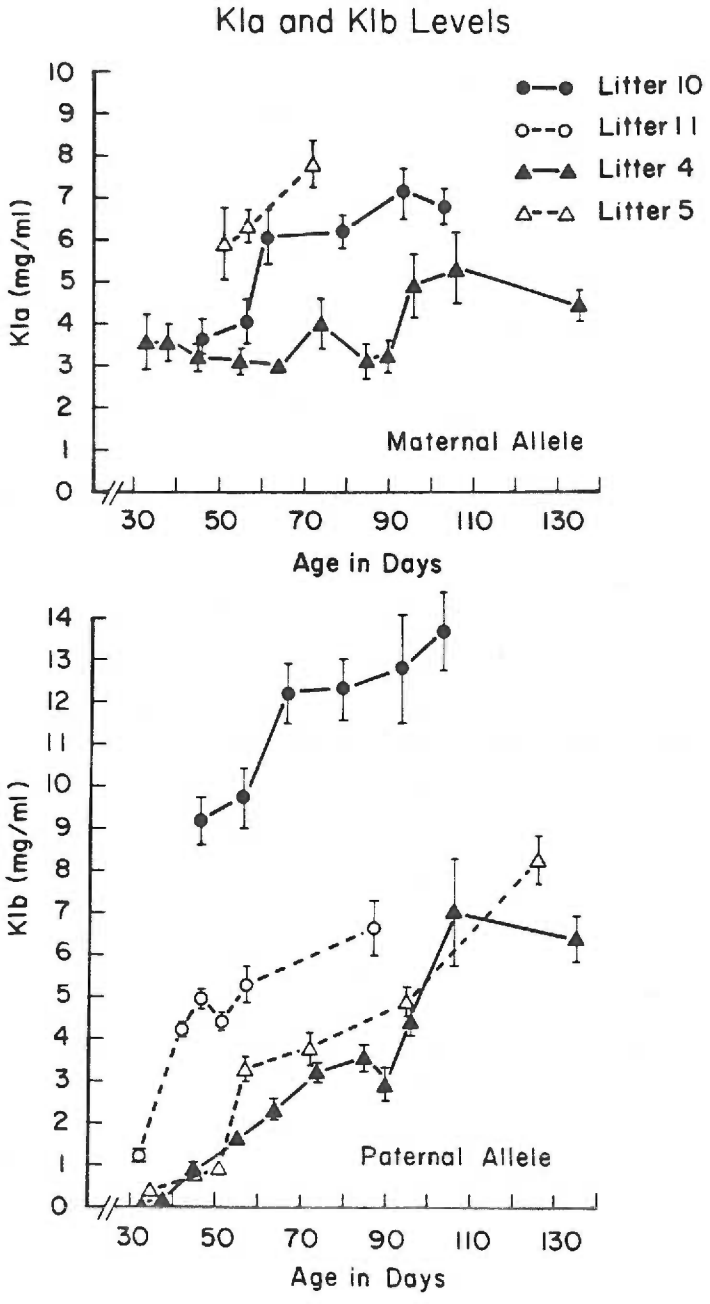

Early Development of Klb Levels
Klb Allotype (mg/ml)
0
1
2
3
4
5
6
30
40
50
60
70
Age in Days
Litter 11
Litter 4
Litter 5

Figure 7. Kinetics of Kla and Klb allotypes in control (Aug x Cop) F1 hybrid, Litters 10 and 11 and maternally suppressed F1 hybrid, Litters 4 and 5. RIA are as described in Materials and Methods.

- ● Control Litter 10 (C)
- ○ Control Litter 11 (C)
- ▲ Experimental Litter 4 (S)
- △ Experimental Litter 5 (S)


Kla and Klb Levels
Litter 10
Litter 11
Litter 4
Litter 5
Kla (mg/ml)
Maternal Allele
Age in Days
Klb (mg/ml)
Paternal Allele
Age in Days

the mother of Litter 6 which was not boosted. It was anticipated that, anti-Klb antibody from the immune mother would cross the placenta into the fetal circulation and/or anti-Klb antibody would be taken up by the gut of the neonate during suckling in sufficient quantities to affect the ontogeny of the Klb allele product. The possibility remains that the Cop IgG antigen might have crossed the placenta and provoked the effects on the Klb ontogeny seen in these experimental litters.

Tables 9 and 10 present the results from maternally suppressed Litters 4 and 5. These results are presented graphically in Figures 6 and 7. The ontogeny of the Klb allotype is strikingly affected in experimental Litters 4 and 5 as compared to control Litters 10 and 11 (Figure 7). Figure 6 chronicles the early period of Klb development between day 30 to day 65 of age in Litters 4 and 5 and control Litter 11. The day 45-46 mean Klb values for control Litter 11 is 4.97 mg/ml ± 0.19, whereas the Klb mean values for experimental Litters 4 and 5 are 0.93 mg/ml ± 0.09 and 0.91 mg/ml, respectively. This control litter value is nearly 5.5 times the mean Klb level of the experimental litters. The control Litter 11 value of 5.8 mg/ml ± 0.44 is still 3.6 times the experimental Litter 4 value of 1.62 mg/ml ± 0.17 and 1.8 times the experimental Litter 5 value of 3.27 mg/ml ± 0.28 in the period of 55 to 57 days of age. Figure 7 demonstrates the relatively long term affects of the maternal immunization method on the Klb allele product. It is clear that experimental Litters 4 and 5 make a slow recovery of the Klb allotype to day 90 followed by a more rapid restoration to normal levels by day 106. The day 87 value of control Litter

Table 9. The development of the Kla and Klb allotypes in maternally suppressed Aug x Cop hybrid Litter 4. The RIA used to quantitate allotypes are as described in Materials and Methods.

