THE THETA ANTIGEN OF MICE AND ITS ANALOG IN RATS

Thesis by

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To Harriet Buhai, Esq., without whose considerable talents this work might not have been possible.

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ABSTRACT

The Thy-1 antigen is a normal cell surface component of mouse fibroblasts, brain cells, and thymus-derived lymphocytes (T-cells). This thesis presents the results of genetic, phylogenetic, developmental, and physiological studies of this antigen and its rat analog, θ-R.

The Thy-1 locus, which controls the expression of the Thy-1 antigen, was previously shown to be located on Chromosome 9 of the mouse. It has been mapped more precisely by following its segregation relative to the linked markers Trf and Mod-1. This group of genes will probably be useful in future genetic studies of this chromosome.

Sublines of the inbred AKR mouse strain have been typed for a variety of genetic markers. Differences were found in Thy-1 antigen, cytoplasmic malic enzyme, kidney esterase-3, principal urinary protein, expression of Murine Leukemia Virus-gs antigen and incidence of leukemias. Those sublines homozygous for Thy-1^{a} appeared to show an increased susceptibility to spontaneous leukemia as compared with those homozygous for Thy-1^{b}.

Thymocytes from rats of 13 different inbred and 2 random-bred populations were found to express an antigen indistinguishable from Thy-1.1. At birth little or none of this antigen was present in rat brain. Soon afterward an approximately logarithmic increase in antigen expression began. This continued until about day 20 when the adult level, which exceeded that in neonatal brain by more than 100-fold, was reached.

Absorption and complement-mediated cytotoxicity tests failed to detect the Thy-1.1-like antigen (θ-R) on rat lymph node, spleen, or bone marrow cells. Pretreatment of immune rat peritoneal exudate cells with anti-Thy-1.1 antibodies in the presence of complement did not abolish their ability to kill tumor cells *in vitro*. These results suggest that the expression of the θ-R antigen by rat thymus-derived lymphocytes not only decreases during maturation, as is the case in the mouse, but ceases altogether.

Contrary to this conclusion is the finding that *Thy-1*^{b}-homozygous mice immunized against rat peripheral lymphocytes made antibodies directed against Thy-1.1. It therefore appears that small amounts of the θ-R antigen are in fact expressed by rat peripheral thymus-derived lymphocytes.

Following suitable absorptions, antisera prepared in rats against thymocytes from mice homozygous for the *Thy-1*^{b} allele could be rendered specific for Thy-1.2. Antibodies directed against one or more other mouse alloantigens could also be detected in these antisera.

The relationship of these results to the current literature on Thy-1 is discussed.

TABLE OF CONTENTS

I. INTRODUCTION

The introduction of foreign substances into the tissues of an animal may have a number of effects. Many such materials can be filtered by the kidneys or removed by phagocytic cells such as those of the liver or spleen. In addition to these relatively nonspecific reactions, higher animals are also capable of making immune responses which are specific for foreign molecules to which the animal has been exposed previously. The altered state of an animal following such a response may be described in terms of three categories; humoral immunity, cellular immunity, and tolerance. In the first case, the immune response is mediated by antibody molecules, which are soluble glycoproteins present in most bodily fluids. Antibodies are capable of specifically combining with the foreign substance which gave rise to the immune response. The formation of these complexes may cause greatly enhanced specific phagocytosis. Also, the attachment of antibodies to the surfaces of foreign cells can result in membrane damage and eventually cytolysis. This is accomplished through the interaction of the attached antibodies with a series of serum components collectively referred to as the complement system (1).

Cellular immune responses differ from humoral responses in that they produce legions of specifically sensitized cells rather than soluble antibodies. Physical contact between immune cells and "target" cells (for example, the cells of an organ transplanted from a genetically disparate individual) can bring about the death of the target cells as well as the release of a variety of biologically active molecules (2).

The third type of immune response does not produce demonstrable humoral or cellular immunity, but rather a state of specific non-responsiveness to future challenges with the same substance. The detailed relationship of tolerance induction to humoral and cellular responses remains relatively obscure although a great deal of work has been done in this area (3).

Substances capable of calling forth an immune response are referred to as immunogens, while those which react specifically with the end products of immune responses (i.e. antibodies or sensitized cells) are called antigens. In practice, the same physical entity can often serve as either an immunogen or an antigen, depending upon whether it interacts with the afferent or efferent arc of the immune response.

The antigens of primary interest in the context of this report are those present on the surfaces of mammalian cells. A number of such antigen systems have been described, each with its own properties and experimental advantages. One example is the Forssman antigen (4), which is widely distributed throughout the tissues of many species. It can be readily assayed by the complement-dependent lysis of sheep red blood cells, and has proven to be amenable to chemical analysis. As a result, the complete chemical structure of this cell surface component has been elucidated (5).

In contrast with the Forssman system, some antigens are restricted to only a single species or tissue. Experiments such as those of Frye and Edidin (6) have shown that species-specific antigens can act as useful labels for the study of the dynamic properties of cell membranes. The discovery of tissue-specific antigens, for example, the thymic

lymphocyte-specific TL (7) or Thy-1 (8) systems of mice, has facilitated the identification, localization, and fractionation of cell populations (9) and aided in the discrimination of tumor cell types (10). Developmental studies of these antigens have provided new information about the revisions in cell surface architecture which accompany the process of cellular differentiation (11).

Cellular antigens may also show genetically controlled variation within a single species. Blood group antigens have become useful genetic markers in a number of species, including the human (12). The analysis of blood group antigens has also contributed to knowledge in the area of systemic as opposed to autonomous gene expression. Although most well-defined cellular antigens are the products of the cells upon which they reside, others such as the J antigen of cattle (13), the R antigen of sheep (14), or the Lewis antigens of the human (15) may be passively adsorbed onto otherwise negative cells.

In addition to learning about the normal structure and function of cells through the study of their surface antigens, it is also possible to study certain pathological states as evidenced by deviations from the normal pattern of antigen expression. The infection of cells with viruses frequently gives rise to distinctive surface antigens (16,17). The emergence of tumor-specific cell surface antigens (18) and the re-expression of fetal antigens (19,20) are frequent concomitants of neoplastic transformation (21). In many cases the antigenic specificity of these tumor antigens reveals whether a given neoplasm was induced by viral or chemical agents (18).

By now it should be apparent that the antigenic composition of a mammalian cell's plasma membrane contains a great deal of information regarding the organism from which it came. The model system which I have studied is the Thy-1 or theta antigen of mice and rats. Several kinds of information revealed by analyses of Thy-1 expression in various cell populations will be considered in the following pages.

The genetic determination of Thy-1 antigenicity in mice, which is treated primarily in chapter 2, forms an underlying basis for the remainder of the work. In chapter 3 the use of Thy-1 as a genetic marker is further illustrated and the issue of its possible association with leukemogenesis is raised. The various genetic, anatomical, developmental, and phylogenetic factors affecting Thy-1 expression are discussed in chapter 4. Finally, it is shown in chapter 5 that antibodies specific for Thy-1 and at least one other genetically controlled mouse cell surface antigen can be produced in rats. This finding is in accordance with expectations based upon the antigenic relationship between the two species outlined in chapter 4.

II. LOCATION OF THE GENE FOR THETA ANTIGEN IN THE MOUSE

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Location of the Gene for Theta Antigen in the Mouse

ELIZABETH P. BLANKENHORN AND TOMMY C. DOUGLAS

THETA (determined by the *Thy-1* locus[25]) is a cell-surface antigen found on mouse thymus-derived lymphocytes and in the brain[17]. Because it is expressed in increasing amounts in developing prenatal thymus and early postnatal brain[15,18], it is a convenient marker for the differentiation state of cells in both tissues. The expression of theta is controlled by an autosomal gene with two known codominant alleles: *Thy-1*^{a} and *Thy-1*^{b}. The mouse strains AKR/J and C3HeB/Fe were used in the reciprocal thymus cell immunizations that produced the original cytotoxic antibody reagents for this antigen[17].

Itakura *et al.*[9] demonstrated linkage between the markers dilute (*d*) and *Thy-1*, placing *Thy-1* in linkage group II. The distance between these two markers was shown to be 16.8 ± 3.8 map units. However, it was not determined on which side of *d* the *Thy-1* gene resided. The purpose of the present study was to define the position of *Thy-1* in linkage group II, which has now been identified as chromosome 9[3].

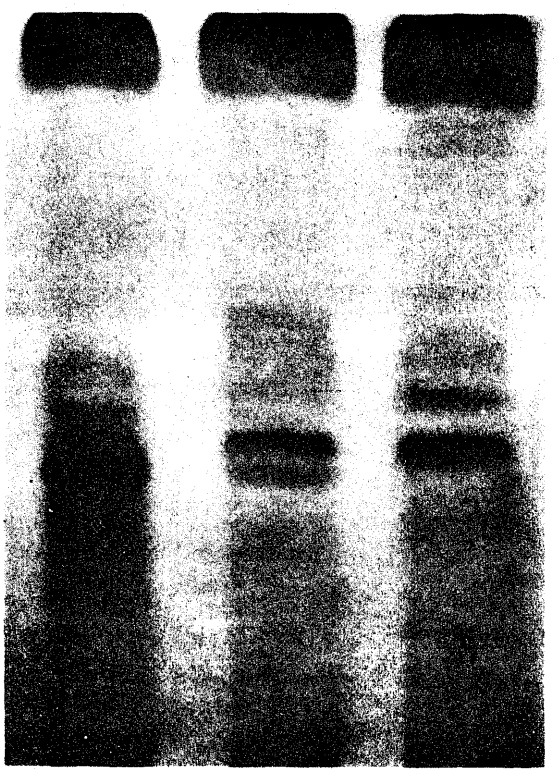
Our experimental design was to measure the recombination between *Thy-1* and *Trf*, the gene controlling an iron-binding serum protein, transferrin. Previously, *Trf* had been placed distal to *d* with respect to the centromere in linkage group II[22]. By observing the frequency of recombination between *Thy-1* and *Trf*, we expected to locate *Thy-1* more precisely. In addition, we followed the segregation at the *H-14* locus, which has not previously been assigned to any linkage group[16,19]. This locus, also known as *Ea-2*, determines a red-blood cell antigen.

During the course of our experiment, Itakura *et al.*[10] reported the linkage of the cytoplasmic malic enzyme locus, *Mod-1*, to *Thy-1* and *d*. We also report data regarding the linkage of this marker, which was segregating in our cross.

Materials and Methods

The F_1 and backcross generations were bred from CBA/J and RF/J mice obtained from The Jackson Laboratory, Bar Harbor, Maine. Samples of blood from the tails of individually numbered mice were typed for both H-14 and transferrin. Red blood cells collected into Gibson's CPD anticoagulant were washed three times in ice-cold saline and tested for H-14 by the PVP hemagglutination method[26]. Serum recovered from depression plates was separated by starch gel electrophoresis and stained with 0.25 percent Coomassie Blue to identify transferrin phenotypes[21,23] (Figure 1). Mice were thymectomized under anesthesia[2] and the thymus cells typed for

FIGURE 1—Patterns obtained by starch gel electrophoresis of sera from mice of transferrin types *b/b* (left), *a/b* (middle), and *a/a* (right). Anode at top of picture.



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theta by direct cytotoxicity using a modification of the method of Amos *et al.*[1]. In later studies, mice were sacrificed and the kidneys and thymus were removed. Kidneys were typed for malic enzyme using the method of Shows and Ruddle[20] with the following modifications: A 12 percent Connaught starch gel was made in 0.008 *M* Tris 0.003 *M* citrate, pH 6.1, and poured into a horizontal Plexiglass template containing 12 upright teeth, which provided sample wells. Kidney preparations were mixed with equal volumes of 2 percent Seaplaque agarose (Marine Colloids, Inc., Rockland, Maine) at 37°C. Samples were added when the gel was cool and run horizontally at 4°C with 0.223 *M* Tris 0.086 *M* citrate (pH 5.6) tank buffer for 5-6 hours at 300 volts (Figure 2).

Results

Reciprocal crosses were made between mice of strains CBA/J and RF/J. The F_1 progeny were then backcrossed to mice of the parental strains. Backcross progeny were typed for theta, transferrin, malic enzyme, and H-14. Mice of strain CBA/J have the genotype *Thy-1*^{b}, *Trf*^{a}, *Mod-1*^{b}, and *H-14*^{z}, while RF/J animals are *Thy-1*^{a}, *Trf*^{b}, *Mod-1*^{a}, and *H-14*^{R}. Because all four of the markers examined in this cross are expressed in a codominant fashion, the genotype of the gamete received from the hybrid parent can be inferred directly. Therefore, in Tables I through III, only those alleles inherited from the hybrid parent are listed. In all cases, the observed phenotypes of the backcross progeny were consistent with the codominant expression of these markers.

