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Abstracts from the Workshop on Immunoglobulin Variable Region Genetics

Genetics of a New IgV_H (T15 Idiotype) Marker in the Mouse Regulating Natural Antibody to Phosphorylcholine. R. Lieberman, M. Potter, E. B. Mushinski, W. Humphrey, Jr., and S. Rudikoff. National Institute of Arthritis and Infectious Diseases, National Cancer Institute, Bethesda, Maryland 20014.

The idiotype present on the Fab of a phosphorylcholine binding IgA myeloma protein TEPC 15 (T15) of BALB/c origin was found in normal serum of BALB/c mice. Molecules carrying the T15 idiotype in normal serum could be absorbed with sepharose-phosphorylcholine beads and R36A pneumococci. The T15 idiotype is absent in germ-free BALB/c but appears when the mice are conventionalized. A survey of normal sera of inbred strains for the T15 idiotype showed it to be present in BALB/c, 129, C57L, C58, and ST but absent or in low levels in CBA, C3H, C57BL/6, C57BL/Ka, C57BL/10, SJL B10.D2, DBA/2, RIII, A, AL, AKR, NZB, and NH inbred strains of mice. The T15 idiotype is associated with some but not all strains carrying the IgC_H allotypes found in BALB/c. Linkage of genes controlling the T15 idiotype in normal serum to the IgC_H locus of BALB/c was demonstrated in F_2 progeny of a BALB/c and C57BL cross, Bailey's recombinant inbred strains, C × BD, C × BE, C × BG, C × BH, C × BI, C × BJ, C × BK, and CB20 congenic strains. Among these strains, only those possessing the IgC_H locus of BALB/c, including the F_2 progeny consisting of BALB/c homozygotes and (BALB/c × C57BL)F_1 hybrids and C × BG and C × BJ recombinants showed the T15 idiotype.

Species-specific binding-site determinants of mouse antiphosphorylcholine antibodies. J. Latham Claflin and Joseph M. Davie. Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110.

The antiphosphorylcholine (PC) antibody response in inbred mice shows a remarkable restriction in class, affinity, and, in some strains, idiotype. Recent studies of binding characteristics for a number of choline analogues have revealed striking differences in binding specificity among anti-PC antibodies of different rodent species but remarkable uniformity among those in 17 inbred strains of mice. Moreover, anti-PC antibodies of all mice, regardless of *H-2* haplotype or heavy chain allotype, showed the same specificity for the choline compounds as does HOPC 8, an IgA PC-binding myeloma protein. To test for the possibility of species-specific binding-site determinants, antisera to HOPC 8 were prepared in rabbits. Site-specific antibodies were selected by hapten elution from a HOPC 8-immunoadsorbent and found, by hemagglutination-inhibition and solid-phase radioimmunoassay, to be idiotypically specific, reactive with HOPC 8 protein but not isolated H and L chains, and greater than 98% hapten-inhibitable. This idiotypic antiserum bound anti-PC antibodies produced in each of the strains studied in the binding-specificity experiments but not anti-PC antibody produced in four other rodents. Moreover, though these mouse strains differed in genetic background, anti-PC antibodies from each carried site-associated determinants which were idiotypically similar, if not identical, to those on HOPC 8. Thus the idiotypic determinant described here appears directly related to binding-site regions on anti-PC antibodies and these regions are regularly expressed in all mouse strains. (Supported by US Public Health Service, NIH grant AI-11635 and several Tobacco Companies.)

Origin of Immunoglobulin Congenic Strains of Mice. E. B. Mushinski and M. Potter, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland 20014.

The IgC_H chromosomal region from C57BL/Ka was introduced onto the BALB/c inbred background by successively mating backcross progeny carrying the unassigned "2" determinant to

BALB/c. In our laboratory the CB-20 strain was developed from progeny of the twentieth backcross, mated to each other; mice homozygous for the "2" determinant were selected as parents for the CB-20 strain. During the development of the CB-20s, heterozygotes were given to other laboratories. Backcross-13 heterozygotes were given to L. A. Herzenberg, Stanford University, from which the BAB-14 homozygous strain was developed at the fourteenth backcross. Backcross-13 mice were given to M. Bosma and E. Weiler at The Institute for Cancer Research, Fox Chase. Our CB-20 colony has now been inbred for five generations. The CAL-9 (derived from the ninth backcross) and CAL-20 (derived from the twentieth backcross) strains also on the BALB/c background carry the AL/N IgC_H region. These were selected by antisera specific for the G^4H^4 determinants. Plasmacytomas are readily induced in these new strains, CAL-20 and CB-20, and these mice are a source of myeloma proteins carrying different allotypes. While the selection process has been made for IgC_H markers, the newly introduced chromosomal region contains a larger segment of DNA, including *Ig*V genes (presumably IgV_H). Our CAL-9 colony has been inbred nine generations and our CAL-20 colony five generations.

Structural and Idiotypic Characteristics of an IgA Phosphorylcholine-Binding Myeloma Protein of CB-20 Origin. J. L. Claflin. S. Rudikoff, M. Potter and J. M. Davie. Washington University School of Medicine, St. Louis, Mo. and National Cancer Institute, Bethesda, Maryland.

The CB-20 strain of mice is congenic to BALB/c but differs from it by carrying the C57BL/Ka IgC_H region and a contiguous segment of DNA. This congenic strain was developed by first crossing BALB/c with C57BL/Ka and then backcrossing the F_1 to BALB/c; backcross progeny producing immunoglobulin with C57BL/Ka allotypes were backcrossed to BALB/c for 20 consecutive generations. The mice (CB-20) were then made homozygous for C57BL/Ka IgC_H genes after 20 generations and bred by brother-sister matings.