Born - 2/17/78

Allotype Levels - mg/ml

Days of Age

Kla	33	38	45	55	64	74	85	90	96	106	135
4-1	6.0	3.8	3.7	3.2	2.9	3.4	2.8	2.1	3.7	6.5	5.1
4-2	3.2	2.3	Died	-	-	-	-	-	-	-	-
4-3	4.6	4.5	Died	-	-	-	-	-	-	-	-
4-4	2.8	2.9	3.2	3.4	2.9	5.2	4.1	4.0	4.1	6.5	4.2
4-5	2.3	3.0	2.4	3.3	3.1	3.0	2.2	2.8	6.5	4.6	4.7
4-6	2.2	4.3	3.4	2.4	3.0	4.5	3.4	3.3	5.5	3.7	3.7
Mean	3.52	3.50	3.20	3.08	3.00	4.00	3.25	3.05	4.95	5.33	4.43
SEM	0.67	0.39	0.32	0.27	0.06	0.58	0.38	0.46	0.74	0.81	0.35

Klb	33	38	45	55	64	74	85	90	96	106	135
4-1	0.04	0.12	0.8	1.5	2.2	2.7	3.5	2.1	3.7	7.9	6.6
4-2	0.03	0.07	-	-	-	-	-	-	-	-	-
4-3	0.04	0.36	-	-	-	-	-	-	-	-	-
4-4	0.05	0.21	0.87	2.1	1.6	3.5	4.2	3.5	5.0	10.1	7.6
4-5	0.03	0.08	1.20	1.6	2.6	3.2	3.0	3.0	4.7	5.1	6.1
4-6	0.04	0.16	0.82	1.3	2.9	3.6	3.6	2.9	4.3	5.2	5.3
Mean	0.04	0.17	0.93	1.62	2.30	3.20	3.60	2.90	4.43	7.08	6.40
SEM	0.006	0.06	0.093	0.17	0.28	0.20	0.28	0.33	0.32	1.38	0.55

Table 10. The development of the Kla and Klb allotypes in maternally suppressed Aug x Cop hybrid Litter 5. The RIA used to quantitate allotypes are as described in Materials and Methods.

Born - 3/27/78

	Allotype Levels - mg/ml Days of Age		
Kla	51	57	72
5-1	1.3	6.5	8.3
5-2	8.8	6.4	10.4
5-3	9.5	7.4	7.7
5-4	7.4	6.5	8.5
5-5	5.3	6.6	7.8
5-6	6.7	7.3	8.1
5-7	5.9	-	-
5-8	4.0	4.2	6.6
5-9	4.6	5.8	5.2
Mean	5.94	6.34	7.83
SEM	0.89	0.38	0.53

Klb	35	46	51	57	72	95	126
5-1	0.46	0.38	0.73	2.9	5.2	4.3	8.8
5-2	0.50	0.89	0.70	2.3	5.0	5.8	7.5
5-3	0.38	0.60	0.88	4.8	3.7	4.5	7.5
5-4	0.32	0.95	0.91	3.0	2.6	5.9	9.9
5-5	0.41	1.10	1.10	2.9	3.2	4.2	8.0
5-6	0.24	1.80	1.50	3.8	4.7	4.1	8.9
5-7	0.57	0.90	0.75	Died	-	-	-
5-8	0.34	1.10	1.10	2.8	3.6	4.9	5.6
5-9	0.43	0.52	0.76	3.7	2.6	5.8	10.3
Mean	0.42	0.91	0.96	3.27	3.82	4.94	8.31
SEM	0.04	0.157	0.09	0.277	0.365	0.29	0.56

11, 6.66 mg/ml ± 0.59, remains over twice the experimental Litter 4 value of 2.9 mg/ml ± 0.33 on day 90 and 1.7 times the experimental Litter 5 value of 3.82 mg/ml ± 0.37 on day 72. Statistical analyses of the mean Klb values of experimental Litters 4 and 5 show them not to be significantly different throughout these studies. In contrast, the Klb values for the experimental litters are significantly different ($p<0.01$) from the mean Klb values of each of the control litters (10 and 11) over the course of these studies.

A comparison of the early Klb kinetics, 30 to 60 days of age, between Litter 1, neonatally suppressed and Litters 4 and 5 maternally suppressed substantiates a very similar development of the Klb allotype in all litters (Figures 4 and 6). The similarity ends beyond 45 days of age since the neonatally suppressed litter increases to control values while the maternally suppressed litters remain lower than controls until approximately 100 days of age.

The kinetics of Kla development in control Litter 10 and maternally suppressed Litters 4 and 5 are shown in Figure 7. An interpretation is hampered by the lack of data on Litter 11 and incomplete information on Litter 5, but some interesting points are apparent. There is a slight difference between Litter 10 and Litter 5 between days 45-75, but it is not statistically significant ($p>0.01$). There is a significant different between the Kla concentration of Litter 4 and either Litter 5 or control Litter 10 ($p<0.01$). The Kla value on day 57 for Litter 5 is nearly twice that of the Litter 4 day 64 value. In like manner, the Kla level determined for Litter 10 on day 61 is 1.8 times the concentration of the Kla allele on day 64 for Litter 4.

