

GENETIC LINKAGE BETWEEN SERUM LEVELS OF THE THIRD
COMPONENT OF COMPLEMENT AND THE *H-2* COMPLEX*

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Fu et al. (1) studied the HLA pedigree of a family with a complement deficiency (C2) and showed evidence for a close linkage between the C2 defect and the HLA loci. Complement (C)-dependent hemolytic activity of mouse serum is also genetically controlled. One locus, Hc (hemolytic complement, with two alleles Hc' and Hc^o) determines the presence or absence of C5 (2-4) and is not linked to the *H-2* major histocompatibility complex (5). In addition, Démant et al. (6) showed that the *S* region of the *H-2* complex, which contains genes controlling serum levels of *Ss* and *Slp* proteins, influences the hemolytic activity of mouse complement. However, they provided no information regarding the specific component involved. We show here that serum levels of C3 in mice are determined by gene(s) linked with the *H-2* complex.

Materials and Methods

Mice. 8-10-wk old, both sexes DBA/2J and AKR/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. 7, 15, 21, and 27-day old AKR/J, DBA/2J, AKD2F₁ (AKR/J × DBA/2J) mice and the progenies of AKD2F₁ × AKR/J and AKD2F₁ × DBA/2J were bred in our own animal facilities.

Bleeding. Mice were bled from the axillary vein and artery in heparin-treated 50 λ capillary tubes. Plasma was stored at -70°C.

Antiserum to Mouse C3. Antiserum was obtained from a rabbit injected with zymosan-mouse C3 in Freund's adjuvant (7). This antiserum did not distinguish between C3, C3b, and C3d, that is, it gave a reaction of identity between the whole molecule and its fragments, both in immunoelectrophoresis and double diffusion in agarose.

Simple Radial Immunodiffusion (8). Plates containing antiserum to mouse C3 were prepared with 1.5% Ionagar in 0.15 M NaCl and 0.01 M Na₂H EDTA pH 7.6. 3 μ l of each mouse plasma were put into wells. Four different dilutions of a pool of normal mouse plasma were included in each plate as standards. After 7 days in a moist box at room temperature, the diameter of the magnified unstained precipitin rings was measured. The area of the rings given by the standards plotted against the respective plasma dilution gave a straight line which was used to estimate the concentration of C3 in the plasma samples included in the same plate. C3 concentrations were expressed as a fraction of the C3 levels of the standard undiluted plasma pool.

Complement-Dependent Release Activity (CRA). We showed previously that immune complexes (bovine serum albumin [BSA]-mouse anti-BSA complement) can be released from the surface of lymphocytes by incubating complex-bearing cells in fresh serum (9). This activity is C3-dependent and proceeds through the properdin pathway. CRA was measured in the plasma of AKR/J and DBA/2J mice as an index of their complement activity. Mouse lymph node lymphocytes coated with [¹²⁵I]BSA-anti-BSA-C were prepared exactly as in (9). All dilutions were made in phosphate-buffered saline (PBS) pH 7.4 (Grand Island Biological Co., Grand Island, N. Y.). A 0.02 ml sample of the

* Supported by National Institutes of Health grants AI 11028 and AI 00392.

‡ Recipient of a fellowship from the Pan American Health Organization.

serum to be assayed was mixed with 0.05 ml of complex-coated cells. The mixtures were incubated at 37°C and 0.010-ml aliquots were taken at 4, 6, and 8 min and mixed with 1 ml of ice-cold PBS. The tubes were centrifuged, decanted, and the supernates and pellets counted for radioactivity. The percent of labeled complexes in the supernate at each time was calculated and plotted vs. time. CRA was estimated by measuring the areas under the release curves between 4 and 8 min by using a computer program. Areas given by controls containing the same cells in PBS were subtracted from experimental values.