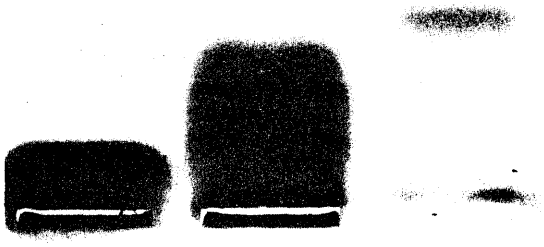

FIGURE 2—Patterns obtained by starch gel electrophoresis of kidney extracts from mice of *Mod-1* types *a/a* (left), *a/b* (middle), and *b/b* (right). Cathode at top of picture.

Table I. Recombination data for *Trf*, *Mod-1*, and *Thy-1*

Hybrid gamete	CR × C*	C × CR	CR × R	R × CR
Parental				
Trf^a *Mod-1*^{b} *Thy-1*^{b}	10	18	21	10
Trf^b *Mod-1*^{a} *Thy-1*^{a}	7	17	19	12
Recombinant, *Trf – Mod-1*				
Trf^a *Mod-1*^{a} *Thy-1*^{a}	0	4	10	1
Trf^b *Mod-1*^{b} *Thy-1*^{b}	4	4	6	1
Recombinant, *Mod-1 – Thy-1*				
Trf^a *Mod-1*^{b} *Thy-1*^{a}	5	12	9	2
Trf^b *Mod-1*^{a} *Thy-1*^{b}	3	4	4	5
Double recombinant				
Trf^a *Mod-1*^{a} *Thy-1*^{b}	0	0	0	0
Trf^b *Mod-1*^{b} *Thy-1*^{a}	0	0	0	0
Total	29	59	69	31
Recombination percentages				
Trf – Mod-1	13.8 ± 6.4	13.6 ± 4.5	23.2 ± 5.1	6.5 ± 4.4
Mod-1 – Thy-1	27.6 ± 8.3	27.1 ± 5.8	18.8 ± 4.7	22.6 ± 7.5
Trf – Thy-1	41.4 ± 9.1	40.7 ± 6.4	42.0 ± 5.9	29.0 ± 8.2

* Strain CBA/J is abbreviated as C, strain RF/J as R, and F_1 hybrids are designated CR

Three-point recombination values for the segregation of *Thy-1, Mod-1,* and *Trf* are given in Table I. The lack of recombinants of the reciprocal types Trf^a-$Mod\text{-}1^a$-$Thy\text{-}1^b$ and Trf^b-$Mod\text{-}1^b$-$Thy\text{-}1^a$ indicates that the gene order is *Trf—Mod-1—Thy-1.*

Additional animals were typed for *Trf* and *Thy-1* only. The results are given in Table II, which includes the data from Table I. The pooled estimate of the recombination frequency for crosses in which the hybrid parent was female, with each estimate weighted by the reciprocal of its variance, is 39.6 ± 3.9 percent. The estimate for recombination in the male, calculated in the same way, is 36.3 ± 5.0 percent. The estimates for the two sexes were tested for homogeneity and pooled in the manner described. The overall recombination frequency between *Trf* and *Thy-1* is 38.3 ± 3.1 percent.

Recombination data from progeny typed only for *Trf* and *Mod-1* are shown in Table III, which also includes the data from Table I. Using the same methods as described for the *Trf—Thy-1* data, estimates of 21.1 ± 3.9 percent and 7.7 ± 2.4 percent were obtained for recombination frequencies between *Trf* and *Mod-1* in the female and male, respectively. When tested for homogeneity, these estimates were significantly different ($P < .01$). The arithmetic average of these percentages is 14.4 percent. No significant difference was found, however, between the female and male recombination frequencies for *Mod-1* and *Thy-1.* The pooled estimate for females is 21.0 ± 4.1 percent, and that for males is 25.4 ± 4.6 percent. The overall estimate of the distance between *Mod-1* and *Thy-1* is 22.9 ± 3.1 percent.

Results from 102 backcross animals failed to indicate linkage between *H-14* and either *Trf* or *Thy-1.* The observed recombination frequencies were: *Trf—H-14,* 46.6 ± 4.9 percent; and *Thy-1—H-14,* 51.0 ± 5.0 percent. This finding is in accord with that of Itakura *et al.*[10].

The gene for albinism (*c,* chromosome 7) and the gene for nonagouti (*a,* chromosome 2) could be followed in our cross. Both *H-14* and the markers of the three-point tests on chromosome 9 assorted independently of both coat-color markers.

Table II. Two-point recombination data for *Trf* and *Thy-1*

Hybrid gamete	CR × C*	C × CR	CR × R	R × CR
Parental				
Trf^b $Thy\text{-}1^a$	15	17	30	12
Trf^a $Thy\text{-}1^b$	19	18	29	10
Recombinant				
Trf^b $Thy\text{-}1^b$	15	8	14	6
Trf^a $Thy\text{-}1^a$	10	16	22	3
Total	59	59	95	31
Recombination percentage	42.4 ± 6.4	40.7 ± 6.4	37.9 ± 5.0	29.0 ± 8.2

* Abbreviations same as those used in Table I

Table III. Two-point recombination data for *Trf* and *Mod-1*

Hybrid gamete	CR × C*	C × CR	CR × R	R × CR
Parental				
Trf^b $Mod\text{-}1^a$	14	38	23	17
Trf^a $Mod\text{-}1^b$	18	51	30	12
Recombinant				
Trf^b $Mod\text{-}1^b$	5	4	6	1
Trf^a $Mod\text{-}1^a$	2	4	10	1
Total	39	97	69	31
Recombination percentage	17.9 ± 6.1	8.2 ± 2.8	23.2 ± 5.1	6.5 ± 4.4

* Abbreviations same as those used in Table I

Discussion

Chromosome 9 of the mouse was reviewed several years ago by Green and Lane[7]. In addition to the standard published linkage map[6], an updated version has been compiled by M. C. Green (personal communication). Several markers have been added to this linkage group since 1967, including *Fv-2*[13] (which influences the response to Friend leukemia virus), *Mod-1*, *Thy-1*, and a new hair deficiency (M. C. Green, personal communication). The 14 identified loci in this linkage group code for isoenzymes, surface antigens, morphological and behavioral effects, a serum protein, and a coat color dilution. Translocations have been useful in determining gene order and centromere position for the chromosome. Klein[11] has reported the location of the breakpoint of one translocation [*T*(**9;17**) *138 Ca*]* to be between curly whiskers (*cw*) and short-ear (*se*). Using another translocation, *T*(**9;19**) *163H*, Lyon *et al.*[14] showed that the centromere is located at the end of the linkage group beyond *cw*.

Itakura *et al.* were able to assign *Thy-1* to linkage group II by virtue of its linkage to dilute (*d*)[9]. In a more recent report by these authors, the gene order *Mod-1—d—Thy-1* was established by a three-point cross[10]. The tentative order *Thy-1—d—Mod-1—lu* was suggested on the basis of earlier two-point recombination data for *d—Mod-1*, and *Mod-1—lu*[8], which had indicated that the *Mod-1* locus resided between the other two markers. Shreffler[22], making use of recombination data for *Trf-d* (12.7 percent) and *Trf—lu* (22.2 percent), located *Trf* distal to *d* with respect to the centromere. If *Thy-1* were also distal to *d*, it would necessarily lie close to *Trf*. Such placement of *Thy-1* was also suggested by the data of Lilly who ordered the loci with respect to *Fv-2*[13]. The order *Fv-2—d—Thy-1* with *Fv-2* proximal to *d*, was based on recombination results from two experiments, one using *Fv-2* and *tk*, and the other *Fv-2*, *d*, and *Thy-1*.

Our study demonstrates that *Trf* and *Thy-1* are not closely linked, as would be predicted from the previous studies and the current map. The observed recombination frequency for *Trf* and *Thy-1* is 36–40 percent. Furthermore, our gene order, *Trf—Mod-1—Thy-1*, may be combined with the order *Mod-1—d—Thy-1*, established by Itakura *et al.*, to provide the unambiguous sequence *Trf—Mod-1—d—Thy-1*. The tentative placement of *Trf* and *Thy-1* on the same side of *d* is therefore unacceptable. It remains uncertain, however, what the orientation of this sequence is with respect to the outside markers ducky (*du*) and luxoid (*lu*).

To resolve this issue with a three-point cross, the outside marker must be chosen carefully, because much of the uncertainty regarding chromosome 9 is due to the nature of the markers themselves. For example, *tk*, *du*, *lu*, and *se* have all been reported to depress viability greatly in the homozygous state. Mice homozygous for staggerer (*sg*), *lu*, and *du* are partially or totally sterile, while low penetrance and poor expressivity present additional problems with *lu*[5,7,24]. The orientation of the *Trf—Mod-1—d—Thy-1* sequence might be resolved by a three-point cross with curly whiskers (*cw*) or another outside marker not affected by the problems mentioned above.

The influence of sex on observed recombination frequency in the region between *d* and *Trf*, exemplified in this report as well as in that of Shreffler[22], should be noted. Significantly higher recombination in females has not been reported in the region proximal to *d;* it is likewise not found in our animals between *Mod-1* and *Thy-1*. The distortion of recombination frequencies by sex differences is found often in mice, but the process by which it occurs is not understood[4]. The chromosomal regions where higher female crossing over is observed fit into no obvious pattern, and even two studies of the same region may not agree on the presence of sex distortion of the recombination fraction. The possibility of such recombination differences, especially where loci are being ordered by two-point crosses, may tend to obscure the true sequence of markers in chromosome 9. The linkage map of this chromosome should be used with some caution until the proper sequence of existing markers is elucidated.

* Chromosome numbers, rather than linkage groups, are used in the symbols for translocations in accordance with the recommendation of the Committee On Standardized Genetic Nomenclature For Mice [3].

Summary

Reciprocal crosses were made between mice of strains CBA/J and RF/J. Segregation of the chromosome 9 markers *Thy-1*, *Mod-1*, and *Trf* was followed by means of backcrosses to the parental strains. These three-point data, combined with two-point data for *Trf-Mod-1* and *Trf-Thy-1*, are consistent with the following map: *Trf*———14———*Mod-1*———23———*Thy-1*. Although the order *Trf—Mod-1—d—Thy-1* is adequately established, the orientation of this sequence with respect to the centromere remains in doubt.

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III. VARIATIONS AMONG SUBLINES OF INBRED AKR MICE

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Variations among Sublines of Inbred AKR Mice

THE AKR inbred line of mice, developed as a strain with a high incidence of leukaemia, has found wide use in the fields of genetics, immunology, virology, and cancer research. While a certain degree of variability is expected among sublines derived from any inbred strain, rather striking differences have been observed among AKR sublines. Because such differences can be crucial to the usefulness of AKR mice for biomedical research, observations regarding some of these AKR sublines have been collected from several laboratories.

Figure 1 shows a partial history of some of the AKR sublines examined here. The AKR strain was originally developed as "Ak" by Jacob Furth between 1928 and 1936[1]. Eventually, descendants of this stock were sent to Oak Ridge National Laboratories by Dr Lynch, where they were maintained by random breeding and brother × sister matings. Furth, incidentally, may have brought some of his own Ak stock from Dallas to Oak Ridge in 1949. This would place the original separation of the AKR/J stock from the Oak Ridge pool of AKR mice at either 1936 or 1948.

Serological test results for mice of the AKR sublines J and Cum are summarized in Table 1[2-6]. Of particular note is the observation that sublines AKR/J and AKR/FuRdA express the Thy-1.1 thymus antigen (formerly called θ-AKR)[7] while AKR/FuA and AKR/Cum (ref. 8) express Thy-1.2 (θ-C3H). All the AKR sublines bearing the Thy-1.2 antigen, including AKR/Ka, were probably obtained from the Oak Ridge collection of AKR mice.

Data for several biochemical markers are given in Table 2. A critical observation is that the sublines differ at *Mod-1*. This gene, which governs the expression of malic enzyme, is located on chromosome 9 (LG II) approximately twenty map units from *Thy-1*[9,10]. Data on the expression of MuLV-gs antigen by several of the AKR substrains are presented in Table 3. The AKR/FuA subline, which has a relatively low incidence of spontaneous leukaemia, also shows decreased expression of the MuLV-gs antigen. In contrast, mice of the high-leukaemic AKR/FuRdA substrain were invariably MuLV-gs$^+$ as judged by immunofluorescence[11] and *in vivo* absorption tests[12,13], even when foster nursed to MuLV-gs$^-$ mothers of strains C3Hf or GR.