Although quantitation of the Kla levels of experimental animals beyond 72 days of age could not be accomplished, a relative comparison of Kla levels was possible using a new Kla RIA. The new Kla RIA was not reliable for quantitation; however, relative comparison of three animals from Litter 5 on days 51, 72, and 126 showed increasing inhibition of the RIA with age. The new Kla RIA inhibition values for 51 days of age were lower than day 72 in accordance with the previous Kla RIA data (Table 10). Kla values for 126 days of age were the same or higher than 72 days of age for all three animals assayed. Based on this relative comparison it appears that the Kla levels of experimental Litter 5 remain high beyond 72 days of age.

Another maternally suppressed litter was obtained from the mother of the first suppressed litter (Litter 4); however, the mother was not immunized with Klb IgG between litters. The results from this group (suppressed Litter 6) are presented in Table 11 and Figure 8. Examination of the data for the Klb allele shows a far less pronounced suppression in the early period between day 36 and 66 in comparison to experimental Litters 4 and 5. Klb concentrations for Litter 4 and 5 are 0.9 mg/ml on day 46 whereas Litter 6 has a day 46 value of 2.97 mg/ml ± 0.14. Although the Klb allotype suppression in experimental Litter 6 (2.97 mg/ml ± 0.14) at 46 days of age is markedly less than experimental Litters 4 and 5, it is still significant relative to the control Litters 10 or 11 Klb values of 9.1 mg/ml ± 0.38 and 4.97 ± 0.19, respectively ($p<0.01$).

Table 11. The ontogeny of the Kla and Klb allotypes in maternally suppressed Aug x Cop hybrid Litter 6. The RIA used to quantitate allotypes are as described in Materials and Methods.

Allotype levels - mg/ml

Days of age

Kla	36
6-1	5.3
6-2	3.5
6-7	4.4
6-9	3.3
6-10	3.5
6-11	3.7
6-12	3.5
6-3	3.2
6-4	3.3
6-5	2.6
6-6	3.9
6-8	3.6
Mean	3.65
SEM	0.19

Klb	36	46	66	73	90	105	115	124	130
6-1	1.3	2.4	4.4	4.4	6.6	4.9	5.0	5.7	5.4
6-2	1.2	3.3	3.6	3.3	4.5	4.7	5.7	5.9	6.1
6-7	1.3	2.9	3.3	3.3	4.0	4.8	4.7	4.9	6.5
6-9	2.3	3.0	3.8	3.8	4.8	5.5	5.2	5.2	6.3
6-10	1.2	2.3	3.3	3.2	4.7	3.9	5.0	3.7	4.9
6-11	1.2	2.6	2.8	4.1	4.2	4.8	4.8	5.6	3.8
6-12	1.9	3.5	3.0	3.6	3.3	4.0	5.2	5.4	5.1
Mean	-	-	-	3.67	4.58	4.8	5.09	5.2	5.44
SEM	-	-	-	0.19	0.42	0.19	0.13	-	-
*6-3	0.68	3.7	3.6	4.8	5.6	4.5	4.8	died	-
6-4	1.7	3.1	4.1	4.4	5.0	4.3	4.9	4.9	6.4
6-5	1.3	2.4	3.6	3.6	4.9	4.9	5.5	4.5	4.3
6-6	1.6	3.5	3.8	4.4	7.3	4.6	4.4	4.8	5.1
6-8	1.6	3.0	3.3	3.3	7.1	3.6	4.9	4.5	6.5
Mean	1.44	2.97	3.60	3.98	5.98	4.38	4.9	4.67	5.57
SEM	0.12	0.14	0.13	0.31	0.57	0.24	0.2	0.22	0.53

*Animals 6-3, 6-4, 6-5, 6-6 and 6-8 received 1 mg of anti-Klb antibody on day 73.

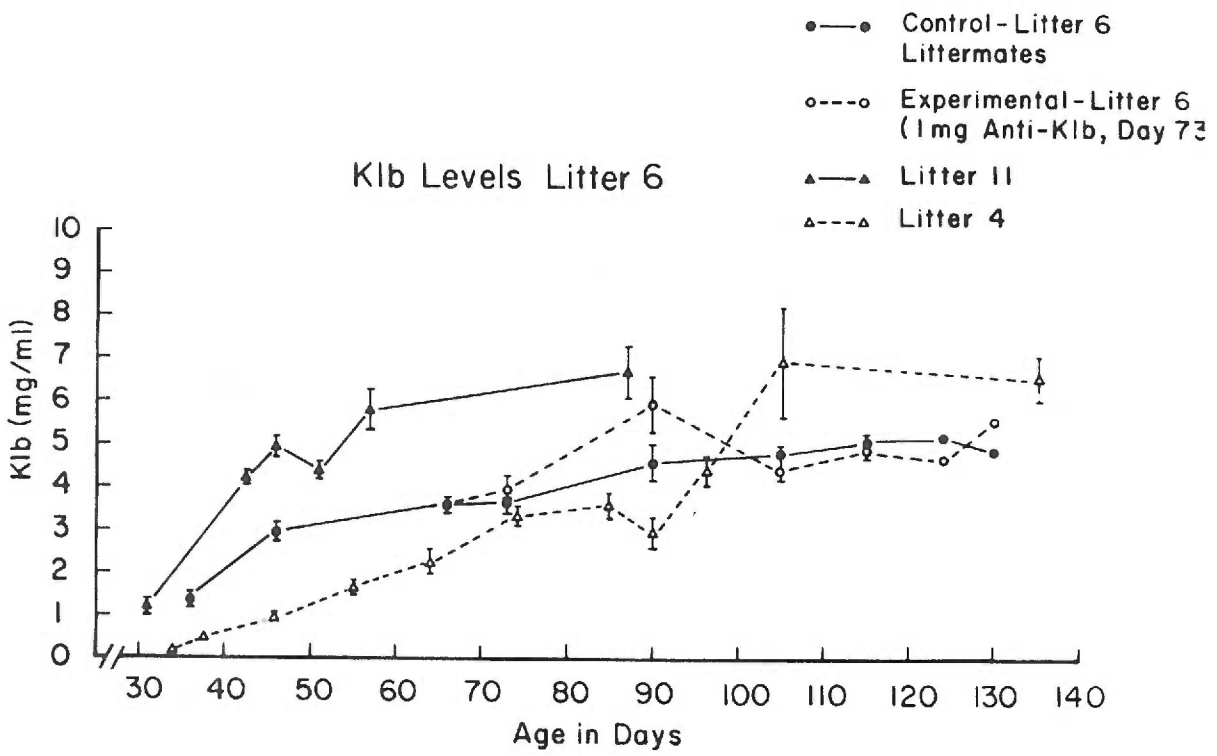
Figure 8. Kinetics of the Klb allotype in maternally suppressed F1 hybrid Litters 6 and 4 and control F1 hybrid Litter 11. Litter 6 experimental group given 1 mg specifically purified anti-Klb on day 73 as described in Materials and methods. RIA is as described in Materials and Methods.