Anti-H-2 Antibody-Mediated Cytotoxicity. Mice were killed by ether inhalation, spleens were taken immediately after bleeding, teased into single cell suspensions, and cytotoxic tests were performed (10). Antisera for detecting the private antigenic specificities 31 and 25 of the *H-2^d* and *H-2^k* haplotypes in the *K* region (serum designation D-31 and D-25 respectively), and the private specificity 4 of the *H-2^d* haplotype in the *D* region (serum designation D-4), were obtained from the National Institutes of Health, Bethesda, Md. A two-stage cytotoxic method was used, and after the second stage residual viable cells were estimated by their ability to exclude trypan blue using a 6,300 A Biophysics cytograph (Bio/Physics Systems Inc., Mahopac, N.Y.).

Results

In Fig. 1, C3 and CRA levels in plasma of DBA/2J and AKR/J mice between 7–28 days of age are compared. Both C3 levels and CRA, which is known to be C3-dependent, are significantly higher at all ages in AKR/J mice. C3 levels of 21-day old backcrosses of AKD2F₁ females and AKR/J or DBA/2J males are shown in Fig. 2. The progeny of these backcrosses were typed for *H-2* and classified as *H-2^{dd}*, *H-2^{dk}*, or *H-2^{kk}*. It is clear that C3 levels of AKD2F₁ fall between the values of the parental strains. The differences between means of parentals and that of F₁ mice are significant ($P < 0.02$). Most important, C3 levels of mice of *H-2^{dk}* type are significantly higher than those with *H-2^{dd}* type ($P < 0.02$) and lower than those with *H-2^{kk}* type ($P < 0.02$). Also, C3 levels of

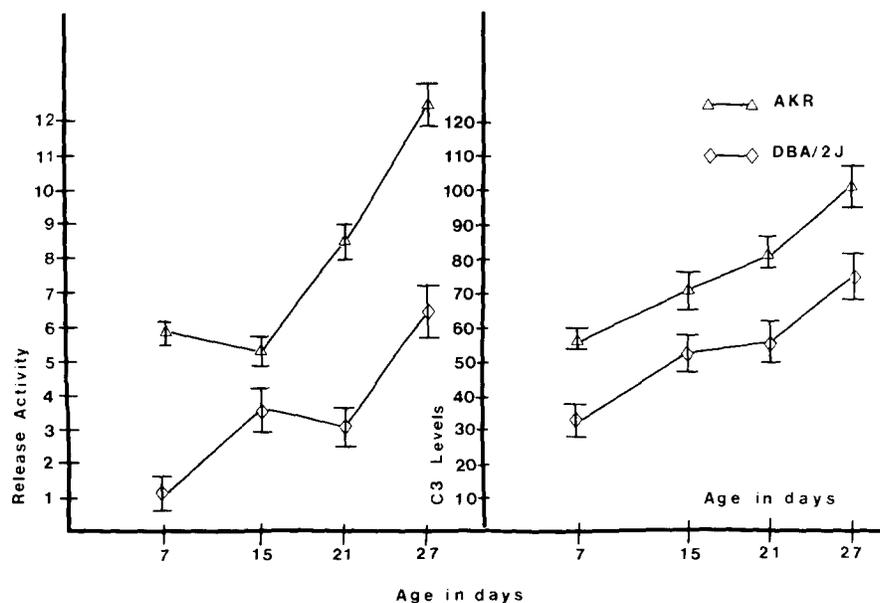


FIG. 1. Complement-dependent release activity and C3 levels of AKR/J and DBA/2J mice. Each point represents the mean \pm SD of one litter of 5–8 mice.