Although the AKR line was originally developed as a high-leukaemic line, the available sublines vary considerably in this respect. For example, the average incidence of leukaemia in mice of subline AKR/FuRdA (generations 5-18) was 92%

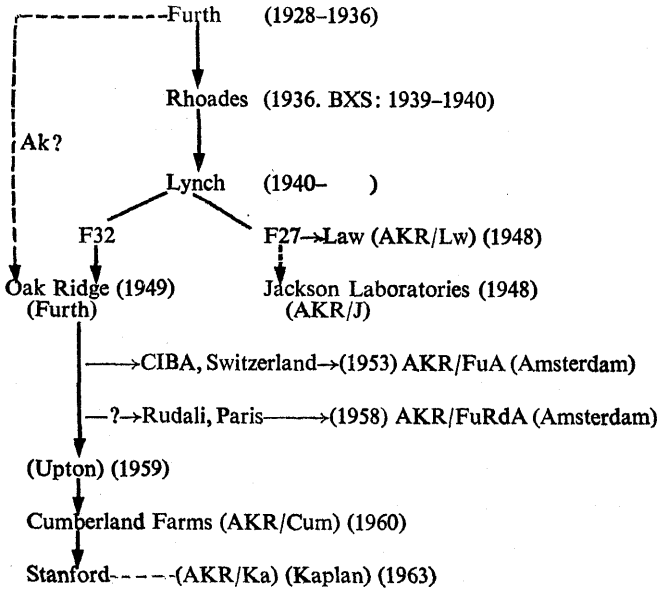

Fig. 1 Lineage of AKR mice.

(273/297) at age 9 months. Since the period 1959-1963, when these data on subline AKR/FuRdA were collected, this subline has continued to show a high incidence of spontaneous leukaemia. On the other hand, mice of subline AKR/FuA, during generations 16-36, showed a leukaemia incidence of only 36% (109/303) at age 11 months. A sister subline, F^m, developed leukaemia at a rate of 20% (45/227) at 12 months for generations 21-35. Preliminary data suggest that the AKR/Cum subline may show a lower spontaneous leukaemia incidence than AKR/J, but larger numbers of mice must be studied over a longer period before this question can be resolved. When combined with the genetic data from Tables 1 and 2, these findings suggest an association between leukaemogenesis and chromosome 9. Such an association has been reported before in the case of Friend virus-induced leukaemias. *Fv*-2, a gene determining susceptibility to this virus, is located on this chromosome[14].

In addition to their variability with respect to spontaneous leukaemias, sublines of AKR also differ in their susceptibility to transplanted leukaemias. Depending on the primary tumour used, resistance to its transplantation may be partial or complete. In an initial experiment, inocula of AKR/J primary leukaemia cells, which were lethal upon transplantation into other AKR/J mice, were successfully rejected by AKR/Cum mice. The only antigen for which the two sublines are known to differ is Thy-1, which may be serving as a histocompatibility antigen. It has been reported that A/Thy-1.1 mice (previously

Table 1 Cell Surface Antigens

Antigen	(LG)*	AKR/J	AKR/Cum
Thy-1.1	(II)	+	—
Thy-1.2		—	+
Ly-1.1	(XII)	—	—
Ly-1.2		+	+
Ly-2.1	(XI)	+	+
Ly-2.2		—	—
Ly-3.1	(XI)	+	+
Ly-3.2		—	—
TL.1,2,3,	(IX)	—	—
H-2^k	(IX)	+	+
$G_{IX}2$		+	+
GCSA		+	+

* LG indicates linkage group.

Table 2 Biochemical Genetic Markers

Marker	(LG)	AKR/ J	AKR/ FuRdA	AKR/ Lw	AKR/ Cum	AKR/ FuA
Hbb	(I)	d	d	d	d	d
Gpi-1	(I)	a	a	a	a	a
Mod-1	(II)	b	b	b	**a**	**a**
Trf	(II)	b	–*	–	b	–
Es-3	(VII)	c	c	c	c	**a**
Mup-1	(VIII)	a	a	a	a	**b**
Gpd-1	(VIII)	b	b	b	b	b
Dip-1	(XIII)	b	b	b	b	b
Id-1	(XIII)	b	b	b	b	b
Pgm-1	(XVII)	a	a	a	a	a
Es-1	(XVIII)	b	b	b	b	b

* Strain not tested.

A/θAKR) reject leukaemias from the congenic strain, A/J, which expresses the Thy-1.2 antigen[15].

Preliminary genetic studies using the progeny of (AKR/Cum ×AKR/J) F1 mice backcrossed to either AKR parent indicate that a relative resistance to another primary transplanted AKR/J thymoma segregates with the *Thy-1*^{b} genotype. F1 and *Thy-1*^{a} homozygous mice die of the tumour in 17.16 ± 0.52 and 17.42 ± 0.60 d, respectively, whereas Thy-1.1 negative mice (*Thy-1*^{b} homozygotes) die of the leukaemia in 23.75 ± 0.97 d (mean ± s.e.). No association was found between resistance and the *Mod-1* locus product in the several animals also tested for malic enzyme type. In a control experiment, small numbers of AKR/Cum mice were preimmunized with either four injections (intraperitoneal) of 10^7 AKR/J liver cells or four injections of 10^7 AKR/J normal thymus cells. Both groups were

Table 3 Quantitative Immunofluorescence Tests for MuLV-gs Antigen in Spleens of Adult Mice of Various AKR Sublines

Subline	Laboratory	Age in months	No. positive/No. tested IFA*	DR†	Titre IFA	ABU d^{-1}† DR
AKR/J	Sloan-Kettering Institute	1–8	22/22	3/3	1/4–1/16	11–>15
AKR/J	Department of Radiobiology, Stanford	6	2/2		1/8	
AKR/Ka	Department of Radiobiology, Stanford	1–6	12/12		1/4–1/16	
AKR/FuRdA	Netherlands Cancer Institute	1–9	32/32	9/9	1/4–1/16	10–>15
AKR/FuRdAfC3Hf‡	Netherlands Cancer Institute	1		1/1		15
AKR/FuRdAfGR‡	Netherlands Cancer Institute	2	3/3		1/4–1/8	
AKR/FuA	Netherlands Cancer Institute	2–5	0/8	3/10		10, 13, 15
AKR/FuA	Netherlands Cancer Institute	11–18	2/6	3/4	1/1–1/4	8, 10, >15

* IFA, Immunofluorescence absorption. The antigen titre refers to one volume of a 17% wet weight extract in 0.85% saline, which absorbs out the immunofluorescence of an equal volume of antiserum, diluted to three double dilutions below the endpoint of the fluorescence (for example, 1/64 if the endpoint of fluorescence is 1/512).

† DR, Disappearance rate. ABU d^{-1} refers to the disappearance rate per day of intraperitoneally injected rat anti-MuLV-gs antibodies from the serum of adult mice as measured by immunofluorescence. A disappearance rate of less that 2 ABU d^{-1} is considered negative.

‡ Foster nursed to MuLV-gs mothers of strain C3Hf or GR, as indicated.

able to reject completely a challenge inoculation of 5×10^6 Thy-1.1 positive AKR/J spontaneous thymoma cells, whereas the unimmunized AKR/Cum mice died of leukaemia within 23 d. Because liver preparations are generally considered Thy-1 negative[2], and because no cytotoxic anti-Thy-1.1 antibodies were found in the sera of animals preimmunized only with liver cells, further work is in progress to determine the effect(s) of factors other than Thy-1 antigenicity in this immune rejection phenomenon.

In summary, sublines of the inbred AKR mouse strain have been found to differ with respect to several traits, including the Thy-1 antigen, malic enzyme, esterase-3, principal urinary protein, expression of MuLV-gs antigen, and incidence of leukaemias. Within the context of these AKR sublines, the data suggest an association between chromosome 9 and leukaemogenesis. Further research in several fields will be required to test this hypothesis. For the present, researchers using AKR mice should be alerted to the subline variations reported.

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IV. OCCURRENCE OF A THETA-LIKE ANTIGEN IN RATS

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OCCURRENCE OF A THETA-LIKE ANTIGEN IN RATS*

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The theta antigen, described by Reif and Allen (1), provides a marker for thymus-derived lymphocytes in the mouse. The uses of this antigen system for studies in numerous fields, including immune system development, cellular cooperation, and the distribution of lymphocyte subpopulations, have recently been reviewed (2). Expression of the theta antigen is governed by a single autosomal locus having two alleles, θ^{AKR} and θ^{C3H} (3).

After immunization with θ-AKR antigen-bearing cells, spleen cells harvested from mice homozygous for θ^{C3H} have been shown to form plaques against thymus cells from several inbred rat strains as well as against those of mice carrying the θ^{AKR} allele (4). The present study was undertaken in order to clarify the relationship between this rat antigen system and the θ-system of mice.

Materials and Methods

Animals.—All inbred rat strains used in this study were obtained from Microbiological Associates, Inc., Bethesda, Md., with the exception of strain DA which was kindly provided by Dr. Joy Palm, The Wistar Institute, Philadelphia, Pa., and strain D which was originated in this laboratory by Dr. Ray D. Owen. AKR/J and C3H/HeJ mice were bought from the Jackson Laboratory, Bar Harbor, Maine, and AKR/Cum mice came from Cumberland View Farms, Clinton, Tenn.

Anti-θ Sera.—Antisera were produced according to the optimal schedule described by Reif and Allen (3). Blood was collected from the tail into heparinized tubes, and the plasma stored at −20°C until used.

Cytotoxicity Assay.—The cytotoxic test employed was similar to the one-step microcytotoxicity assay described by Amos (5), except for the substitution of Hanks' balanced salt solution (HBSS)[1] for barbital buffer in the thymocyte (TC) suspension, and of 0.3% trypan blue in HBSS for 0.25% trypan blue in saline. Test cells were preincubated with antiserum for 15 min at room temperature before complement addition. Test plates (No. 3034; Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.) were then further incubated for 20 min at 37°C in a 6.3% CO_2 atmosphere. Exclusion of trypan blue dye was then used as the measure of test cell viability.

Complement.—Rabbit serum diluted 1/3 in HBSS (from which Ca^{++} and Mg^{++} had been omitted) was absorbed with purified agarose (Bio-Rad Laboratories, Richmond, Calif.) at 0°C for 30 min in the presence of 0.01 M ethylenediaminetetraacetic acid (EDTA) disodium

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[1] *Abbreviations used in this paper:* HBSS, Hanks' balanced salt solution; TC, thymocyte.

salt (6, 7). After filtration to remove the agarose beads, divalent cations were restored by the addition of $CaCl_2$ in an amount equimolar to the previously added EDTA. The pH of the absorbed serum was brought to approximately 7.4 with 0.1 M NaOH. 0.5-ml aliquots of absorbed sera were stored at −80°C until shortly before use.

Sera from rats of strains WF or F344 were used unabsorbed and were diluted 1/4 in HBSS before storage at −80°C.

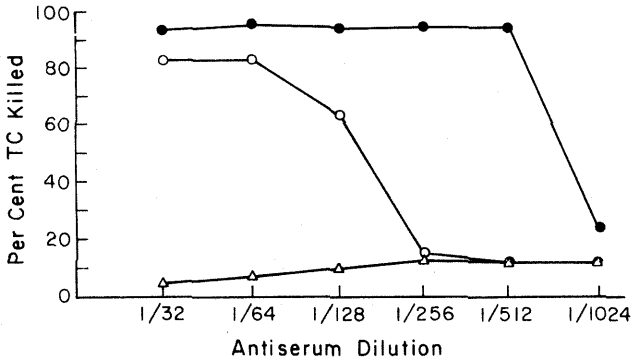

FIG. 1. Killing of AKR/J TC by C3H/HeJ anti-AKR/J serum. 100-μl aliquots of serum diluted to 1/32 in HBSS were absorbed with the following numbers of thymocytes: none (●—●), 1×10^7 DA rat TC (○—○), 1×10^7 AKR/J TC (△—△). Complement source: 1/12 agarose-absorbed rabbit serum.

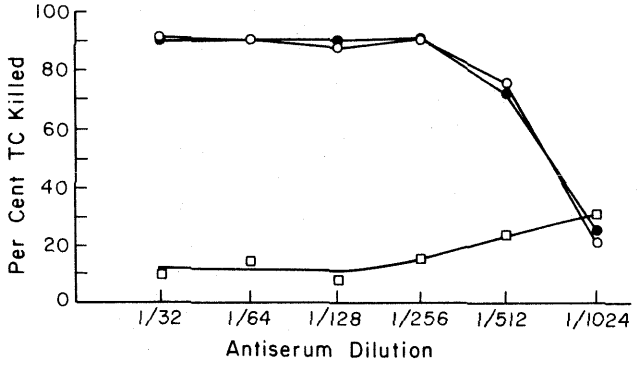

FIG. 2. Killing of C3H/HeJ TC by AKR/J anti-C3H/HeJ serum. Symbols and procedure same as in Fig. 1. Absorption with 1×10^7 C3H/HeJ TC (□—□).