● Control Litter 6 (no injection day 73)

○ Experimental Litter 6 (1 mg anti-Klb day 73)

▲ Control Litter 11 (C)

△ Experimental Litter 4 (S)


Klb Levels Litter 6
Control - Litter 6 Littermates
Experimental - Litter 6 (1 mg Anti-Klb, Day 73
Litter 11
Litter 4
Klb (mg/ml)
0
1
2
3
4
5
6
7
8
9
10
30
40
50
60
70
80
90
100
110
120
130
140
Age in Days

In an attempt to modulate further the Klb allele product one mg of anti-Klb antibody which had been specifically purified for use in the Klb RIA was injected into the peritoneum of five Litter 6 animals on day 73, as indicated in Table 11. The animals were assayed at day 90, two and one half weeks post injection. The results show those animals which received passive anti-Klb have significantly higher mean Klb levels than control littermates. This elevation is particularly striking in two animals, 6-6 & 6-8 (Table 11, day 90). It should be noted that rat 6-1, which did not receive anti-Klb antibody on day 73 also shows an increase in Klb values on day 90. Fifteen days later (105 days of age) 80% of the Litter 6 anti-Klb recipients had Klb values below the mean of the uninjected littermates. Little difference is seen between injected and control groups after 105 days of age. It is apparent from these data that the passive anti-Klb treatment of experimental Litter 6 did not result in the re-establishment of the suppressed state, even though some minor fluctuations were noted.

The contrast between the maternally suppressed Litters 4 and 6 does correlate well with a drop in their mother's antiallotype antibody. This fall in anti-Klb antibody was assessed by inhibition of the Klb assay with serum from their mother, August 3-5.

Serum from August 3-5 was obtained one month before the birth of Litter 4 and three days before the birth of Litter 6. Serum which was obtained one month before the birth of Litter 4 inhibited the Klb RIA 98% at a dilution of 1:10, whereas that which was obtained three days before the birth of Litter 6 caused only a 67% inhibition at a dilution of 1:10. An even greater difference in the levels of antiallotype

in these sera might be obtained if the two samples were repeated at higher dilutions.

DISCUSSION

This research was designed to investigate rat kappa chain allotypes as a model system for allotype suppression. The salient points investigated include: 1) the detection and quantitation of the K allotypes by radioimmunoassay, 2) the ontogeny of the K allotypes in F1 heterozygous hybrids, 3) the effects of passive administration of anti-allotypic antibody on F1 heterozygous neonates, and 4) the effects of maternal immunization against the paternal allotype on F1 heterozygous progeny. The data presented above confirm that antisera to the kappa chain allotypes can be produced by reciprocal immunization of inbred rat strains. Previous studies of the K allotypes had utilized exclusively bacterial antigen-antibody complexes as the immunogen to elicit an anti-allotypic response (1, 5, 22, 23, 67). This research documents the first apparent use of Group A Streptococcal vaccine-anti group carbohydrate antibody complexes in raising antisera to the allotypes. This thesis also demonstrates the successful application of a second method of antiallotype antisera production in the rat. The second method has been widely used in producing antisera to the murine allotypes (27, 30, 66). This method uses an immunogen which contains antibody to the recipient's tissue components, i.e. the MHC. Previous studies have shown in the mouse that this method of immunization produces a greater percentage of recipients responding to the immunogen as well as more antibody to the allotype than groups given bacterial immune complexes (27, 66). A more recent adaptation

of this protocol has used specific immune anti-MHC (H-2) antibody of the allotype to which the antiserum was to be directed to immunize mice of the appropriate H-2 haplotype (30). This adaptation has been effective at producing antisera to allotype which had previously produced very weak antisera (66). Based on the qualitative reactions in gel diffusion and passive hemagglutination of antibody sensitized SRBC, this method also produced antisera in the rat that were equivalent or stronger than that produced by bacterial immune complex immunization. Investigators in the murine system speculate that use of anti-tissue antibodies as the immunogen serves to focus the immune response to the small allotypic differences between strains by forming complexes in vivo (27). These complexes might be formed with dermal tissue or immune system cells recruited to the immunogen depot.