The plasmacytoma CB-20 ABPC-2 was induced in a CB-20 mouse that had been injected intraperitoneally with 0.5 ml pristane and 40 days later with Abelson Virus (MLV-A). This plasmacytoma produced an IgA myeloma protein that precipitated the phosphorylcholine (PC) containing antigen LB-4. The CB-20 ABPC-2 protein was purified by affinity chromatography on sepharose-phosphorylcholine columns.

Sequence analysis of the L chain through the first hypervariable region has shown this chain to be identical to that of HOPC 8, a phosphorylcholine binding myeloma protein of BALB/c origin. The heavy chain of ABPC-2 is also identical to that of HOPC 8 through the first hypervariable region, with the exception of substitutions at positions 14 and 16. Moreover, as measured by inhibition of precipitation between the myeloma proteins and pneumococcal C polysaccharide using PC, glycerophosphorylcholine, acetylcholine, and choline, both proteins have the same specificity and this specificity is clearly distinguishable from that of other BALB/c PC-binding myeloma proteins that have known sequence differences from HOPC 8.

Similarities in the combining site of HOPC 8 and ABPC-2 were further assessed with a rabbit antiserum, which was idiotypically specific, exclusively, for binding site determinants of HOPC 8. This antiserum reacts with HOPC 8 and TEPC 15 (another BALB/c anti-PC myeloma, which is idiotypically identical to HOPC 8), but not with PC-binding myeloma proteins of a different binding specificity. The antiserum also binds ABPC-2, and the idiotypic determinant on ABPC-2 is serologically identical to that on HOPC 8, indicating that the combining sites of these two proteins are antigenically similar if not identical. Idiotypic antisera to HOPC 8, prepared in A/J mice, which reacts with nonbinding site determinants, does not bind ABPC-2. Thus the ABPC-2 protein, though carrying the heavy chain allotype of C57BL/Ka shows structural similarity and, by the criteria of antibody specificity and a site-associated idiotype, shows combining site identity with HOPC-8. This suggests that BALB/c and C57BL/Ka mice may have the potential for producing antibody with the same combining site for PC. (Supported in part by the following companies: Brown and Williamson Tobacco Corporation; Larus and Brother Company, Inc.; Liggett and Myers Incorporated; Lorillard, a Division of Loews Theatres, Incorporated; Philip Morris, Incorporated; R. J. Reynolds Tobacco Company; United States Tobacco Company; and Tobacco Associates, Inc.)

Linkage Analysis of the Dextran Response Gene. Roy Riblet, Melvin Cohn and Martin Weigert. The Salk Institute, La Jolla, California and The Institute for Cancer Research, Philadelphia, Pa.

The study of the immune response of mice to α-1,3 linked dextran stems from the characterization of two BALB/c myeloma antibodies with antidextran specificity. These prototype antibodies are J558, an IgAλ protein, and MOPC - 104E, an IgMλ. These two myeloma proteins have identical light chain sequences and are similar but not identical in their heavy chain V region sequences, their combining site specificities and idiotypes (B. Blomberg, *et al.*: *3rd Int. Convoc.*

Immunol., pp. 285-293, Karger, Basel, 1973). The appearance of this related pair and of similar sets with other antibody specificities suggested that these proteins might be representative of the normal BALB/c antibody response to their respective antigens. This in fact is the case at least for the responses to α-1,3 dextrans and phosphoryl choline, and other antigens are under study. When a BALB/c mouse is immunized with an α-1,3 linked dextran, the resulting antibodies resemble the myeloma prototypes in containing λ chains, which in the mouse occur with low frequency, and in having a cross-reactive idiotype. Most mouse strains with heavy chain allotypes like those of BALB/c respond in the same manner, while nearly all allotypically dissimilar strains, such as C57BL/6 or A/He, respond with a lower titer of antibody. This antibody contains κ rather than λ light chains and does not react with the anti J558 anti-idiotype serum. This response difference was shown in a survey of many inbred, congenic and recombinant inbred strains to be due to a single locus linked to the heavy chain allotype loci (B. Blomberg, *et al.*: *Science 177*: 178, 1972.). This gene we presume is a structural gene coding for the heavy chain variable region of the antidextran antibody; we shall refer to it as V_H-*dex*. In BALB/c mice the heavy chain product of this gene (V_H-*dex*$^+$ allele) combines with λ light chain to yield an antidextran antibody resembling the myeloma prototypes. C57BL/6 mice presumably have either a deletion of this gene or an altered allele (V_H-*dex*$^-$) whose product cannot assemble with λ chain to give an antidextran specificity.

We have extended the genetic analysis of this response difference with three backcrosses to determine the recombination distance between the response gene, V_H-*dex*, and the IgG_{2a} allotype locus, *Ig-1*. These crosses are all of the form (R × NR)F_1 × NR where R is a BALB-like strain giving a high-titer J558-idiotype positive response to dextran immunization, while NR is an A-like strain giving a low-titer idiotype negative response. The BALB response pattern is dominant in the F_1 and segregates in the backcross as a single Mendelian factor closely linked to allotype. In a (129 × A)F_1 × A backcross we found no recombinants among 179 progeny, and in a (BAB/14 × A)F_1 × A cross there were no recombinants among 140 progeny. A third backcross was set up between congenic strains on a BALB/c background to eliminate all genetic differences between the strains except those at and near the heavy chain allotype loci. In this cross (BAB/14 × C.AL_9)F_1 × C.AL_9, of 102 progeny one had the BAB/14 allotype but gave a very low, idiotype negative response characteristic of the C.AL_9 parent. This mouse was test mated to C.AL_9 and its recombinant genotype verified. The progeny are being inbred to yield a strain congenic to BALB/c and having a recombinant heavy chain chromosome.