RESULTS

Using AKR/J test cells and C3H/HeJ anti-AKR/J TC sera, rat thymocytes showed an intermediate absorptive capacity, relative to AKR/J TC (Fig. 1). However, using AKR/J anti-C3H/HeJ sera, negligible amounts of activity against C3H/HeJ cells were removed by rat thymocytes while C3H/HeJ TC showed strong absorption (Fig. 2). Control absorptions with TC of the serum donor's strain were also negative. Cytotoxic autoantibodies (8) were not observed in this system.

These absorption results were confirmed by the observation of direct killing of rat TC by mouse anti-θ serum in the presence of rat complement (Fig. 3). C3H/HeJ anti-AKR/J and AKR/Cum anti-AKR/J TC sera were both strongly cytotoxic for rat TC while C3H/HeJ and AKR/Cum normal sera were not. Also negative against rat TC were AKR/J anti-C3H/HeJ TC and AKR/J anti-AKR/Cum TC sera.

The complement source used to demonstrate the presence of θ-like antigen on rat thymocytes was of critical importance, as may be seen from Figs. 4 and 5. With rabbit complement, C3H/HeJ anti-AKR/J TC serum showed only a small differential killing effect. Using rat complement, however, this difference was striking.

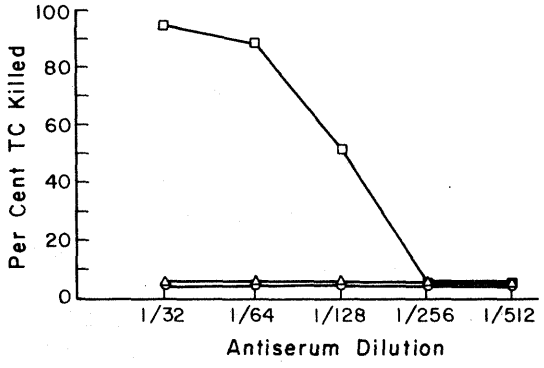

FIG. 3. Complement-mediated cytotoxicity of WF rat TC by anti-θ serum. Sera: C3H/HeJ anti-AKR/J TC (□—□), AKR/J anti-C3H/HeJ TC (△—△), C3H/HeJ normal serum (○—○). Complement source: 1/4 WF rat serum.

Preliminary tissue distribution experiments indicated that rat thymus and brain had the highest absorptive capacities for C3H/HeJ anti-AKR/J TC serum while rat liver, heart, testis, lung, skeletal muscle, erythrocytes, Peyer's patches, spleen, and peripheral white cells showed substantially less absorption. Based upon this observation, a developmental study of θ-like antigen in the rat nervous system was undertaken.

Rats were killed with chloroform and perfused with 0.85% NaCl. The brains were dissected out, weighed, and homogenized in saline so as to make suspensions containing 100 mg of tissue wet weight/ml of homogenate. All homogenates were made using Potter-Elvehjem type tissue grinders. Brain suspensions were stored at $-20°C$ until used, at which time they were thawed and rehomogenized. Measured aliquots of homogenate were centrifuged at approximately 2150 g for 5 min and the supernatants discarded. 0.05-ml aliquots of AKR/Cum anti-AKR/J TC serum diluted $1/256$ in HBSS were then added. Absorptions were carried out for 30 min at room temperature with frequent stirring. The absorbed sera were centrifuged at 2150 g for 5 min, and

the supernatants tested for residual cytotoxic activity against AKR/J TC. Absorptive capacities were determined from von Krogh plots (Fig. 6 [9, 10]).

Fig. 7 shows that an approximately logarithmic increase in the rat brain's theta-absorptive capacity occurs between birth and approximately day 20, at

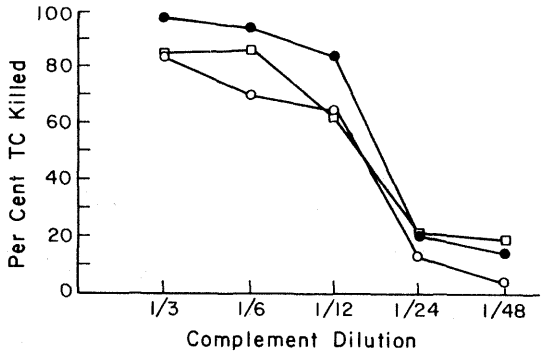

FIG. 4. Cytolysis of WF rat thymocytes by anti-θ serum and agarose-absorbed rabbit complement. Cells were incubated with or without anti-θ serum diluted 1/32 in HBSS. C3H/HeJ anti-AKR/J serum (●—●), AKR/J anti-C3H/HeJ serum (○—○), no serum (□—□)

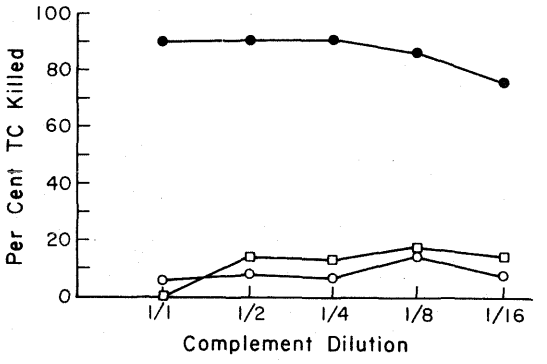

FIG. 5. Cytolysis of WF rat thymocytes by anti-θ serum and unabsorbed rat complement. Symbols same as for Fig. 4.

which point the slope of the curve rapidly declines. No significant difference in θ-absorptive capacity was observed between male and female rats.

To date, eight inbred rat strains have been tested for the presence of theta-like antigen. By both absorption and direct cytotoxic tests, thymocytes from rats of the ACI, BN, BUF, D, DA, F344, LEW, and WF strains all expressed antigenic determinants cross-reactive with the θ-AKR mouse antigen (Table I). No significant cross-reactivity with the θ-C3H antigen was observed. Wistar and Holtzman random-bred rats also gave similar results.

DISCUSSION

Quantitative absorption tests using mouse anti-θ sera and mouse thymocytes as target cells showed that rats of the inbred strains tested all expressed an antigen which was strongly cross-reactive with the θ-AKR, but not the θ-C3H, specificity of mice. The same pattern of cross-reactivity was seen when direct

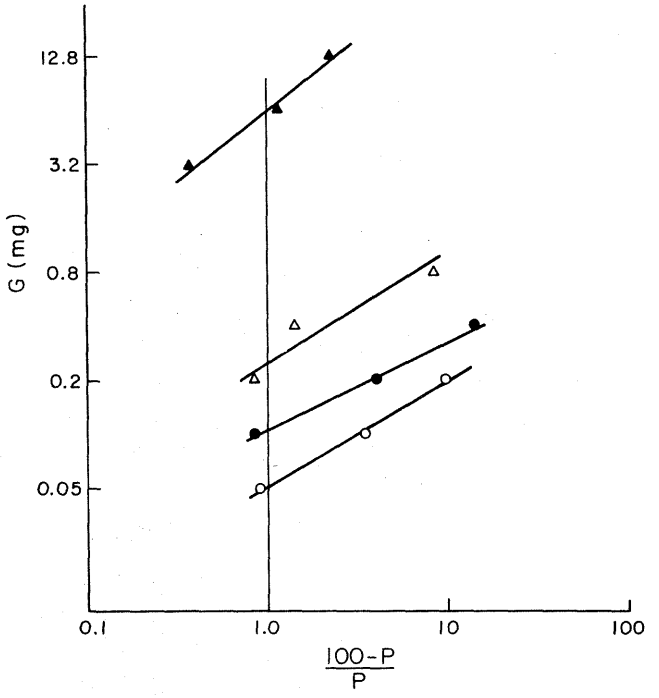

FIG. 6. Determination of the theta-absorptive capacities of WF rat brain homogenates Postnatal age: 8 days (▲—▲), 18 days (△—△), 33 days (●—●), 271 days (○—○). Serum: AKR/Cum anti-AKR/J TC diluted 1/256 in HBSS. Complement source: 1/18 agarose-absorbed rabbit serum.

cytotoxic tests were performed using mouse anti-θ sera with rat thymocyte target cells.

The AKR/Cum mouse subline has been shown to be similar to the AKR/J subline with reference to several alloantigenic markers (Dr. E. A. Boyse, personal communication) while differing at the θ-locus (11). The cross-reactivity of AKR/Cum anti-AKR/J sera with rat antigens thus argues strongly that the specificity detected is indeed θ and not some other antigen.

As has been observed with rat lymphocytes (12) as well as with those of other species (13), the complement source used in cytolytic assays can be of critical importance. Although rat strains WF and F344 differ at three of the

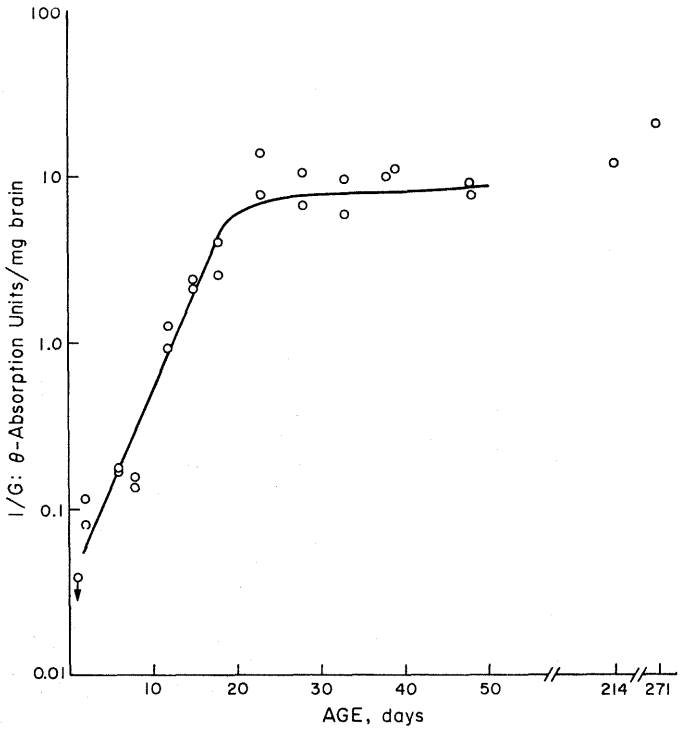

FIG. 7. Developmental appearance of θ-like specificity in WF rat brain. Absorptive capacities are expressed as θ-absorptive units = reciprocal of the number of milligrams of brain homogenate required to produce 50% inhibition of cytotoxicity. Serum: same as for Fig. 6. At 1 day, theta-absorptive capacity <0.04.

TABLE I

Per Cent Rat TC Killed by Mouse Anti-θ Sera

Rat strain	Serum							
	None*	AKR/ Cum NS	AKR/ Cum anti-AKR/J	C3H/ HeJ NS	C3H/ HeJ anti-AKR/J	AKR/J NS	AKR/ J anti-C3H/HeJ	AKR/ J anti-AKR/Cum
ACI	18	12	58	11	77	17	19	15
BN	20	8	60	7	78	22	27	19
BUF	24	6	41	7	81	18	24	17
F344	11	6	63	5	86	11	7	11
LEW	13	9	64	3	90	11	9	12
WF	14	10	77	10	81	16	16	14

All sera used at 1/32 dilution in HBSS. Note that this serum concentration does not necessarily represent a "cytotoxic plateau" for the respective sera, but merely gives a qualitative index of cytotoxic activity. Complement source: WF serum diluted 1/4 in HBSS.

* Preincubated with HBSS alone.

well-defined genetic loci for rat cellular antigens (14), their sera gave very similar results when used as complement sources for the immune cytolysis of thymocytes from six different strains of rats. The presence of rat natural antibodies (15) apparently does not pose a problem in this system, and rat serum provides a convenient source of complement for such testing.

In addition to exhibiting serological cross-reactivity with θ-AKR, the rat theta-like antigen also parallels the θ-antigen of the mouse in its tissue distribution and developmental kinetics (1, 16). Of particular interest is its occurrence at high concentration in brain tissue. In both species, neonatal brain expresses little or no antigen. During the first few weeks of postnatal life, however, expression of the antigen rapidly rises to the adult level.

The existence in rats of an antigenic system similar to the theta system of mice provides a potentially valuable resource for structural and functional studies of both the immune system and the nervous system. It is recommended that the new rat antigen be designated θ-R based upon its homology with the mouse θ-antigen. If allelic variants are found, they may be designated θ-R^1, θ-R^2, etc.