This thesis re-emphasizes the value of inducing a serous accumulation in the peritoneum or ascites when producing antiserum in the rat. Utilizing a modification of the procedure of Tung et al., previous studies have established the similarity between serum and ascites on the basis of total protein, immunoelectrophoresis and specific antibody content (47). The large volumes of ascites which can be obtained from individual animals render this procedure especially useful in the production of antiallotype antiserum. This is especially evident from inspection of one antiserum group. The Aug anti-Klb (anti-MHC) group produced a pool of high antiallotype activity serum with a total volume of 17 ml, whereas this group's pool of high titered ascites had a total volume of 120 ml.

Specificity of the radioimmunoassays

The specificity of the antiallotype antibody used to develop a radioimmunoassay (RIA) for each allotype as well as the reproducibility of each assay were rigorously investigated. A survey of inbred rat strains revealed that the antigen(s) detected by one alloantiserum were present in some but not all strains tested (Table 2). Those strains negative for one allele product proved to be positive for the alternate allele product and agreed with published reports of kappa allotype distribution among rat strains with one exception (1, 5, 23, 54). Two separate studies examined the allotypic phenotype of over 125 individuals of the outbred Sprague-Dawley rat strain (1, 5). All animals were reported as homozygous Klb. Evaluation of the allotypic phenotype of 14 Sprague-Dawleys for this research revealed 3 of the 14 to be homozygous Klb and the remainder homozygous Kla. All of the Sprague-Dawley rats bred for responsiveness to Group A Streptococcal carbohydrate examined for the allotypes were assayed homozygous Kla. It can be argued that the uniformity of the anti-SACHO SD colony could be due to a founder's effect; that is, the animals originally selected for the first brother sister matings, by chance, all happened to be homozygous Kla. This type of effect should not be seen in examination of a large outbred colony, like that examined by previous investigators (1, 5). This finding indicates that the degree of inbreeding in SD rats may vary from one source to the next and may be of importance if a randomly outbred animal is absolutely necessary.

Localizing the specificities detected by the antiallotype antisera to the kappa light chains was accomplished by inhibiting the radioimmunoassays with fragments of immunoglobulin molecules (Table 3). The RIA to both allotypes were fully inhibited by IgM fractions as well as IgG when the appropriate inbred strain was used. This indicated that the alloantisera recognized either an antigenic specificity present on both classes of immunoglobulin, i.e. the L chain,or recognized multiple specificities. When the Fc portion of the IgG molecule was removed by digestion with pepsin the resulting $F(ab')_2$ fragments continued to be completely inhibitory in the proper RIA; therefore the allotypic specificity had to be present on the L chain and/or the Fd fragment. The specificity of each RIA was localized to the L chain by isolation of H and L chains and analyzing these preparations with the allotype RIA. The L chain preparations from appropriate strains showed over 10 times the inhibition of the H chain preparations. The partial inhibition of the RIA was the result of contaminating L chains. This conclusion is supported by the work of previous investigators, who have shown a similar inhibition with H chain preparations due to an approximate 2% admixture of L chains (54, 67). A greater inhibition of the RIA with the L chain preparations was obtained using a higher concentration of sample. Finally, no direct binding of one radiolabelled allotype to the alternate alloantiserum could be detected over a wide range of concentration indicating that the antisera did not share any common specificities.

Neonatal allotype suppression

Passive infusion of anti-Klb antiserum into allotypically heterozygous neonates produced a clear modulation in the early development of the Klb allotype in comparison to normal control littermates (Figure 5). This modulation in the ontogeny of the Klb allotype is shortlived with experimental animals restoring the Klb allotype to control levels by about 50 days of age. The restoration of Klb levels in experimental animals may reflect a recovery in the number of Klb positive cell clones, their escape from a regulatory signal or the catabolism of anti-Klb antibody below a critical level.

The only previous description of neonatal passive allotype suppression in the rat was reported by Rokhlin *et al.* (54). These investigators used a single subcutaneous injection of 0.1 mg of "pure rabbit anti-RL-2 (Kla) antibodies" to establish the suppression in (Klb x Kla) F1 heterozygotes. Based on the binding of radiolabeled gamma globulin to "specific rabbit anti-allotypic antibody" immunoadsorbants, an estimation of the Kla suppression was made. The data from this study show very little Kla molecules at one month of age which is in good agreement with the Klb neonatal studies of this thesis, which represent the first studies of Klb allotype suppression in the rat. A further comparison of the two studies is hampered by Rokhlin's lack of data at two months of age.

The modulation in Klb ontogeny seen after passive exposure to anti-Klb antibody was reminiscent of that observed by Herzenberg *et al.* in heterozygous F1 mice (other than Balb/c x SJL), i.e. (C57Bl/10 x

Balb/c)(25). F1 mice exposed to antibody to the paternal Balb/c allotype showed a delay in the initial synthesis of the paternal allotype to 21 days of age. With the onset of production of the suppressed allotype, serum levels climbed at the same rate as normal hybrids and reached control values at three months of age. A similar early development of the suppressed allotype was seen in the experimental rats in Litter 1. (Figure 5) These animals have little detectable Klb through 36 days of age followed by a rapid recovery in Klb levels to essentially normal values at 53 days of age.

Passive allotype suppression in the rat bears little resemblance to its counterpart in the rabbit. Passive administration of antibody to the paternal allotype in neonatal rabbits produces, in certain animals, a total abrogation in synthesis of the paternal allotype lasting several months to years (40). If the suppression does break a markedly diminished level of the allele product may last for the entire lifetime of the animal.