Taylor and Bailey's recombinant inbred strains and Potter and Herzenberg's allotype congenic strains give additional data on recombination in this genetic region. In the derivation of the C.B_{20}, C.AL_9, BAB/14, CXB and AKXL strains there occurred the equivalent of 109 opportunities for recombination between V_H-*dex* and *Ig-1* and one crossover event happened. The BAB/14 strain was derived from C57BL/Ka (*Ig-1*^{b}, V_H-*dex*$^-$) and BALB/c (*Ig-1*^{a}, V_H-*dex*$^+$) by breeding the *b* allotype onto the BALB background to make a congenic strain. Apparently, in the last stages of this process a crossover occurred between *Ig-1* and V_H-*dex*, resulting in BAB/14 which is *Ig-1*^{b} but V_H-*dex*$^+$. The interpretation of BAB/14 as a recombinant rather than as a mutation or other event is reinforced by its reversal in the creation of the recombinant obtained from the (BAB/14 × C.AL_9) F_1 × C.AL_9 cross. That is, the *Ig-1*^{b} gene from C57BL/Ka was first recombined with the V_H-*dex*$^+$ gene from BALB/c in the creation of BAB/14 and then recombined with V_H-*dex*$^-$ gene from C.AL_9 in the backcross experiment. When all the data are pooled, the resulting recombination frequency or map distance between *Ig-1* and V_H-*dex* is 2 of 530 or 0.4%. (Supported by a Leukemia Society of America Special Fellowship to R.R. and NIH grants GM-20964 to M.W. and A-105875 NIH and CA-05213 (Training - NIH) to Dr. Melvin Cohn.)

Separate V_H gene loci that specify the idiotypes of antibodies to phenylarsonate and to Group A streptococcal carbohydrate in mice. K. Eichmann and A. Nisonoff. Institute for Genetics, University of Cologne, Cologne, and Department of Biological Chemistry, University of Illinois Medical Center, Chicago, Illinois 60680.

In strain A mice a fortunate situation has developed in that two distinct idiotypes have been described, both of which are linked to the strain A C_H allotype *Ig-1*^{e}. Idiotype ARS is associated with antibodies to the *p*-azophenylarsonate hapten produced in all strain A mice, whereas idiotype A5A is associated with antibodies to Group A streptococcal carbohydrate (A-CHO) produced in 94% of all strain A mice. Neither idiotype is expressed in antibodies of BALB/c mice (C_H allotype *Ig-1*^{a}.) As both idiotypes have been shown to be linked to the same C_H allele, a study was performed to investigate the linkage between the three markers *Ig-1*^{e}, A5A and ARS in (A × BALB/c) F_1 × BALB/c backcross (ABB) mice. The absence of idiotypic cross-reactivity between antibodies

to phenylarsonate and antibodies to A-CHO enabled us to successively immunize mice with both antigens and to determine both idiotypes in each individual mouse. Among 19 ABB mice, 8 were *Ig-1^a/Ig-1^a* homozygous and were negative for both idiotypes and 11 were *Ig-1^a/Ig-1^e* heterozygous and were positive for both idiotypes. This confirms the linkage between the three markers within the strain A H-chain linkage group. A genetic dissociation between idiotypes A5A and ARS was observed in the progeny of a phenotypically recombinant ABB mouse (BB ♂ 7), which was previously reported to possess the A5A idiotype in association with an *Ig-1^a/Ig-1^a* homozygous genotype. Mating this mouse to BALB/c resulted in 13 *Ig-1^a/Ig-1^a* homozygous progeny, 6 of which were A5A positive but all of which were negative for the ARS idiotype. This suggests a crossover between the loci encoding idiotypes A5A and ARS, respectively, and permits a mapping of the *ARS+* locus between *A5A+* and *Ig-1^e*. The case for a separate V_H gene locus for each of these idiotypes is further supported by the observation that different inbred strains may express either both idiotypes (strains A and A/He), or ARS alone (strains AL/N and CAL-20), or A5A alone (strain RF). (Supported by Sonderforschungsbereich 74 and by NIH Grant Al-10220.)

A New V-Region Genetic Marker in Strain A Mice. A. Nisonoff. Department of Biochemistry, University of Illinois, Chicago, Illinois 60680.

Anti-idiotypic antiserum was prepared in a rabbit against the antikeyhole limpet hemocyanin (anti-KLH) antibodies of an individual A/J mouse. For immunization an IgG fraction of the ascites fluid of the KLH-immune A/J mouse was used. The resulting rabbit antiserum was absorbed with a crude globulin fraction of normal A/J serum and with whole A/J serum. Tests for anti-idiotypic antibody were carried out by radioimmunoassay using ^{125}I-labeled specifically purified anti-KLH antibody of the donor antibody as ligand. Binding by the absorbed rabbit antibody was inhibited by hyperimmune serum of the donor mouse. An amount of serum containing 0.05 μg of anti-KLH antibody caused 85% inhibition of binding of 0.01 μg of labeled ligand. Two milligrams of nonspecific A/J IgG or 1 milligram of A/J antiovalbumin were noninhibitory. Removal of anti-KLH antibody from the donor serum with an immunoadsorbent removed essentially all inhibitory capacity. Each of 33 antisera from individual KLH-immune A/J mice caused inhibition of binding. The amount of antibody required for 50% inhibition ranged from 0.03 to 1.5 μg, with a median value of 0.30 μg. Sera from 5 A/WYSn mice immunized against KLH were similarly inhibitory; the median value for the amount of anti-KLH antibody needed for 50% inhibition was 0.32 μg. For 8 A.BY/Sn congenic mice, with the *histocompatibility-2* haplotype of the BY strain on a strain A background, the median value was 0.40 μg; all 8 sera were inhibitory. Each of 7 antisera from CAF_1/J hybrid mice similarly caused at least 50% inhibition, with a median value of 0.68 μg antibody required. Five to 7 mice of each of the strains listed below were immunized with KLH and the antisera tested for inhibition of binding in the same assay system; the volume of serum tested contained 25 μg of anti-KLH antibody. All sera caused less than 30% inhibition; the median value for individual strains ranged from 0% to 15% inhibition. Strains tested: AKR, SWR/J, RF/J, NZB, BALB/c, C57BL/J, CBA, and B10.A (having the *H-2* haplotype of strain A on a C57BL background). Although data from additional immunized mice of the apparently negative strains are required, the results so far strongly suggest that A/J anti-KLH idiotype will prove a useful genetic marker for the V-region (A. Tung and A. Nisonoff: unpublished data).