Other rat thymus (17–20) and brain (21, 22) antigens have been reported. Potworowski and Nairn (17, 18) used BALB/c mouse anti-Lister hooded rat TC microsome sera whereas Waksman and his collaborators (19, 20) prepared anti-rat TC sera in rabbits. Because BALB/c mice are homozygous for θ^{C3H}, anti-θ-R-type antibodies might be expected to occur in sera raised against rat TC. The relationship between θ-R and the antigens described by Waksman are somewhat more difficult to assess. Activity paralleling that of anti-θ serum has been observed with rabbit anti-mouse TC and rabbit anti-mouse brain sera, but such reagents have not distinguished the genetic variants revealed by alloantisera (23–26).

SUMMARY

A rat antigen system parallel to the mouse theta system has been described. All rat strains tested expressed an antigen cross-reactive with the θ-AKR specificity of mice while none cross-reacted strongly with θ-C3H. The rat antigen may be demonstrated by either absorption or direct complement-mediated killing of rat thymocytes. Patterns of organ distribution and developmental appearance in the nervous system of rats also parallel those previously reported for theta in mice.

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IV. ADDITIONAL DATA

Additional studies of the inter- and intraspecific distribution of Thy-1-like antigens have been undertaken. Absorption experiments indicated an absence from guinea pig brain tissue of antigens cross-reactive with either Thy-1.1 or Thy-1.2. The thymocytes and brain of the degu (Octodon degus, a South American rodent)[1] were also tested for the presence of Thy-1.1 by absorption. The results were negative even though an amount of Thy-1.1 equal to one-fourth that expressed by mouse tissues would have been readily detected.

It has not yet been possible to exploit the θ-R system as a genetic marker in the rat because no genetic polymorphism in this antigen has been discovered. In an attempt to find such a polymorphism, microcytotoxicity tests were performed using the thymocytes of five inbred strains of rats not included in earlier experiments (WF/fz, COP, A/2, AUG, BN.B2).[2] As in the cases previously described, all five of these strains were found to express θ-R. No cross-reactivity with Thy-1.2 was found. The number of thymocytes from a given rat strain required to absorb a standard amount of anti-Thy-1.1 antibody varied from 2.2×10^5 to 4.3×10^5, suggesting that no large quantitative differences in θ-R expression exist among these five strains. These values are comparable with 2.8×10^5 which was observed for thymocytes of strain WF.

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[1] Degu tissues were a gift from Dr. David Boraker, University of Vermont School of Medicine.

[2] These rats were a gift from Dr, Joy Palm, Wistar Institute, Philadelphia.

If a genetic polymorphism for θ-R is eventually found, the most probable candidates for linkage studies will be those markers already known to be linked to Thy-1 in the mouse. Several genetically controlled transferrin variants have been reported in Rattus rattus (22) but no similar variation has been found in Rattus norvegicus. Electrophoresis[3] of serum samples from the 13 inbred and 2 random-bred Rattus norvegicus populations referred to above revealed no variation in their serum transferrins.

Extracts of kidneys from the same rats were screened for electrophoretic variants of the cytoplasmic malic enzyme. Using the buffer system described in chapter II, the rat enzyme migrated anodally while the products of Mod-1^a and Mod-1^b of the mouse migrated cathodally at slower rates. No variation in the electrophoretic mobility of the enzyme was observed among the 15 rat populations tested. As an additional check for possible variation, the buffer system of Richmond (23) was also tried. Under these conditions of electrophoresis both rat and mouse enzymes migrated anodally, the latter moving relatively faster. No differences were observed among the following strains: A/2, AUG, BN, BN.B2, COP, LEW, WF, WF/fz.

Additional data relating to the expression of θ-R antigen by various rat lymphoid cell populations have also been gathered. Comparative results of cytotoxic and absorption tests performed on thymus, spleen, lymph node, and bone marrow cells of WF rats (24) and AKR/J mice are presented in Table 2. Although Thy-1.1$^+$ cells in AKR/J

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[3]See chapter II for a description of experimental details.

mouse spleen and lymph node preparations were killed using the same antiserum, no significant cytolysis of the corresponding rat cells was observed. The differing levels of θ-R expression suggested by these results were confirmed by the greatly diminished absorptive capacities of rat lymph node and spleen cells relative to thymocytes.

Although the general pattern of decreased θ-R expression on rat lymph node and spleen cells parallels that reported for Thy-1 in mice (8,25) an important difference between these systems is apparent. In rats, θ-R expression on peripheral thymus-derived lymphocytes (T-cells) drops below the level of detectability using the absorption procedures described above. Acton (26) has obtained similar results using quantitative absorption and radioimmunoassay techniques. In contrast, the indirect membrane immunofluorescence data of Micheel et al. (27) indicate the presence of $\theta\text{-R}^+$ cells in Wistar rat lymph node and bone marrow. The experiments described in the remainder of this chapter were designed to shed additional light upon the question of θ-R antigen expression by rat peripheral T-cells.

The first approach to this question was based upon the hypothesis that anti-Thy-1.1 antibodies in the presence of complement might interfere with rat peripheral T-cell function, even though they did not cause cytolysis. The particular function chosen for study was the cell-mediated immune destruction of EL4 (mouse) leukemia cells.

Ten to sixteen days before cell-mediated immunity was to be assayed, mice or rats were injected intraperitoneally with 5×10^6 or 2×10^7 EL4 cells, respectively. On the day of the experiment,

peritoneal exudate cells (PEC) were collected into Dulbecco's Modified Eagle Medium (DMEM, Grand Island Biological Co,) according to the method of Berke et al. (28).

EL4 ascites leukemia cells were maintained by serial intraperitoneal passage in C57Bl/6J mice. Twenty to thirty million washed EL4 cells suspended in 1 ml of DMEM were incubated with 100 microcuries of $Na_2{}^{51}CrO_4$ for one and one-half hours at 37°C, then washed five times in 3 ml aliquots of the same medium prior to use. The relative proportion of ^{51}Cr released from these prelabeled tumor cells can be used as a quantitative measure of cell death, and hence cell-mediated immune function, after a correction has been applied for the relatively slow rate of spontaneous ^{51}Cr release (29).

One-tenth ml aliquots of washed PEC at a concentration of 2.5×10^7/ml in DMEM were placed into 10 x 75 mm glass culture tubes. Following the addition of 10 microliters of whole mouse normal serum or antiserum the tubes were mixed, covered with plastic film, and incubated for 15 minutes at room temperature. Forty microliters of a suitable dilution of whole guinea pig serum (Figure 8) or 20 microliters of undiluted guinea pig serum (Table 3), which had been stored at -70°C until shortly before use, were added as a source of complement. The tubes were then mixed, covered, and incubated for 20 to 30 minutes at 37°C. At the end of this period the volume of each tube was brought to a total of 1.0 ml by the addition of DMEM. Following centrifugation at 360 x g for 10 minutes, the supernatants were carefully aspirated and discarded so as to minimize the transfer of unbound antibodies or

other serum components. The pellet from each tube was resuspended by trituration in 0.9 ml of DMEM supplemented with 10% fetal calf serum (DMEM + 10% FCS, see reference 28) and transferred to one well of a Disposo-Tray (Linbro Plastics).

When all of the treated PEC from a single experiment had been transferred to the coded wells of a tray, 0.1 ml of ^{51}Cr-labeled EL4 leukemia cells at a concentration of 5×10^5/ml in DMEM + 10% FCS was added to each well. The entire tray was covered with plastic film and incubated on a rocking platform (30) under an atmosphere of 94% air - 6% CO_2 for 6 hours. At this point 1 ml of DMEM + 10% FCS was added to each well. The contents of each well were lightly triturated and transferred to a disposable 10 x 75 mm glass culture tube. After centrifugation at 360 x g for 10 minutes, 1 ml (one-half the total volume) of the supernatant from each tube was removed for counting in a Nuclear-Chicago Model 4218 well type gamma counter. The remaining contents of each tube were also counted. The percentage of ^{51}Cr released was computed using the following formula:

$$\text{Percent } ^{51}\text{Cr Released} = \frac{2 \times (\text{supernatant} - \text{background})}{\text{remainder} + \text{supernatant} - 2 \times \text{background}} \times 100$$

where "supernatant" = counts/10 minutes in the 1 ml supernatant sample, "remainder" = counts/10 minutes in the tube containing the pellet plus the other half of the supernatant, and "background" = counts/10 minutes with no sample in the counting chamber. The factor of 2 in the numerator of this expression takes account of the fact that only one-half of the counts released from the labeled cells are actually sampled for counting in the "supernatant" tube.

The results of an experiment using immune AKR/J PEC are shown in Figure 8. Immune PEC pretreated with AKR/J mouse normal serum uniformly caused maximal tumor cell lysis. In contrast, the cytotoxic activity of PEC treated with serum containing antibodies specific for Thy-1.1 (θ-AKR) was completely abolished. The degree of PEC inactivation observed falls off with decreasing complement concentration, strongly suggesting that the inactivation process is complement-dependent.

Data from a similar experiment with immune WF rat PEC are presented in Table 3. Each sample of immune PEC was pretreated with either AKR/Cum normal serum, AKR/Cum anti-AKR/J thymocyte serum (containing anti-Thy-1.1 antibodies), or AKR/Cum anti-BN rat spleen serum.[4]

Rat PEC pretreated with AKR/Cum anti-AKR/J thymocyte serum showed anti-tumor activity equivalent to those treated with normal serum, but pretreatment of these cells with AKR/Cum anti-BN spleen serum resulted in a complete inhibition of their cytotoxic activity. Thus, the ability of these antisera to interfere with the function of WF rat peritoneal cytotoxic cells is correlated with the presence of species antibodies, and not of those directed against Thy-1.1. This is quite different from the pattern observed for AKR/J immune PEC, which are strongly inactivated by either AKR/Cum anti-AKR/J thymocyte serum or AKR/Cum anti-BN rat spleen serum, but not by AKR/Cum normal serum.

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[4] This serum also contains anti-Thy-1.1 antibodies in addition to antibodies directed against rat species-specific antigens. The properties of this antiserum are discussed further in connection with Figure 9.

The effect of a similar set of antisera on WF rat PEC viability was determined in a separate experiment. One-tenth ml aliquots of PEC were pretreated with antibody and complement as described above. The viability of the cells in each group was then determined by the Trypan Blue exclusion method. It is apparent from the data presented in Table 4 that the inactivation of rat PEC by AKR/Cum anti-BN rat spleen serum is the result of complement-mediated cytolysis. The partial decline in the viability of cells treated with either AKR/Cum normal serum or anti-Thy-1.1 antibody[5] also correlates well with the partial loss of effector cell activity observed in similarly treated cell populations (Table 3). On the basis of these data plus those presented in Table 2, it seems unlikely that anti-Thy-1.1 reagents can be used for the selective elimination of rat peripheral T-cells from mixed lymphoid cell populations, as has been done successfully in the mouse.

The final approach taken in search of θ-R on rat peripheral T-cells was based upon the hypothesis that small amounts of this material might be immunogenic even though they did not present an adequate target for complement-mediated cell damage. In order to evaluate this possibility, AKR/Cum mice were injected with BN rat spleen cells according to the previously described protocol for raising anti-Thy-1.1 antibodies. The results of direct complement-dependent cytotoxic tests of these sera against thymocytes from mice of strains AKR/Cum, AKR/J, and C3H/HeJ are shown in Figure 9. The specific killing of Thy-1.1^{+}

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[5] C3H/HeJ anti-AKR/J thymocyte serum rather than AKR-Cum anti-AKR/J thymocyte serum was used in this experiment because of its higher titer.

thymocytes indicates the presence of anti-Thy-1.1 antibodies in this antiserum, and hence the presence or θ-R on rat spleen cells.