A few of the numerous variables that affect the development of the Klb allotype are exemplified by the suppression data obtained from Litter 1. Further investigation of passive allotype suppression in the rat:1) using large quantities of specifically purified anti-allotypic antibody, 2) varying the timing of the injection, 3) establishing the optimal site of neonatal inoculation and 4) suppressing other F1 hybrid crosses would develop this system into an exciting model for the study of the mechanism(s) of antibody regulation. Studies of passive allotype suppression in the rabbit have established that the duration

of the suppression is highly dependent on the dose of antiallotypic antibody and the timing of injections (16). There also appears to be a correlation between the persistence to 21 days of age of detectable circulating antibody to the suppressed allotype and the degree of suppression obtained in the rabbit (9).

Speculation on the mechanism(s) of suppression in the neonatally induced rat hinges on previous studies in the mouse and rabbit. In the rabbit two periods of suppression have been postulated (9). The first period is marked by the ability to neutralize the suppression with small quantities of the suppressed product. The second period is delineated by the failure of this procedure to neutralize the suppression. It is theorized that the first stage is merely a "holding operation"; that is, the passively acquired antiallotype antibody is 1) binding all positive receptors present on B cells preventing release of newly synthesized allotype and/or, 2) combining with circulating positive molecules and thus clearing them from the neonates circulation. The second phase is postulated to be controlled by suppressor T cells. The investigators believe the second mechanism to be a distortion of normal homeostasis which controls levels of all serum immunoglobulins, but in the suppressed animal are chronically influenced in favor of the non-suppressed allotype.

In neonatally suppressed F1 mice which exhibit a relatively short term suppression, a mechanism similar to that proposed for the first stage in the rabbit has been discussed. Due to some unknown factor the suppression does not result in the generation of a cellular mechanism for long term maintenance of the suppressed state.

The study in the rat of neonatally induced allotype suppression presented here tends to favor the humorally based mechanism presented for the above suppression models. Antibody to the Klb allotype delays the onset in detectable Klb synthesis at least 5-7 days, based on the extrapolation from normal hybrid animals. These type of results are in good agreement with a humoral mechanism which wanes relatively soon as the antibody is cleared from the animal.

Maternally induced allotype suppression

The suppression of a gene product (allotype) by maternal isoantibodies was first observed in neonatal rabbits from mother's immune to a paternal allotype (15). The application of this scheme to rats has produced a more lasting effect on the Klb allotype levels than that produced in the neonatal suppression study. The maternally suppressed Litters 4 and 5 show little detectable paternal Klb allotype at 32-33 days of age (Figure 7 and Tables 7 and 8). After this age, the suppressed litters gradually recover reaching control levels by 100 days of age.

The normal ontogeny of the Klb allotype is demonstrated by the normal Aug x Cop hybrids, Litters 10 and 11. It can readily be seen however, that there are significant differences in the levels of both allotypes between normal groups. Despite the inconsistency between normal Litters 10 and 11 it is obvious that their Klb development does not resemble that of the suppressed litters. This is especially evident by examination of the Klb values on days 45-46 of the four groups.

The similarity between the rat studies of maternally induced allotype suppression and studies of maternally suppressed F1 hybrid mice other than (Balb/c x SJL) is especially striking (25). A typical hybrid litter from a mother immune to a paternal allotype had no detectable paternal allotype 22 days after birth. This was followed by a rapid upsurge in paternal allotype levels, more pronounced than that observed in the rat, until approximately 50 days of age. Following day 50 the animals stage a gradual recovery in the paternal allotype much like that seen in the rat reaching normal values around 90 days of age.

Rabbits can be rescued from allotype suppression before 50 days of age if minute quantities of the suppressed allele product are injected into the animal (9). This neutralization of the suppression results in a gradual restoration of this allotype to control levels by four months of age. Animals older than two months are refractory to this treatment and maintain suppressed levels. A plot of suppressed allotype values from a rabbit which has had the allotype suppression neutralized resembles those curves generated in the rat and mouse models recovering from maternal suppression.

The third maternally suppressed litter, Litter 6, demonstrated a far less pronounced affect on the K1b allotype than the previous suppressed litters (Figures 7 and 8, Table 9). The mean K1b value of Litter 6 on day 46 is over three times the value seen in Litters 4 and 5. This decreased severity in suppression correlates well with the decrease in antipaternal allotype antibody detected in the mother of

Litters 4 and 6. Studies in rabbits on allotype suppression have established the sensitivity of this phenomena to the dose of eliciting antibody. When smaller quantities of antibody to the allotype were used less suppression of the target allotype was seen. The results of Litter 6 confirm this type of relationship in maternal suppression of the Klb allotype of the rat.

In an attempt to regain the Klb suppression five animals from Litter 6 were given one mg each of specifically purified anti-Klb antibody on day 73 (Figure 8 and Table 9). This treatment had, if anything, a short stimulatory affect on Ig synthesis in these animals. This may have been the result of too little anti-allotypic antibody being administered to the animals, or the animals were refractory to this type of treatment due to fully mature homeostatic mechanisms. Similar work in the rabbit has shown that suppression can be re-established in animals which are "breaking" by the injection of large quantities (10 mg) of anti-allotype antibody (16).