Suppression of Immunologic Memory for a Cross-Reactive Idiotype. A. Nisonoff. Department of Biochemistry, University of Illinois, Chicago, Illinois 60680.

In another investigation it was found possible to suppress immunologic memory for the idiotype of anti-*p*-azophenyl-arsonate antibody in A/J mice (L. L. Pawlak, *et al.*: *Eur. J. Immunol.*, in press). Previous investigations had shown that the appearance of the characteristic idiotype can be suppressed for prolonged periods of time by administration of rabbit anti-idiotype antibodies to adult A/J mice prior to immunization with KLH-*p*-azophenylarsonate (KLH-Ar). In the present study, mice were primed with the antigen in saline (day −28). On days −14 and −11, two-tenths and three-tenths ml of anti-idiotypic antiserum was administered. A secondary challenge with antigen was given on day 0 and mice were bled 7 days later. They were inoculated again, i.p., with 0.5 mg KLH-Ar in complete Freund's adjuvant on days +14 and +28 and bled again on day +35. Marked suppression of idiotype was seen at day +7; twenty-nine of 30 control mice given normal rabbit serum at days −14 and −11 produced the cross-reactive idiotype, whereas twenty-eight of 30 mice given a total of 0.6 ml of anti-idiotypic antiserum failed to produce significant quantities of the idiotype. (Control experiments in which only one injection of antigen was given, showed that a secondary response was produced as a result of a second challenge. At day +35, twenty-one of 26 remaining mice that had received 0.6 ml of anti-idiotype antiserum were still suppressed; the sera of

24 mice that had received normal rabbit serum all contained the cross-reactive idiotype. Suppression of idiotype is probably brought about by the action of anti-idiotypic antibodies on B-cell receptors.

Inheritance of Fine-Specificity in Mouse Anti-hapten Antibodies. T. Imanishi and O. Mäkelä. Department of Serology and Bacteriology, Helsinki University, 00290 Helsinki 29, Finland.

Fifteen mouse strains studied produced approximately equal amounts of antibody to NP (4-hydroxy-5-nitrophenylacetyl) but the specificity of anti-NP was different in different strains. CBA and C57BL/6 are examples of extreme strains: the C57 antibody is strongly heteroclitic (higher affinity for NIP and NNP than for NP) while CBA anti-NP is "normal." These strain differences could be demonstrated by three methods: inhibition of PFC, inhibition of Farr binding, and inhibition of NP-T4 phage inactivation. Strain differences between these antibodies have also been revealed by isoelectric focusing. The strain characteristics were demonstrable in anti-NP raised by two different carrier proteins and also in nonimmune sera (natural antibodies). They were demonstrable in IgG, IgA and IgM classes. To study the inheritance of these characteristics, approximately 40 (CBA × C57BL/6)F_1 mice were immunized with NP-CG. Most of these had anti-NP similar to the C57BL parent. They were backcrossed to the recessive CBA parent, and approximately 60 backcross animals were similarly tested. Those heterozygous for the C57BL H chain allotype were similar to C57BL and F_1 while mice homozygous for the CBA allotype were indistinguishable from the CBA. Such single factor inheritance was true only for the primary response. Mice homozygous for the CBA allotype were still like CBA in the secondary response but allotype-heterozygous backcross mice had a distribution different from that of F_1 mice in the secondary response. Fine specificity of anti-NP was thus exclusively controlled by one H-chain linked gene in the primary response and in natural antibodies, but in the secondary response an additional gene(s) seemed to be involved.

Anti-NP of 15 inbred strains was studied. Those belonging to the IgC_H allotype group a^1 (CBA, BALB/c, C3H, MA/J, C57L, ST/bJ and IAH) or a^4 (A/J) had anti-NP similar to that of CBA. Strains of group a^2 (C57BL/6, C57BL/10, LP/J) had anti-NP similar to anti-NP of C57BL/6. SJL/J also belonging to this group had a different anti-NP. Members of group a^3 (RF/J and DBA/2) may have intermediary anti-NP. A similar polymorphism could be demonstrated in anti-NBrP (4-hydroxy-3-bromo-5-nitrophenylacetyl). In this case even the secondary response was controlled exclusively by (1) gene(s) linked to the H-chain allotypes. Preliminary evidence suggests that the two polymorphisms are controlled by different V genes.

Inheritance of Genes Coding for an Antibody Showing Characteristic Isoelectric Spectrum. A. J. McMichael and A. R. Williamson. National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

Antibodies raised against NP in C57BL/6 and CBA mice were examined by isoelectric focusing with development by ^{131}I-NIP-aminocaproate. The sera used were supplied by Imanishi and Mäkelä and were the same as those in which they found the C57BL/6 heteroclitic antibodies. The primary IgG NIP-binding antibodies made by either CBA or C57BL/6 mice showed restricted isoelectric spectra corresponding to a small number of monoclonal spectra in each serum. This simplicity made it possible to see that almost all C57BL/6 sera have a common monoclonal isoelectric spectrum (NP-clone). This spectrum correlated with the heteroclitic antibody demonstrated by Imanishi and Mäkelä. By contrast there is no repetition of the clonal spectra between different CBA sera.