As a test for the presence of θ-R on other peripheral T-cells of the rat, groups of AKR/Cum mice were immunized with WF rat cells taken from the mesenteric lymph node, bone marrow, Peyer's patches, peritoneal cavity, or blood leukocyte fraction of WF rats. Thymocytes and spleen cells were used as positive control immunogens. Preliminary tests indicated the presence of anti-Thy-1.1 antibodies in all groups.[6]

The principal objection to this type of experiment has been raised by Komuro *et al.* (31). These authors found that mice lacking the TL antigen sometimes produced anti-TL antibodies following immunization with spleen cells from TL^+ donors. This observation was puzzling because TL has been reported to be present only on thymocytes, and not on peripheral mouse T-cells (7). Mice thymectomized as adults failed to give the anomalous response to TL although they responded normally to Thy-1. This finding was interpreted to mean that the original spleen cell inoculum contained (TL^-, $Thy\text{-}1^+$) stem cells which differentiated into TL^+ cells under the influence of the host's thymus. These differentiated cells then evoked an immunological response to the TL antigen. Experiments designed to evaluate the possibility that this is happening in the θ-R system are currently being pursued in collaboration with Andrew Dowsett.[7]

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[6] Andrew Dowsett, personal communication

[7] Undergraduate, California Institute of Technology

TABLE 2

Expression of Thy-1.1 Antigen by WF Rat and AKR/J Mouse Lymphoid Cells

	WF Rat Cells[a]		AKR/J Mouse Cells	
Cell Type	Cytotoxic Index	Absorptive Capacity[b]	Cytotoxic Index[c]	Absorptive Capacity[d]
Thymus	93	100	97	100
Spleen	10	<3	57	5.0
Lymph Node	10	<4	69	5.7
Bone Marrow	8	ND[e]	ND	<1.5

[a] Data from reference (24), which is included as Appendix I
[b] Relative to an equivalent number of thymocytes
[c] Unpublished data
[d] Determined by Reif and Allen (8), using a somewhat different absorption procedure
[e] Not done

TABLE 3

In Vitro Killing of EL4 Leukemia Cells by Immune WF Rat Peritoneal Exudate Cells (% ^{51}Cr Released)

	Experiment Number						
Pretreatment of PEC	1	2	3	4	5	6	Average
AKR/Cum Normal Serum	35.3	36.4	32.5	36.2	30.7	28.2	33.2
AKR/Cum anti-AKR/J Thymocyte Serum	29.0	34.2	35.5	35.1	30.4	32.9	32.9
AKR/Cum anti-BN Rat Spleen Serum	15.4	15.4	14.8	12.7	14.9	14.2	14.6
None	51.4	48.5	50.7	49.1	ND	ND	49.9
Background ^{51}Cr Release	15.1	12.9	17.7	14.4	14.8	13.0	14.7

TABLE 4

Complement - Dependent Cytolysis of WF Rat Peritoneal Exudate Cells by Mouse Antiserum

Pretreatment of PEC	Viability (%)
AKR/Cum Normal Serum	71
C3H/HeJ anti-AKR/J Thymocyte Serum	73
AKR/Cum anti-BN Rat Spleen Serum	3
None	93

FIGURE LEGENDS

Figure 8. In vitro killing of EL4 leukemia cells by immune AKR/J mouse peritoneal exudate cells. The percentage of ^{51}Cr released from labeled EL4 cells by immune PEC pretreated with either AKR/J normal serum (O) or AKR/Cum anti-AKR/J serum (□) was measured. The amount of label released following incubation with no PEC (background release) or untreated PEC is shown by (X) and (Δ), respectively. Each point represents the average of three determinations, except those marked [a], for which only 2 measurements were made. Error bars show the range for each value. "Complement dilution" refers to the conditions under which immune PEC were pretreated (see text).

Figure 9. Cytotoxic tests of AKR/Cum anti-BN rat spleen serum against mouse thymocytes. Target thymocytes were from strain AKR/Cum (Δ), C3H/HeJ (□), or AKR/J (O). The microcytotoxicity procedure described earlier in this chapter was used.

FIGURE 8

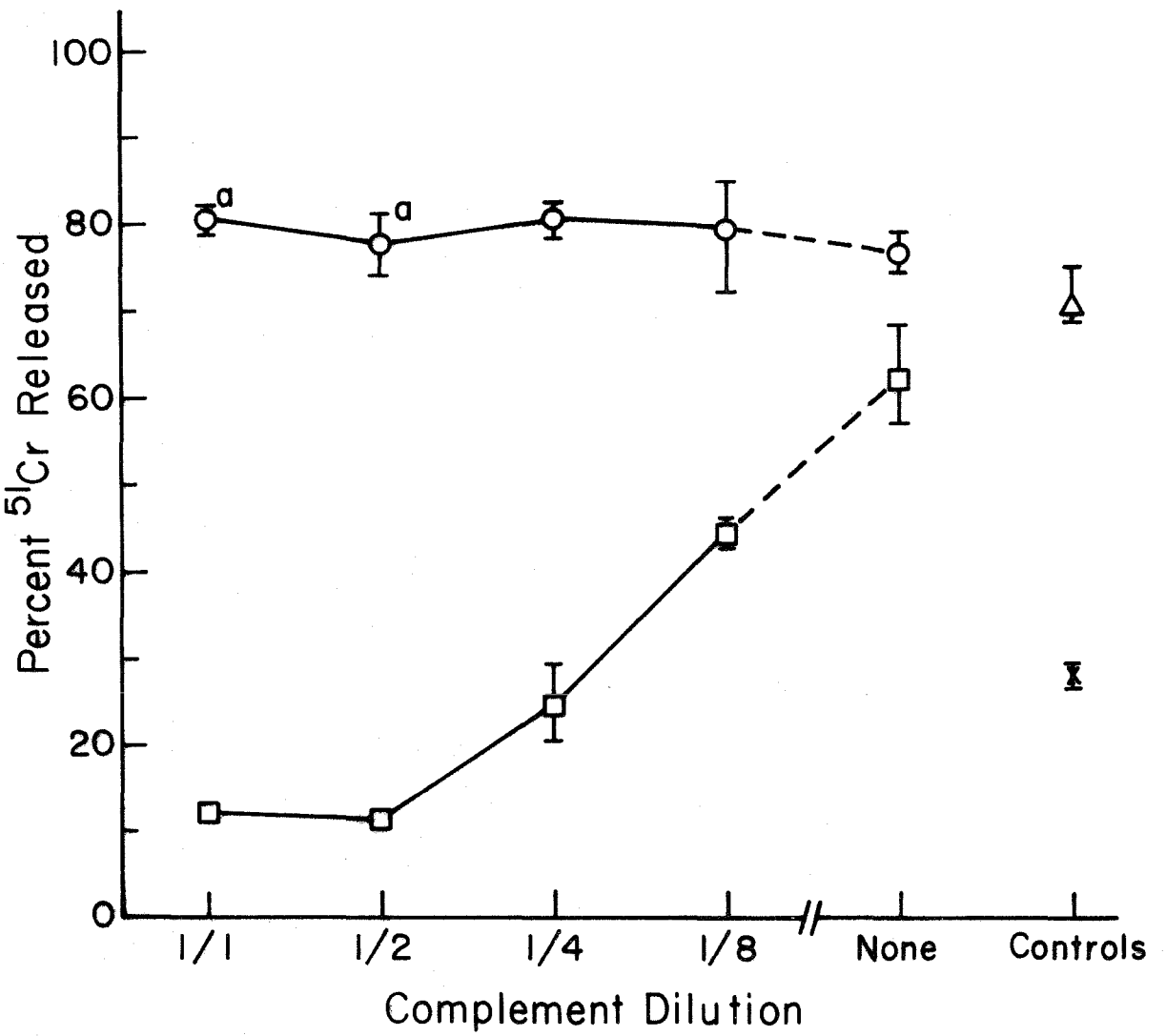
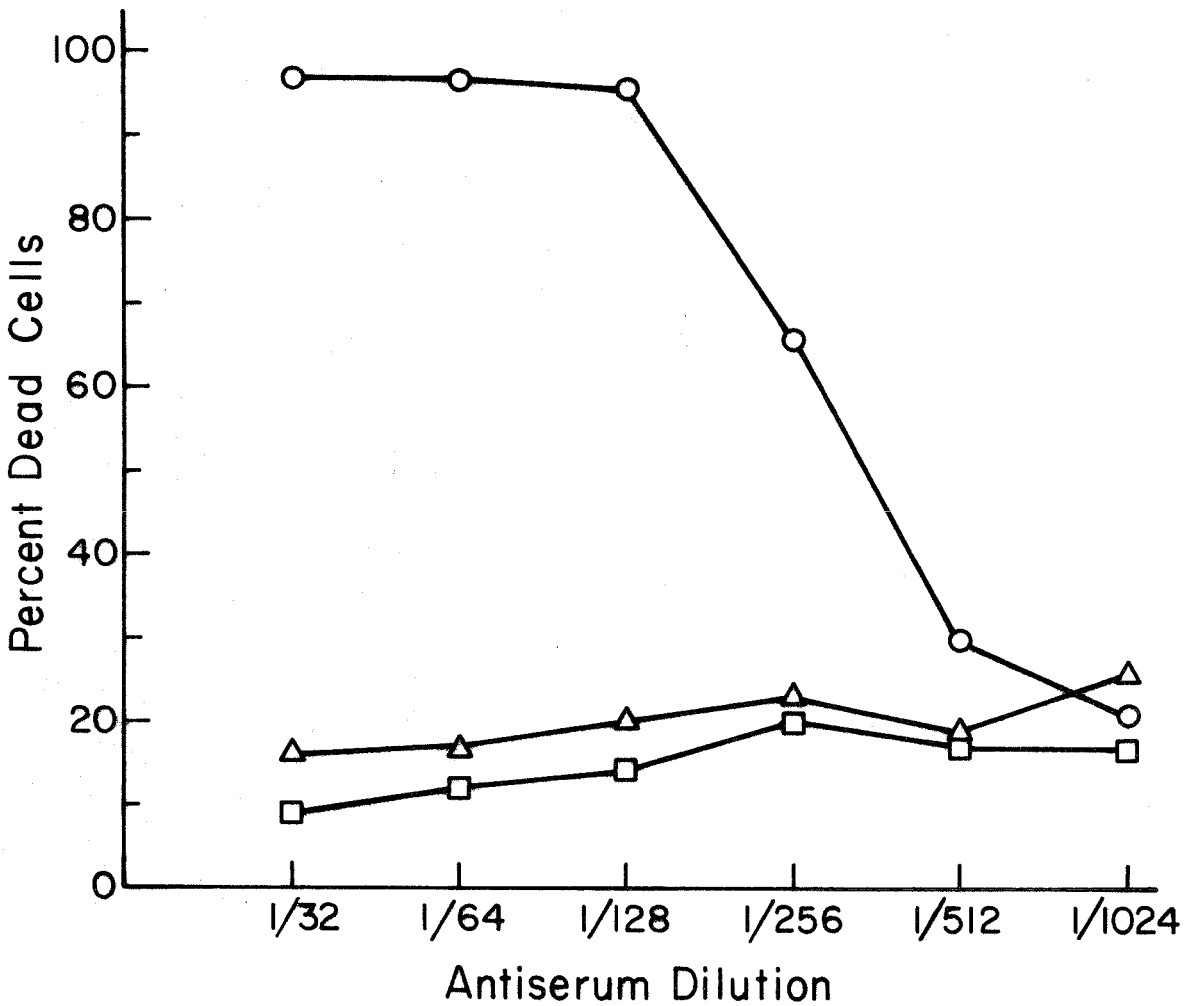

100
80
60
40
20
0
Percent ^{51}Cr Released
a
a
1/1
1/2
1/4
1/8
None
Controls
Complement Dilution

FIGURE 9


100
80
60
40
20
0
Percent Dead Cells
1/32
1/64
1/128
1/256
1/512
1/1024
Antiserum Dilution

V. GENETIC VARIATION IN MOUSE THYMOCYTE ANTIGENS DETECTED BY HETEROANTISERA

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GENETIC VARIATION IN MOUSE THYMOCYTE ANTIGENS DETECTED BY HETEROANTISERA[1]

The θ antigen of the mouse (*16*) provides a cell surface marker for thymus-derived lymphocytes (*15, 18*). The allelic variants of this antigen, Thy 1.1 and Thy 1.2, are expressed by animals carrying the *Thy-1*^{a} or *Thy-1*^{b} allele, respectively (*20*). Both specificites are expressed by mice heterozygous at the *Thy-1* locus (*17*). For the purposes of this report, the three known *Thy-1* genotypes are designated *a/a*, *a/b*, and *b/b*.

With one exception (*6*), the genetically determined θ specificities have been distinguishable only by appropriate alloantisera (prepared by intraspecies immunizations). The finding that rats express an antigen cross reactive with Thy 1.1 (*7, 8, 13*) suggested that immunization of rats with mouse thymocytes bearing the Thy 1.2 antigen might yield a reagent specific for Thy 1.2. Mice of types *b/b* (A/J, AKR/Cum, CBA/J, C3H/HeJ), *a/a* (AKR/J, RF/J, A/Thy-1.1), and *a/b* (CBA/J × RF/J) were used to test this hypothesis.

Inbred female WF rats received 1×10^7 AKR/Cum or C3H/HeJ (*b/b*) thymocytes suspended in Hanks' balanced salt solution i.p. each week for 6 weeks. Blood was obtained by cardiac puncture at the end of weeks 1, 5, and 6. Although antibodies against mouse thymocytes were demonstrable by dye exclusion microcytotoxicity tests[2] at the end of the 1st week of immunization, the later bleedings showed much higher titers, and were pooled for further experiments. Preimmune sera from the individual rats used were all negative in the cytotoxic test.