Speculation on the mechanism(s) responsive for the Klb suppression in the maternally suppressed rat draws from the two phased mechanism discussed for the rabbit. It appears that a humoral mechanism establishes the nearly full Klb suppression seen through 50 days of age. The gradual recovery of the Klb to control levels by 100 days of age may reflect the dwindling influence of a cellular mechanism which in normal non-suppressed animals controls the levels of the Ig classes. During the humoral suppression period the homeostatic mechanism is chronically perturbed. The normal balance in this cellular homeostatic control is finally restored by 100 days of age.

There are important differences between the maternally induced and neonatally induced allotype suppression which may account for the different kinetics observed. These differences center about the length of exposure to antiallotype antibody and the effective dose of antiallotype achieved with the two methods. The neonatally induced animals were administered undiluted antiserum over 14 days. Systematic problems such as, leakage of the antiserum from the peritoneum and fluctuation in dose taken up within the experimental group may favor the use of specifically purified anti-Klb and a different inoculation site. The maternally induced system provides: 1) a more uniform administration of anti-Klb in the experimental litter; 2) an earlier exposure to antibody due to placental passage of anti-Klb and 3) a longer effective dose with neonates receiving anti-Klb via suckling for approximately 20 days after birth.

There are significant differences between the severity and duration of allotype suppression in the rabbit and that presented here for the rat. The rabbit provides a system in which suppression can be easily established by maternal isoantibodies or passively by neonatal inoculation and in some cases the suppression remains throughout the lifetime of the animals. The rat provides an inbred model that is less well defined and produces a more short term suppression. An inspection of the allotypes themselves, through amino acid sequence studies, provides some insight into the differences observed between these animal models. The "b" series allotypes (b4, b5, b6, b9) are present on the rabbit kappa light chains. They are readily suppressed by antiserum made in rabbits homozygous for "b" allotypes other than the target

allele product (33). The antiserum produced to the "b" allotypes is strongly precipitating due in part to the multi-determinant nature of the target allotype (39). Indeed, sequence studies by Farnsworth et al. have shown that the constant regions of "b" molecules differ by as much as 35% in amino acid sequence (17). This great sequence dissimilarity is thought to constitute the largest amino acid differences ever recorded between "alleles". This is emphasized further by the fact that the sequence difference between the constant region of mouse and human kappa chains is 40% (78). Yet these species are thought to have diverged about 75 million years ago. Amino acid sequence studies of the rat kappa chain allotypes have shown a 15% sequence difference between alleles (21). It is probable then that the differences in severity and duration of allotype suppression between these two species may be a function of the eliciting antibody. Further studies in the rat may demonstrate a more chronic state of suppression through timing of the hybrid pregnancy to correlate to peak anti paternal antibody levels in the mother or by coupling maternal and neonatal methods of establishing the suppression.

SUMMARY

This research was designed to investigate the rat kappa chain allotypes as a model system for allotype suppression. The salient points investigated include: 1) the detection and quantitation of the K allotypes by radioimmunoassay, 2) the ontogeny of the K allotypes in F1 heterozygous hybrid rats, 3) the effects of passive administration of antiallotypic antibody on F1 heterozygous neonates, and 4) the effects of maternal immunization against the paternal allotype on F1 heterozygous progeny.

The data presented confirm that antisera to the kappa chain allotypes can be produced by two apparently novel methods. The allotype levels in the serum were quantitated with sensitive RIA developed for each allotype. The results demonstrated that neonatal administration of anti-K1b antiserum caused a short term suppression in the K1b ontogeny; whereas, maternal immunization against the paternal allotype produced a longer lasting effect on the K1b development.

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Appendix A: The ontogeny of serum proteins in control Aug x C F1 hybrids and maternally suppressed Aug x Cop F1 hybrids.

Several workers have reported that allotype suppression in the rabbit results in a compensatory increase in serum levels of the non-suppressed allotype (9, 16, 39, 40). This homeostatic compensation maintains serum Ig levels at control values, despite the depletion or total absence of one allotype. This type of mechanism is not seen in either the short term or chronic allotype suppression described in the mouse (25, 30).

In order to determine if total serum gamma globulin changes in maternally allotype suppressed rats, the percentage of serum proteins in the gamma region was determined by cellulose acetate electrophoresis. Tables I-IV show the distribution of the serum proteins among the electrophoretic regions for control Litters 10 and 11 and maternally suppressed Litters 4 and 5. It can be seen that maternal suppression of allotype results in a depletion of the serum proteins found in the gamma region and that this depletion closely followed the suppression measured at the same times by the K1b RIA (Tables III & IV). It appears from these data that the K1b allotype suppression seen in the maternally suppressed rat does not cause a compensatory increase in the K1a allotype or lambda class which would keep serum Ig near normal.

Table I. The distribution of serum proteins and total protein values as determined by CA for control Aug x Cop F1 Litter 10

Animal	Age (Days)	Total Protein mg/ml	% Albumin	% Alpha	% Beta	% Gamma
10-1	56	51	43.8	23	19.3	13.8
	93	58	44.4	22.7	19.7	13.3
	103	60	42.9	23.2	19.1	14.6
10-2	56	44	48.3	23.2	17.1	11.0
	93	53	44.4	22.7	19.7	13.3
	103	30	53.2	20.9	14.4	11.3
10-4	56	26	48	22.9	17.2	12.0
	93	70	43.7	21.6	20.0	14.5
	103	39	46.3	23	17.9	12.6
10-5	56	57	42.9	26.2	20.9	10.0
	93	26	43.8	27.1	17.9	10.9
	103	52	42.5	26.9	19.1	11.1

Table II. The distribution of serum proteins and total protein values as determined by CA, for control Aug x Cop F1 Litter 11