The sera from (CBA × C57BL/6)F_1 hybrids and backcrosses used to follow inheritance of the heteroclitic antibody were screened by isoelectric focusing. Most F_1-mice showed the characteristic NP-clonal spectrum and there was complete correlation with the presence of heteroclitic cross specificity. The expression of this clone is clearly dominant. In the backcross F_1 × CBA, the NP-clone was expressed in heterozygotes (*Ig-1^b/Ig-1^a*) at the same high frequency as in F_1 mice, The NP-clone was not seen in any *Ig-1^a/Ig-1^a* serum. In the heterozygote backcross sera there was a good but not an exact correlation between heteroclitic properties of the sera and the presence of the NP-clone.

The inheritance of the NP-clonal antibody as followed by its isoelectric spectrum (or as followed by its heteroclitic properties) is apparently controlled by a single gene linked to the H-chain constant region. The inheritance of the fine-specificity in an IgG antibody indistinguishable by isoelectric focusing raises the question of the origin of the L-chain contributing to the NP-clone. In the F_1 mouse there could be preferential selection of the C57BL/6 L-chain by the characteristic H-chain. However, in the backcross the incidence of the NP-clone is higher than

expected for coinheritance of unlinked genes. This imples the presence in the CBA repertoire of L-chain genes of a gene controlling the structure of a chain which can complement the C57BL/6 H-chain to give the NP-clonal spectrum.

Genetic Control of Levels of Natural Antibodies to Chicken Erythrocytes in Mouse Serum: Association with the *Ig-1* locus. B. A. Taylor, Marianna Cherry, D. W. Bailey, and L. S. Shapiro. The Jackson Laboratory, Bar Harbor, Maine 04609.

Levels of chicken hemagglutinins in unimmunized mouse serum are strikingly strain dependent. Two sets of recombinant inbred (RI) lines derived by continuous brother-sister mating from the F_2 generation of strain crosses, were used to determine the mode of inheritance of this trait. The first set, designated AKXL, consists of twenty partially inbred lines derived from the cross of strains AKR/J and C57L/J. A statistical analysis of the data suggests that agglutinin levels are controlled by two loci acting additively. The AKR/J strain carries the alleles for low agglutinin levels, while the C57L/J strain carries the alleles for high agglutinin levels. F_1 hybrids are intermediate on a log scale but closer to the high parent. A strong association between mean chick hemagglutinin titers and the immunoglobulin allotype locus (*Ig-1*) is evident among the AKXL lines. Two-thirds of the genetic variance in agglutinin titers is associated with the *Ig-1* locus. There was no association with the *H-2* complex. The 7 CXB RI strains derived from the cross of BALB/cBy and C57BL/6By have also been characterized for chick hemagglutinins. Six strains are low like the C57BL/6By progenitor strain, while one strain CXBG, is consistently high like the BALB/cBy strain. Chick hemagglutinin levels segregated with the *Ig-1* locus in each case except strain CXBJ, which carries the BALB/cBy allele at *Ig-1* but resembles C57BL/6By with respect to mean agglutinin titer.

Agglutinin activity is eliminated by treatment of the serum with mercaptoethanol, suggesting that the responsible antibodies belong to the IgM class. Packed red blood cells and a homogenate of liver and spleen from AKR/J mice failed to absorb the hemagglutinins from C57L/J serum. The genes determining agglutinin levels may control the immune response to some ubiquitous antigen(s) such that the antibodies formed cross react with one or more antigens present on the cell surface of chicken erythrocytes. (Supported by Research Contract NO1 CP 33255 within the Special Virus-Cancer Program of the National Cancer Institute, grant CA-12663 from the National Cancer Institute, and grant GW 7506 from the National Science Foundation.)

The Genetic Control of the Humoral Immune Response to H-2D Alloantigenic Specificities. Ian F. C. McKenzie. Transplantation Unit and the General Surgical Services, Massachusetts General Hospital and the Department of Surgery, Harvard Medical School, Boston, Massachusetts 02114.

The humoral antibody response to H-2 alloantigenic specificities appears to be under the control of at least two genes. One is *H-2* linked (J. H. Stimpfling and T. Durham: *J. Immunol. 108*:947, 1972; F. Lilly *et al.*: *Transplant Proc. 5*:193, 1973) and has been localized to the *I* region of the *H-2* complex. The other gene is not *H-2* linked but may be linked to the *Ig* loci. The evidence is as follows: a) B10.AKM ($H\text{-}2^m$) can reject B10.BR ($H\text{-}2^k$) skin grafts but will not make an anti-H-2.32 antibody. A similar combination (AKR.M anti-AKR) with the same H-2 differences but a different genetic background can reject skin grafts and make antibody. Therefore AKR.M is a "responder" and B10.AKM is a "nonresponder." b) "Responsiveness" is a dominant trait, as the (B10.AKM × AKR.M)F_1 hybrid makes antibody. c) A single gene appears to be involved, as the (B10.AKM × AKR.M)F_1 × B10.AKM cross (which is not a segregating generation for *H-2*) gives a 1:1 ratio of responders:nonresponders. d) As BALB/c is a responder and C57BL/6 a nonresponder, the Bailey recombinant inbred lines can be used as a preliminary test for linkage. Crosses of the type (CXB × B10.AKM)F_1 were immunized with B10.BR tissues. Only CXBG and CXBJ were responders, whereas CXBD, CXBE, CXBH, CXBI, and CXBK were nonresponders or gave a much lower titer of antibody than the responders. This pattern of reaction is identical for that given from the allotyping data on these strains. e) Again, BALB/c is a responder but BALB/c-$Ig\text{-}1^b$ is not. In strain CB-20, Dr. M. Potter has put the C57BL/6 *Ig* genes on to the BALB/c background and this has converted responsiveness to H-2.32 to nonresponsiveness. Formal linkage studies are now in progress but it seems likely that the *Ir* gene affecting the antibody response in these studies is *Ig* linked. Other *H-2D* specificities (H-2.2, 4, 30) are subject to the same genetic control. The two genes, which effect the humoral response to H-2D alloantigens, provide an example of genetic control in two distjnctive cell populations: a) the *H-2* linked *Ir*-gene, which controls responses in T cells, and b) the *Ig* linked *Ir*-gene, which presumably controls a B-cell response.