Antisera raised against thymocytes of inbred female *b/b* mice (AKR/Cum or C3H/HeJ) were complement inactivated by heating at 56 C for 30 min and diluted to 1:10 in Hanks' balanced salt solution. The cytotoxic titer of rat anti-AKR/Cum serum was 1:640–1:1,280 whether tested against *a/a* or *b/b* thymocytes. Rat anti-C3H/HeJ titers were 1:320 and 1:640–1:1,280, respectively, for the two cell types. These data reflect the presence of rat antimouse species antibodies in these unabsorbed heteroantisera.

Three-milliliter aliquots of 1:10 diluted sera were absorbed twice with AKR/J (*a/a*) red blood cells, then twice again with pooled AKR/J lymph node, spleen, and thymus cells. One milliliter of each of the absorbed antisera was then absorbed four times with pooled RF/J (*a/a*) lymph node, spleen, and thymus cells. Each absorption was carried out for 30 min at 0 C, and used either 0.3 ml of packed, washed erythrocytes or one-fourth of the pooled lymph node, spleen, and thymus cells from one donor.

The results of microcytotoxicity tests using these multiply absorbed rat antisera are shown in Figure 1. Thymocytes of the *b/b* strains (A/J, AKR/Cum, CBA/J, C3H/HeJ) are selectively killed relative to those of the *a/a* strains (AKR/J, RF/J). Cytolysis is also observed when thymocytes from heterozygous (CBA/J × RF/J)F_1 mice are used as target cells. The absorbed antisera fail to kill thymocytes from mice of the congenic stock A/Thy-1.1, which differs from the A/J line at the *Thy-1* locus. While this last observation illustrates the presence of antibodies specific for the Thy 1.2 antigen, it does not prove the absence of other antibodies, because the gene(s) for the corresponding antigen(s) might not be present in the A/J background.

As a further test of specificity, thymocytes from 10 progeny of the cross (CBA/J × RF/J) × RF/J were tested against the absorbed rat antisera and also typed for θ using standard alloantisera[3] (Fig. 2). This group of mice included two recombinants involving the linked *Thy-1* and *Trf* loci, one of each θ type. The rat anti-AKR/Cum serum clearly distinguished two classes of thymocytes (Fig. 2A). These classes are in complete accord with the θ typing results, i.e., Thy-1.2-positive cells are readily killed whereas Thy-1.2-negative cells are not. Considered in terms of genetic mapping, these results

[1] Supported by Atomic Energy Commission Contract AT (04-3)-767 and United States Public Health Service Grant GM 00086.

[2] The dye exclusion microcytotoxicity test is a modification of that described by Amos *et al.* (*1*). Details are given in reference 7.

[3] The standard θ typing sera are AKR/Cum anti-AKR/J (anti-Thy-1.1) and AKR/J anti-C3H/HeJ (anti-Thy-1.2).

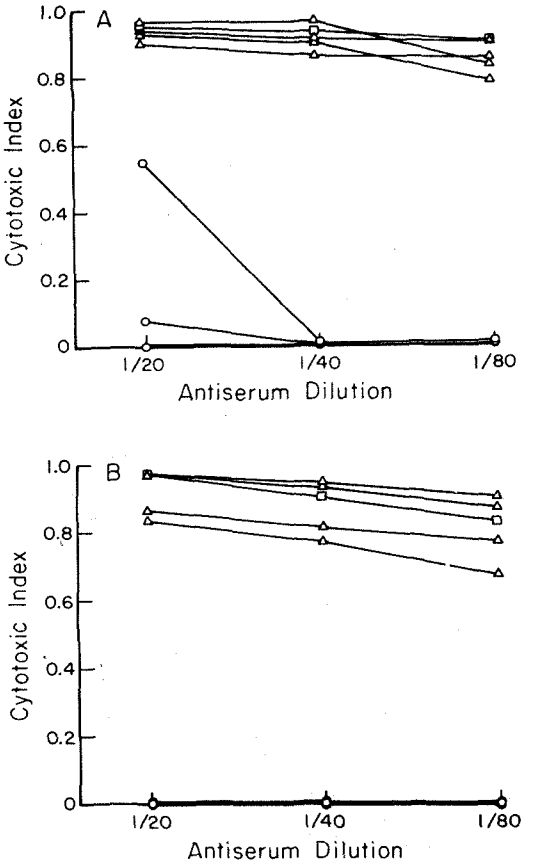

FIGURE 1. Immune cytolysis of thymocytes from inbred and F_1 hybrid mice by absorbed rat anti-AKR/Cum (A) and rat anti-C3H/HeJ (B) reagents. Target cells of genotypes *a/a* (AKR/J, RF/J, A/Thy-1.1: ○), *a/b* (CBA/J × RF/J: □), and *b/b* (A/J, AKR/Cum, CBA/J, C3H/HeJ: △) were used. The complement source was unabsorbed WF rat normal serum diluted 1:6 in Hanks' balanced salt solution. Cytotoxic index = (% stained cells in test well − % stained cells in control)/(100 − % stained cells in control).

strongly suggest that the gene controlling thymocyte reactivity with this antiserum resides on chromosome 9 in the general area of *Thy-1* (*2, 10, 11*). The evidence therefore indicates that a reagent specific for Thy 1.2 can be prepared, by appropriate absorption, from the rat anti-AKR/Cum serum.

θ Types are also distinguished by the absorbed rat anti-C3H/HeJ (also *b/b*) serum, but in this case the presence of antibodies directed against at least one additional thymocyte surface component is indicated (Fig. 2B). Thymocytes from two of the six *a/a* segregants were not killed by this serum, but those of the other four fell into an intermediate class. This result suggests the presence of one or more genetic factors not closely linked to *Thy-1*, contributed by the CBA/J parent. On the basis of its apparent strain distribution (i.e., presence in CBA/J but absence in AKR/J and RF/J), the antigen giving rise to this effect cannot be identified with any of the known specificities controlled by the *Tla*, *Ly-2*, or *Ly-3* loci (*3, 4*). The antigen Ly-1.1 cannot be ruled out on these grounds. The presence of antibodies to Ly-1.1 is further suggested by the difference between the two rat antisera. Anti-Ly-1.1 antibodies might be ex-

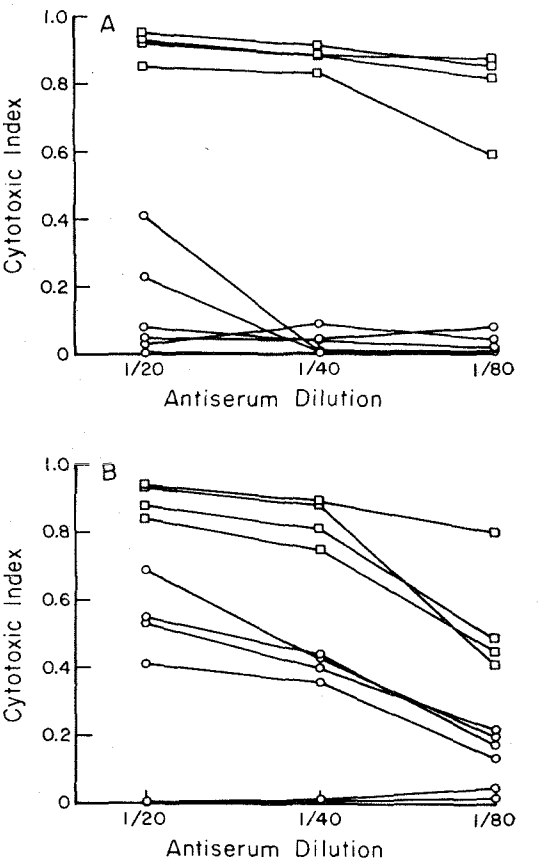

FIGURE 2. Immune cytolysis of thymocytes from 10 (CBA/J × RF/J) × RF/J backcross mice by absorbed rat anti-AKR/Cum (A) and rat anti-C3H/HeJ (B) reagents. Symbols and procedure are as in Figure 1. Each curve represents the results of cytotoxic assays using target cells from one mouse.

pected to occur in antisera raised against C3H/HeJ (Ly-1.1$^+$), but not in those raised against AKR/Cum (Ly-1.1$^-$). Further experiments will be necessary to establish the identity of these antibodies.

In addition to the antigen-antibody systems already discussed, the characteristics of the absorbed rat antisera described in this report can also be distinguished from H-2 (*12*). Little or no anti-H-2 activity is expected in these sera because strains AKR/Cum and C3H/HeJ, against which the sera were raised, as well as strains AKR/J and RF/J, with which the sera were absorbed, are all homozygous for the *H-2*^{a} allele at the major histocompatibility locus. This expectation is supported by the differences in reactivity observed between strains A/J and A/Thy-1.1, both of which are homozygous for *H-2*^{a}.

Although antisera raised in rabbits against mouse thymocyte membranes (5) or brain tissue (*6*, *9*) have been reported to show specificity for thymus-derived lymphocytes, direct cytotoxicity and absorption analyses of these sera have generally failed to demonstrate antibodies specific for the Thy-1.1 or Thy-1.2 antigens (*5*, *9*). Similar negative findings have suggested that the immunosuppressive effects of in vivo absorbed rabbit antimouse lymphocyte antisera could not be attributed to anti-Thy-1 activity per se (*19*). In contrast, Clagett et al. (*6*) reported a weak specificity for Thy-1.2 in rabbit anti-CBA/ST mouse brain serum. These investigators also observed that rabbit antirat brain sera contain antibodies reactive with one or more antigenic components (possibly equivalent to Thy-1.1) present in the brain and thymus of AKR/J mice (*a/a*), but not of eight *b/b* strains. Other antigens, apparently unrelated to Thy-1, are shared by rat and mouse thymocytes (*6*). Antibodies directed against these determinants are probably relevant to the immunosuppressive effect of rabbit antirat lymphocyte serum on C3H mice (*14*).

The strain distribution and genetic segregation data presented here show that reagents specific for the Thy-1.2 antigen (θ C3H) of the mouse can be prepared by the absorption of heteroantisera raised in rats. It seems likely that the capacity of rats to produce specific anti-Thy-1.2 antibodies is associated with their possession of a self antigen indistinguishable from Thy-1.1. In any case, rat antisera now appear to be a ready source of reagents for the detection of at least one class of lymphocyte alloantigens in the mouse.

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LITERATURE CITED

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VI. CONCLUSIONS

The Thy-1 antigen of mice and its counterpart θ-R in rats may be viewed from several different perspectives. It may be considered as a genetic marker, a species-specific antigen, or an indicator of differentiation at the level of the whole tissue or the individual cell. The following discussion will deal with Thy-1 as it appears from each of these viewpoints.

The Thy-1 locus has become a useful genetic marker for several reasons. Because the allelic alternatives at this locus are expressed in a codominant fashion it is possible to distinguish heterozygotes from either homozygote without the necessity of progeny testing. Disadvantages such as inviability, sterility, incomplete penetrance, and poor expressivity which plague some of the other markers on chromosome 9 (LG II) of the mouse do not appear to present a problem in this case. Partly as a result of our work the location of Thy-1 with respect to three of the other most useful markers on this chromosome (Trf, Mod-1, d) has been established. It seems probable that this group of linked genes, which is some 38 map units in length, will provide a useful landmark for future genetic studies of this chromosome.

In its capacity as a genetic marker the Thy-1 system has been applied to the analysis of the genetic variation among sublines of the inbred AKR mouse strain. Unexpectedly, some of these sublines were found to express the Thy-1.2 antigen (32). This is probably related to their common origin from the Oak Ridge stock, in which this marker may have been segregating. Within the context of these sublines, animals

homozygous for Thy-1^a seem to show an increased susceptibility to spontaneous leukemia as compared with those homozygous for Thy-1^b. If this association is not merely fortuitous because of the limited number of strains tested, it may reflect either linkage disequilibrium between Thy-1 and leukemia susceptibility factors or direct participation of the Thy-1 system itself in the process of leukemogenesis.

Historically, the genetic determinants of alloantigen systems have been first described as simple di- or triallelic series. As these systems were analyzed more closely, additional genetic complexity was often discovered (33). A case in point is the H-2 region of the mouse which has been described as "one of the most complex genetic systems known" (34). Data suggesting the occurrence of new Thy-1 alleles among inbred mouse strains have been presented recently (35). If these observations are correct, our understanding of Thy-1 may already be on the well-traveled road to complexity.