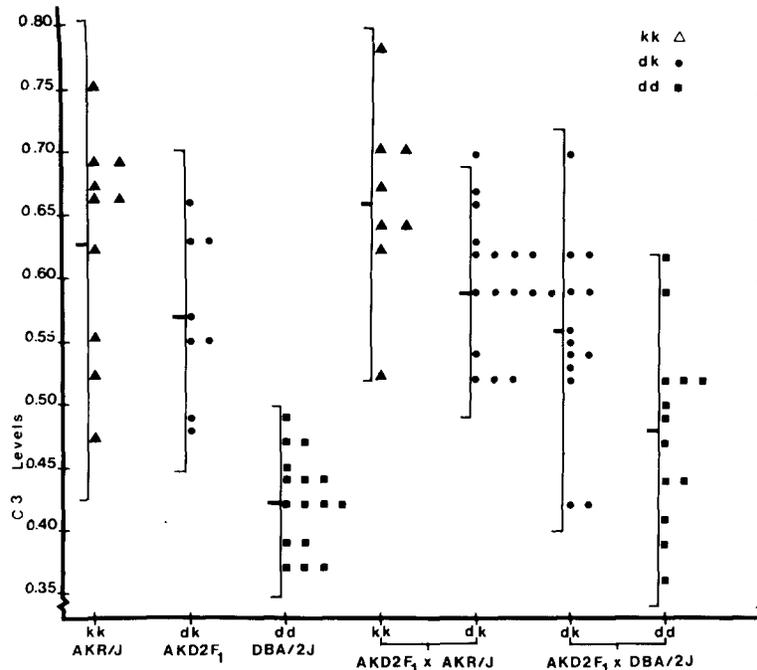


FIG. 2. C3 levels at 21 days of age of AKR/J, DBA/2J, AKD2F₁ mice and the progeny of AKR/J × AKD2F₁ and DBA/2J × AKD2F₁ mice. Animals were typed for *H-2* and classified as dd, dk, and kk. Each point represents the C3 levels of individual animals. Bars represent the mean ± SD.

backcrosses of $H-2^{dk}$, $H-2^{dd}$, and $H-2^{kk}$ types are not significantly different from those of AKD2F₁, DBA/2J, and AKR/J respectively.

Discussion

Although phenotypic variation of serum C3 levels is relatively large, the present results show a clear association between serum C3 levels and *H-2* complex in mice. In general, $H-2^{kk}$ mice have higher C3 levels at 21 days of age than homozygous $H-2^{dd}$ or heterozygous $H-2^{dk}$ mice. Since F₁ mice have levels of C3 intermediate between parentals, codominance is postulated. Additivity of the effects of the loci is also suggested by the observation that the mean of the backcrosses is intermediate between the respective parentals and F₁ hybrids. Although the mean of C3 levels of parental strains differs significantly, the standard deviation of one of them (AKR/J) is relatively large as compared to the other (DBA/2J), and there is some overlap. This suggests that nongenetic factors have substantial influence on individual C3 levels. In these circumstances it is difficult to draw inferences as to the number of independently segregating pairs of alleles involved. However, between 25–50% of the offspring of backcrosses between AKD2F₁ and DBA/2J have C3 levels which are parental-like, and as few as one or two loci may be involved (11).

A gene, or cluster of genes controlling C levels is found within the *S* region of the *H-2* complex (6). It would be of interest to determine whether the locus which influences C3 levels is also located in the *S* region, whose function seems to be

quite distinct from those of other *H-2* regions (12). It may well be that the *S* region controls C activity either directly if it contains structural genes for some key complement components, or indirectly through a multistage regulatory pathway. In any case, the present findings and those of Fu et al. (1) demonstrate that differences in serum levels of two C components are associated with the major histocompatibility complex, and add a new level of complexity to the functions of this relatively small segment of the chromosomes which is primarily concerned with immune functions (12).

Summary

AKR/J (*H-2^{kh}*) mice have higher serum C3 levels than DBA/2J (*H-2^{dd}*). The F₁ hybrids have intermediate levels. Analysis of the progeny of backcrosses at 21 days of age shows that C3 levels in mice of *H-2^{kh}* type are significantly higher than those with *H-2^{dd}* type and lower than those with *H-2^{kh}* type. In addition, mice of *H-2^{kh}*, *H-2^{dk}*, and *H-2^{dd}* types have C3 levels not significantly different from those of AKR/J, AKD2F₁, and DBA/2J respectively. These findings demonstrate linkage between a gene controlling C3 levels and the *H-2* complex.

The authors are grateful to Gertrude Fastaia and Jean Weiner for their skillful technical assistance.

Received for publication 25 November 1974.

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