Identification and Genetics of a Cross-reacting Marker Associated with IgG_{2a}, Myeloma Proteins and Normal Serum of BALB/c Mice. Rose Lieberman, Michael Potter, and William Humphrey, Jr. National Institutes of Health, Bethesda, Maryland 20014.

Antisera prepared in SJL/J mice separately to two different BALB/c γ2aλ myeloma proteins HOPC1 (NIH origin) and Y5444 (Salk Institute origin) identified allotypic specificities (γ2a-Fc), idiotypic specificities (unique for each immunizing protein) and cross-reacting idiotypic specificities (found on both γ2a myeloma proteins). The antisera made specific for the cross-reacting idiotype by absorption with a γ2a, κ myeloma protein were then reacted with the nonimmunizing cross-reacting γ2aλ myeloma protein coupled to sheep red blood cells (SRBC) in the hemagglutination-inhibition system, using normal BALB/c serum as the inhibitor. Normal BALB/c serum had an inhibitory titer equivalent to that of 38 to 75 μg/ml of HOPC1 or Y5444. A survey for the HOPC1-Y5444 idiotype in normal serum from other inbred and hybrid strains was made. Three phenotypes, high (log 2 mean titer 6-8), intermediate (log 2 mean titer 1-3), and low (log 2 mean titer <1) were found. In the high phenotype were BALB/c and the Bailey RI strains CxBG and CxBJ; in the intermediate group were CBA, RF, DBA/1, I, A and AKR, and in the low group were BRSUNT, C57BL/10, C57BL/6, LP, RIII, NZB, CE, BAB14, CB20, CXBD, CXBE, CXBH, CXBI, and CXBK. The cross-reacting HOPC1-Y5444 idiotype was associated with some but not all strains in the three IgC_H (allotype) groups a^1, a^3 and a^4, indicating an unusual strain distribution of this phenotype. The presence of the high HOPC1-Y5444 idiotype in the Bailey CXBG and CXBJ strains, which are of a^1 allotype and the low level in the congenic CB20 and BAB14, which are of a^2 allotype and in all of the Bailey RI strains of a^2 allotype strongly suggest linkage of the genes controlling the HOPC1-Y5444 idiotype to the IgC_H gene complex. Accordingly, the sera of (BALB/c × C57BL/6)F_1 × C57BL/6 progeny were examined for HOPC1-Y5444 idiotype. Thirty-nine homozygous a^2a^2 (allotype of C57BL/6) mice lacked the idiotype while 39 a^1a^2 progeny (heterozygous for BALB/c and C57BL/6 allotype) had intermediate levels of idiotype. This result further supports the linkage of genes controlling the HOPC1-Y5444 idiotype and IgC_H allotypic specificities, although it suggests incomplete penetrance of the trait in heterozygotes. Anti-HOPC1-Y5444 idiotype antisera were partially inhibited by other λ-type myeloma proteins, including a I $\mu\lambda_1$ (MOPC 104E), an $\alpha\lambda_1$ (J558) and a λ_1 Bence-Jones protein (RPC-20) but not by an $\alpha\lambda_2$ (MOPC 315). Further investigation of the λ-related specificity is underway.

Genetic Correlation of a Mouse Light Chain V-Region Marker with a Thymocyte Surface Antigen. Paul D. Gottlieb. Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Edelman and Gottlieb have described a genetic marker in the V-region of immunoglobulin light chains from inbred strains of mice. This genetic marker, called the I_B-peptide marker, is detected by peptide mapping as a set of hexapeptides derived from the first cysteinyl residue of the V-region (Cys^{23}). This set of peptides is expressed in the four inbred strains AKR/J, C58/J, RF/J, and PL/J and is not expressed in most other strains. In I_B-positive strains, the marker represents approximately 5% of the light chains. F_1 hybrids between I_B-positive and negative strains express about half as much I_B-peptide marker as the I_B-positive parent, and expression of the marker segregates in the F_2 generation. The inbred and congenic strain distributions of various genetic markers were compared with that of the I_B-peptide marker in an attempt to identify the locus or linkage group responsible for its expression. The I_B-peptide marker was found to be linked to the genetic locus causing expression of the Ly-3 thymocyte cell surface antigen in linkage group XI on chromosome 6 of the mouse. If the I_B-peptide marker represents a true difference between inbred strains in structural genes coding for light chain V-regions, then such structural genes may be linked to the *Ly-3* locus. Alternatively, a genetic locus linked to *Ly-3* may control the expression of light chain V-region genes encoded elsewhere, causing expression of the I_B-peptide marker in some inbred strains but not in others.

H-2 *Linked* Ir *Genes Controlling Immune Responses to BALB/c IgG (γ2a)κ and IgG (γ2a)λ Type Myeloma Proteins.* Rose Lieberman, Michael Potter, William E. Paul, and William Humphrey, Jr., National Institutes of Health, Bethesda, Maryland 20014.