In addition to its genetic specificity, Thy-1 also shows species specificity. The Thy-1.1 antigen is shared by rats and mice but not degus. Thy-1.2 has been detected only in mice, and neither antigen has been found in hamsters (27) or guinea pigs. This pattern of species specificity has made it possible to use defined mouse alloantisera to study the rat θ-R antigen. It probably also underlies the successful attempts to produce antisera specific for Thy-1.2 in rats. To date it has not been possible to raise rat antibodies against θ-R; neither qualitative nor quantitative variation in the expression of this antigen has been found among inbred rat strains.

In terms of their tissue specificity and developmental expression, the Thy-1 antigen of the mouse and θ-R of the rat show strong similarities. The antigens are expressed most plentifully in brain, thymus, and embryonic fibroblasts (36) of both species. In the mouse Thy-1$^+$ cells first appear in the thymus about day 15 of gestation, increasing in number as the organ becomes progressively lymphoid (11). Later, as thymocytes differentiate into peripheral T-cells the concentration of Thy-1 on the cell surface decreases in counterpoint with the rise in H-2 antigen expression. The early stages in the appearance of θ-R in embryonic rat thymus have not been investigated, but the developmental sequence of antigenic expression during later T-cell differentiation appears to be similar. Although some doubt remains as to whether the θ-R antigen is expressed at all on rat peripheral T-cells, it can be tentatively concluded that the antigen is present in small amounts.

The temporal changes in antigenic expression during brain development in the rat and mouse are virtually identical. At birth little or no Thy-1 (or θ-R) is present. Soon afterward an approximately logarithmic increase in antigen expression begins. This continues until about day 20, when the adult level, which exceeds that in the neonate by more than 100-fold, is reached.

With each new piece of knowledge about the specificity with which Thy-1 or θ-R is expressed, the question "What is the function of this antigen?" is more forcefully implied. The answer in this case, as well as in that of almost all cell surface antigens, is as brief as

the question. "Nobody knows." Looking into the future, two avenues of exploration show particular promise in this connection. First, it may be true that Thy-1 serves as a marker for specific classes of cells in the nervous system, much as it does in the immune system (37,38). If this is so, the specialized functions of the cells which carry the antigen may provide valuable clues as to the physiological role of the antigen itself. Second, the chemical analysis of Thy-1 antigen molecules solubilized from cell membranes is beginning to progress (39-41). The structure of the molecule(s) which carries this specificity may be suggestive as to its function. In any case it seems likely that the Thy-1 antigen system will continue to be of interest in several areas of biological research for some time to come.

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VIII. APPENDIX I

A RAT ANALOG OF THE MOUSE THETA ANTIGEN

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A Rat Analog of the Mouse Theta Antigen

By T. C. Douglas

THE θ antigen of the mouse[1] is of considerable interest as an indicator of cellular differentiation. For example, the rapid appearance of theta in the thymic rudiments of 14- to 18-day embryos corresponds to the development of the lymphoid elements of the thymus.[2] In adult mice, this antigen provides a marker for thymus-derived lymphocytes.[1,3,4] Developmental changes in theta antigen expression have also been observed in the nervous system. During the first weeks following birth, the mouse brain's θ antigen content rises from near zero to a plateau level comparable to that expressed by thymocytes.[1,5]

The two known genetic variants of the θ antigen, θ-AKR and θ-C3H, are products of the alleles Thy-1^a and Thy-1^b, respectively.[6-8] Anti-θ sera are routinely raised by the reciprocal injection of thymocytes from inbred strains homozygous for different Thy-1 alleles, but matched at the H-2 locus.[6] Fuji, Zaleski, and Milgrom[9] have reported that spleen cells from mice immunized against the θ-AKR antigen are capable of forming direct plaques against rat thymocytes as well as against those of mice expressing θ-AKR. The purpose of the experiments reported here was to define the extent to which rat thymocyte antigens are analogous to the θ antigen of mice. Absorption and trypan blue dye exclusion microcytotoxic techniques used in this study have been described elsewhere.[10]

SEROLOGICAL SPECIFICITY

The data shown in Fig. 1 demonstrate the absorption of anti-θ-AKR cytotoxic antibodies by rat thymocytes. When similar absorptions were performed using an anti-θ-C3H serum of comparable titer, no significant suppression of cytotoxicity was observed (Fig. 2). These absorption data were confirmed by the finding that anti-θ-AKR sera were cytotoxic for rat thymocytes in the presence of complement while anti-θ-C3H sera and normal C3H mouse sera were not (Fig. 3). Absorption and cytotoxic tests with thymocytes from rats of eight inbred (ACI, BN, BUF, D, DA, F344, LEW, WF)[11] and two random bred (Wistar, Holtzman) stocks all yielded similar results. The presumed common rat thymic antigen responsible for this specific immunological cross-reactivity is designated θ-R.

TISSUE DISTRIBUTION

In contrast to the results obtained with thymus, homogenates of other rat organs such as liver, lung, testis, cardiac muscle, and skeletal muscle showed little or no absorption of anti-θ-AKR cytotoxic antibodies. The only marked exception to this pattern was brain, which showed a high absorptive capacity.

The θ-R antigen also exhibited a distinctive distribution on cells of the lymphoid organs. The results of cytotoxic and absorption tests using WF rat thymus, spleen, lymph node, and bone marrow cells are shown in Table 1. Although θ-bearing cells in AKR/J mouse spleen and lymph node preparations were killed using the same antiserum, no significant cytoloysis of the corresponding rat cells was observed. The different levels of θ-R antigen expression suggested by these results were confirmed by the greatly diminished absorptive capacities of lymph node and spleen cells relative to thymocytes. Although the general pattern of decreased θ-R expression on rat

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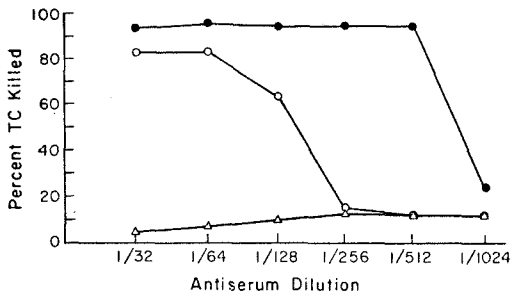

Fig. 1. Killing of AKR/J thymocytes by C3H/HeJ anti-AKR/J serum. Of serum diluted 1/32 in Hanks' balanced salt solution 0.1-ml aliquots were absorbed with the following numbers of thymocytes: none (closed circles), 1×10^7 DA rat (open circles), 1×10^7 AKR/J (triangles). Complement: 1/12 agarose-absorbed rabbit serum.

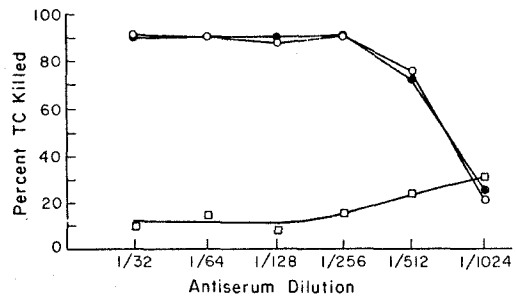

Fig. 2. Killing of C3H/HeJ thymocytes by AKR/J anti-C3H/HeJ serum. Symbols and procedure same as in Fig. 1. Absorption with 1×10^7 C3H/HeJ thymocytes (squares).

lymph node and spleen cells parallels that reported for the θ antigen of mice,[1] an important difference between these systems is apparent. Anti-θ sera have been widely used for the selective elimination of thymus-dependent lymphocytes from heterogenous cell populations.[4] The failure to observe significant killing of rat spleen and lymph node cells by anti-θ-AKR sera suggests that this strategy may not be of use with rat cells.

DEVELOPMENTAL ANTIGEN EXPRESSION IN THE BRAIN

Of anti-θ-AKR serum diluted 1:256 in Hanks' balanced salt solution 0.05-ml aliquots were absorbed with 2150 g pellets from saline homogenates representing known wet weights of brain tissue. The absorptive capacity of a given sample was defined as the reciprocal of the number of milligrams of brain homogenate (G) required to produce a 50% inhibition of cytotoxicity using this standard serum and AKR/J target cells.[12,13] As may be seen in Fig. 4, the θ-absorptive capacity of WF rat brain increases rapidly from birth (day 0) through about day 20 of postnatal life. Complex patterns of cellular proliferation,

Table 1. Expression of θ-R Antigen on WF Rat Cells

Cell Type	Cytotoxic Index (%)	Absorptive Capacity
Thymus	93	100
Spleen	10	<3
Lymph node	10	<4
Bone marrow	8	ND*

*No data.

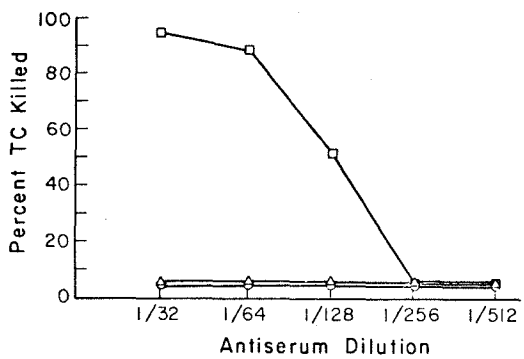

Fig. 3. Complement-mediated cytotoxicity of WF rat thymocytes by anti-θ serum. Sera: C3H/HeJ anti AKR/J (squares), AKR/J anti C3H/HeJ (triangles), C3H/HeJ normal serum (open circles). Complement: ¼ WF rat serum.

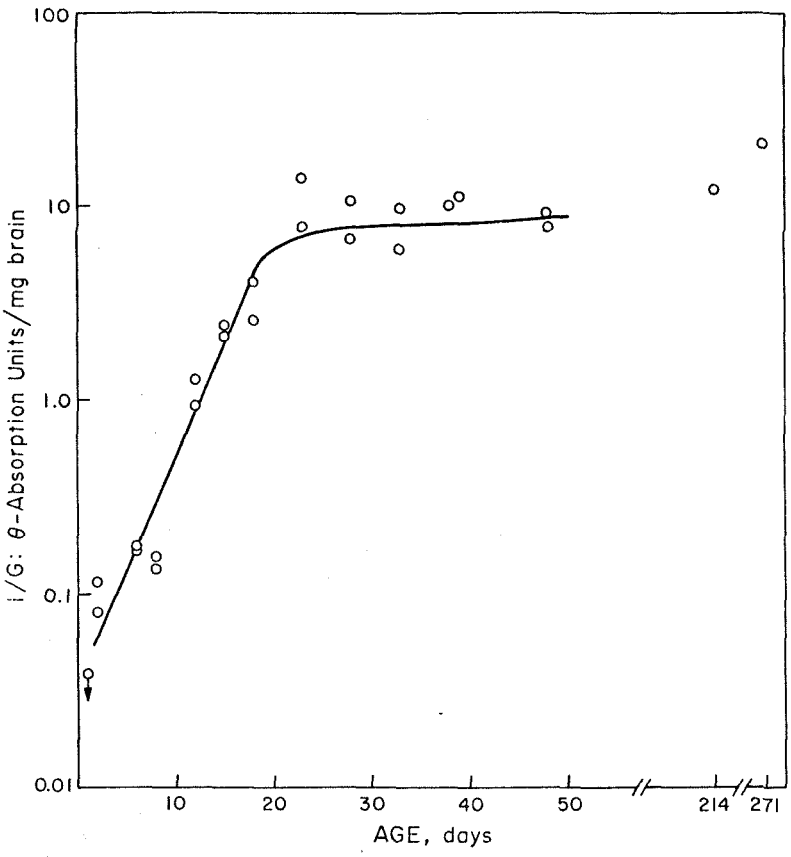

Fig. 4. Developmental appearance of θ-R specificity in WF rat brain. Serum: AKR/Cum anti-AKR/J.[16] At age 1 day, absorptive capacity <0.04.

migration, and differentiation contribute to the process of neurogenesis during this developmental period.[14] Further data relating the expression of θ-R antigen to these changes may prove to be of great interest.

SUMMARY

The occurrence of a rat antigen, designated θ-R, has been reported. This antigen resembles the θ antigen of mice in several respects. Absorption and direct cytotoxic tests showed specific cross-reactivity between θ-R and the θ-AKR, but not the θ-C3H specificity. Except for the brain and thymus, which are rich in θ-R, the other rat organs tested showed little or none of this antigen. The reduced expression of θ-R on spleen, lymph node, and bone marrow cells relative to thymocytes also resembles the distribution of θ. Finally, the developmental kinetics of θ-R in the rat brain closely parallel those reported for θ in the mouse. These characteristics of θ-R clearly place it in the category of the "differentiation antigens,"[15] and point to the potential usefulness of this system for studies of neurophysiological as well as immunological development in the rat.

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