Animal	Age (Days)	Total Protein mg/ml	% Albumin	% Alpha	% Beta	% Gamma
11-2	42	63	47.5	26.8	18.9	6.8
	54	40	61.2	13.0	14.7	11.0
11-4	42	55	51.3	21.1	20.4	6.6
	57	36	64.2	13.4	12.3	10.2
11-5	42	51	53.7	23	17.3	6.2
	57	35	63.2	13.8	14.4	7.7
11-7	42	57	53.4	15.4	22.7	8.7
	57	47	54.5	17.7	17.0	10.0

Table III. The total protein and distribution of the serum proteins as determined by CA for maternally suppressed Aug x Cop F1 Litter 4

Animal	Age (Days)	Total Protein mg/ml	% Albumin	% Alpha	% Beta	% Gamma
4-1	33	53	57.7	22.6	15.0	4.5
	45	73	54.4	23.6	18.3	3.7
	64	50	48.8	26.2	18.4	6.1
	135	41	43.8	23.1	18.4	14.4
4-4	33	52	54.1	25	16.6	4.2
	45	72	51.7	23.9	19.4	4.6
	64	55	49.8	24.5	16.4	8.9
	135	53	42.2	24.8	20.0	12.9
4-5	33	53	60.3	21.5	14.7	3.4
	45	76	54.8	25.4	15.5	4.1
	64	46	50.4	24.6	18.1	6.7
	135	41	47.1	24.6	16.4	11.9
4-6	33	52	53.8	21.5	18.1	6.3
	45	56	57.1	22.9	17.3	3.4
	64	58	44.9	28.0	19.1	7.7
	135	51	44.9	26.1	18.9	9.9

Table IV. The distribution of serum proteins and total protein as determined by CA for maternally suppressed Aug x Cop F1 Litter 5

Animal	Age (Days)	Total Protein mg/ml	% Albumin	% Alpha	% Beta	% Gamma
5-1	35	59	57.8	22.4	15.1	4.2
	51	52	52.5	25.6	16.5	5.3
	72	74	43.9	27.8	18.9	8.5
5-2	35	49	54.8	25.0	16.2	3.8
	51	51	54.6	24.4	15.4	6.0
	72	108	36.9	33.0	20.3	9.8
5-3	35	61	47.9	26.8	19.1	5.2
	51	51	55.2	23.3	16.6	4.5
	72	102	49.5	25.0	18.3	7.0
5-4	35	50	55.3	23.3	16.8	4.0
	51	54	53.2	24.5	16.4	5.6
	72	62	44.8	27.8	19.1	8.3

Appendix B. Serum immunoglobulin values of control Aug x Cop F1 hybrid Litter 10 as determined by RID.

Litter 10 serum Ig levels were measured by RID in order to gain information on the accuracy of the K allotype RIA. Previous examination has revealed that the rat has 95% of its Ig light chains of the K type (28); thus measurement of the K allotypes should measure 95% of total Ig. RID of Litter 10 samples is shown in Table I. Two animals had all classes and subclasses to which antisera was available measured by RID. It is apparent that the RIA closely measure the total Ig for these animals.

Table I. Serum immunoglobulin values of control Aug x Cop hybrid Litter 10 as determined by RID

Animal	Age	μ	γ1	γ2a	γ2b	γ2c	Total Ig by RID	Total kappa (1a+1b) by RIA
10-1	46	2.4	1.6	5.5	7.2	1.5	18.2	14.9
	56	2.1	2.1	5.5	5.2	0.75	15.7	16.3
10-2	46	2.0	1.9	4.6	5.6	.54	14.6	13.8
	56	2.8	1.9	4.4	4.4	.32	13.8	16.3
10-3	46	2.4		5.8				
	56	3.2		5.8				
10-4	46	2.1		4.6				
	56	2.1		3.9				
10-5	46	2.8		2.7				
	56	2.1		3.9				
10-6	46	3.3		3.0				
	56	2.8		4.4				

Appendix C. The immune response of a normal Aug x Cop F1 hybrid litter to GASV as measured by the K allotype RIA.

A normal Aug x Cop F1 hybrid litter was bred and immunized with GASV following the protocol described in Materials and Methods. Table I summarizes the K allotype values of this litter before and during immunization. A ratio of K1a to K1b allotypes reveals that the prevalence of K1b allotype demonstrated before immunization lessens under immunization with GASV. This is in agreement with the data of Roklin and Nezlin (54) who showed that the K1b prevalence of F1 hybrid rats before immunization diminished with successive immunization in some hybrid groups.

Table I. Kla and Klb levels in the serum of a normal Aug x Cop hybrid litter immunized with GASV as described in Materials and Methods.

		Allotype levels mg/ml		
Kla	Animal Number	PB*	Wk 1	Wk 6
	1	2.8	4.7	29.8
	2	2.9	-	-
	3	1.9	2.7	8.1
	4	3.3	-	-
	5	3.0	4.4	18.0
	6	3.1	6.6	19.5
	7	2.7	-	-
	8	2.0	2.8	13.4
	9	2.4	5.2	16.3
Mean		2.67	4.4	17.5

Klb	Animal Number	PB	Wk 1	Wk 6
	1	8.5	14.1	26.2
	2	7.7	-	-
	3	10.5	10.9	25.2
	4	13.6	-	-
	5	17.6	11.3	20.2
	6	8.6	8.6	26.1
	7	5.0	-	-
	8	4.8	5.2	19.4
	9	5.8	7.4	14.9
Mean		9.1	9.5	22

*Animals were first immunized at 60 days of age.