It has been shown in the mouse that the immune response to a BALB/c γ2aκ type myeloma protein (MOPC173) is controlled by the immune response gene *Ir-IgG*. In mice with IgC_H allotypes different from BALB/c, the *Ir-IgG* gene was found to be linked to *H-2* haplotypes *b, bc, p, r, s,* and *v* and not to *a, d, k,* and *q*. The response of mice of the $H\text{-}2^b$ haplotype and lack of response of $H\text{-}2^a$ mice permitted us the opportunity to map the *Ir-IgG* gene in the *H-2* complex by investigating the immune response of the $H\text{-}2^a/H\text{-}2^b$ recombinants that were available. These recombinants included B10.A (1R), B10.A(2R), B10.A(3R), B10.A(4R), and B10.A(5R). It was found that 3R, 5R, and 4R gave a high response while 1R and 2R did not. On the basis of prior mapping of other *Ir* genes, e.g., *Ir-1* and *Ir-IgA* it was shown that *Ir-IgG* was located between the *Ir-IgA, Ir-1*

complex and *Ss-Slp*. In that study we postulated that the allotype present on the C_H (Fc) region was recognized by specific thymus-derived (T) lymphocytes and that an immune response to the idiotype present on the Fab could only be elicited when the allotypes of the immunized mouse differed from BALB/c. On these premises, irrespective of the differences in the V_H regions (idiotypes), the same *H-2* linked *Ir* gene should be involved in responses to all myeloma proteins of the same class. To test this view, the immune response to six different BALB/c γ2a myeloma proteins was investigated in inbred *H-2* congenic and recombinant strains. Four of the γ2a myeloma proteins are of the kappa type (MOPC173, LPC1, UPC10 and RPC5); two are of the lambda type (HOPC1 and Y5444). All six myeloma proteins have the same C_H region allotypic determinants $G^{1,6,7,8}$. Each of the four kappa type proteins has its own unique idiotypic specificity which does not cross-react with that of any of the others. The two lambda types in addition to possessing unique idiotypic specificities also have a cross-reacting idiotypic determinant. Among the inbred strains of various *H-2* types and Ig allotypes the immune response for the six γ2a myeloma proteins was remarkably consistent. No antibody response was observed in mice with the same allotype as BALB/c, including one strain of the $H\text{-}2^b$ haplotype. In strains of mice with allotypes different from BALB/c, a high immune response (mean log 2 HI titer 8 - >12) was found in *H-2* types *b, r, s,* and *v* for 2 or 3 of the k types and for 1 or both of the lambda types. A low or absent immune response was found in *H-2* haplotypes *a, d, k,* and *q* for at least two of the four kappa types and for one of the lambda types. One notable exception was the high response of RF ($H\text{-}2^k$) to UPC10, a kappa type. The results in the B10 congenic strains and $H\text{-}2^a/H\text{-}2^b$ recombinant strains were similar for all the kappa types but indicated differences between the immune response to kappa- and lambda-type proteins. Among the kappa types a high response was found in $H\text{-}2^b$ (B10) and a low response in $H\text{-}2^a$ (B10.A). Among the $H\text{-}2^a/H\text{-}2^b$ recombinants, 1R and 2R gave a low response, while 4R and 5R gave a high response. The response of 4R, while present, was always less than that of 5R or B10. The data indicate that regardless of the V_H region of the myeloma protein, the same *Ir* gene (*Ir-IgG*) is employed in the immune response to κ type γ2a BALB/c myeloma proteins.

The findings with the two λ type γ2a proteins was very different. First, the results on repeated experiments revealed a marked variability among individuals within a strain, a finding not observed with the κ type proteins. For Y5444, all B10 mice gave a high response, whereas for HOPC1 only 3 of 22 gave a high response in three separate experiments using different preparations of HOPC1. Both the Y5444 and HOPC1 gave low responses in the recombinants 2R, 4R, and 5R. However, there were some inconsistencies in these latter groups. Among the 4R, two mice out of 18 gave a good response and among the 5R, two out of 23 gave a good response to HOPC1. That the immune response to HOPC1 was linked to *H-2* was shown by the B10.M strain, all of which individuals gave a very high immune response (log 2 H-1 mean titer >12).

The data support the concept that the genetic control of responsiveness to $\gamma G(\gamma 2a)\kappa$ myeloma proteins involves recognition of constant region allotype associated determinants. This most likely involves antigen recognition by T lymphocytes, but our data do not *establish* such a role for T lymphocytes. However, one strain (RF) responded to UPC10 but not MOPC173 or LPC1, suggesting in this case a recognition limited to a unique antigen of UPC10. The situation of the λ γ2a proteins is much less clear, although it seems possible that an *Ir* gene distinct from, although linked to, that controlling responsiveness to κ γ2a proteins may be involved.

Amino Acid Diversity in the Mouse Heavy Chain Family and its Correlation with Hapten Binding Specificities. P. Barstad, M. Weigert, M. Cohn, and L. Hood. California Institute of Technology, Pasadena, California, The Salk Institute, La Jolla, California.

Amino acid sequences of the N-terminal 20 residues of 13 BALB/c heavy chains are compared with 15 others taken from the literature. Seventeen of the 28 variable regions of the heavy chain (V_H regions) differ by one or more amino acids. These V_H regions can be divided into four distinct sets whose prototype (average) sequences differ from one another by 40%-50% (Fig. 1). The largest set, which contains 18 proteins, can be further subdivided into at least three subsets by the presence of two or more linked residues for each subset. These data suggest that at least seven distinct germ-line V_H genes must be postulated to explain the diversity seen in this set of sequences. The degree of diversity noted in these sequences is only a lower estimate of the actual V_H sequence diversity since most heavy chains were selected because the immunoglobulins from which they were derived bound to various simple haptens. There is a striking correlation between hapten binding specificity and the N-terminal sequence of these heavy chains (Fig. 1). The heavy chains derived from immunoglobulins binding to dinitrophenyl form one major set, those from immunoglobulins binding to 1-3 α dextran form a second set, and those from an immunoglobulin binding to 5-acetyluracil form yet another set. Generally even the linked subsets within the major set correlate with specific hapten binding properties.

Tumor Number	Ig Class	Light Chain Type	1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 20	Activity
Prototype V_{HI}			E V Q L Q E S G P S L V K P S Q T L S L	
M460	IgA	κ	———————————————————	DNP
S23	IgA	κ	———————————————————	DNP
M315[a]	IgA	λ	D ———————— G ———————— S ———	DNP
Prototype V_{HII}			E V Q L Q E S G P E L V K P G A S V K M	
J558	IgA	λ	———————————————————	1,3D
MOPC 104E	IgM	λ	———————————————————	1,3D
Prototype V_{HIII}			E V K L L E S G G G L V Q P G G S L K L	
Y5476	IgA	κ	———————————————————	L
W3434	IgA	κ	———————————————————	1,6D
W3129	IgA	κ	——— V I * ———————— * ———————	1,6D
M173[c]	IgA	κ	———————————— P ———— L ———	U
2020	IgA	λ	—— Q — V ——————————— * ———————	U
Y5444	IgG2a	λ	—— M — V ——————————————————— * —	U
S10[d]	IgA	κ	———————————————————	1,6G
X24[d]	IgA	κ	——————————————————— * —	1,6G
X44[d]	IgA	κ	———————————————————	1,6G
T191[d]	IgA	κ	———————————————————	1,6G
J539[d]	IgA	κ	———————————————————	1,6G
J1[d]	IgA	κ	——— I ———————————————	1,6G
H-8[e]	IgA	κ	——— V ——————————————— R —	PC
T15[e]	IgA	κ	——— V ——————————————— R —	PC
S107[e]	IgA	κ	——— V ——————————————— R —	PC
M603[e]	IgA	κ	——— V ——————————————— R —	PC
M167[e]	IgA	κ	——— V V ————————————— R —	PC
Y5606	IgG_3	λ	D — Q — V ———————— * ——— Z ——	TMA
M-21A[a]	IgA	κ	D — Q — V Q ——————————— M ——	U
406[b]	IgA	κ	D ——— Q ————————————————	AM
W3082	IgA	κ	——— E ——————————————— M ——	L
J606	IgG2a	κ	——— E ——————————————— M ——	L
Prototype V_{HIV}			E V Q L Q Z S G T V L A R P G S S L K M	
S176 major	IgA	λ	S ——————————————— * ———	5AU
S176 minor	IgA	λ	——————————————————— * ———	5AU

Fig. 1. The N-terminal 20 amino acids from BALB/c myeloma heavy chains. N-terminal sequences of BALB/c heavy chains. The one letter code of Dayhoff is used. Ig indicates immunoglobulin; DNP designates dinitrophenyl; 1,3D indicates α1,3 dextran; 1,6D designates α1,6 dextran; U indicates unknown specificity; L designates levan; AM designates N-acetyl-D-mannosamine; 1,6G indicates β1,6 galactan; PC indicates phosphorylcholine; TMA designates trimethylamine; and 5AU is 5 acetyluracil. a is from Francis, S., Leslie, R., Hood, L. & Eisen, H. (1974) Proc. Nat. Acad. Sci. USA, in press; b is from Capra, J.D., personal communication; c is from Bourgois, A., Fougereau, M. & De Preval, C. (1972) J. Biol. Chem. *24*, 446; d is from Rudikoff, S., Mushinski, E., Potter, M., Glaudemans, C. & Jolly, M. (1974) J. Exp. Med., in press; and e is from Barstad, P., Rudikoff, S., Potter, M., Cohn, M., Konigsberg, W. & Hood, L. (1974) Science *183*, 962. *indicates uncertainty about amide or residue assignment.

The Rabbit Ig Heavy Chain Linkage Group. Rose G. Mage, Ph.D. Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20014.

Dray and coworkers (Dray *et al.*, Ann. Immunol. (Inst. Pasteur) 125 C; 41, 1974) have summarized some of the known linkage relationships between genes that control antigenic determinants associated with the V_H and C_H regions of rabbit Igs. Examination of these "allogroups" gives some insight into the types of recombinations that may now be observed between different linked V_H markers as well as between V_H and C_H markers in the mouse. The actual order of genes shown in Table 1 is not known. Furthermore, there is little doubt that additional allogroups exist.

Nevertheless, examination of the allogroups in Table 1 suggests that in addition to the two reported recombination events between V_H and $C\gamma$ markers observed during laboratory breeding (Mage *et al.*, Nature New Biol. (London) 230, 63, 1971; Kindt and Mandy, J. Immunol. 108, 1110, 1972), other recombination events, including intra-V_H region recombinations, may have occurred in rabbit populations.

Table 1. Some Known Heavy Chain Allogroups of Rabbits[a]

Allogroup designation[b]	V_H	C_μ	C_γ	C_α
I	a^1, x^-, y^-	n^{81}[c]	$de^{12,15}$	f^{73}, g^{74}
II	a^1, x^{32}, y^{33}	n^-	$de^{12,15}$	f^{71}, g^{75}
III	a^1, x^-, y^{33}	n^-	$de^{11,15}$	f^{72}, g^{74}
IV	a^1, x^-, y^{33}	n^-	$de^{12,14}$	$f^-, g^{74'}$ [d]
X	a^2, x^{32}, y^{33}	n^{82}	$de^{12,15}$	$f^-, g^{74'}$ [d]
XI[e]	a^2, x^{32}, y^{33}	n^{82}	$de^{12,14}$	$f^-, g^{74'}$ [d]
XII	a^2, x^{32}, y^{33}	n^{82}	$de^{12,15}$	f^{71}, g^{75}
XX	a^3, x^{32}, y^-	n^-	$de^{12,15}$	f^{71}, g^{75}
XXI	a^3, x^{32}, y^-	n^-	$de^{11,15}$	f^{72}, g^{74}

[a]Data from Dray *et al.*, Ann. Immunol. (Inst. Pasteur) 125 C; 41, (1974), Mage *et al.*, *In* the Antigens, pp. 299, Academic Press, New York, (1973), and unpublished observations of S. Dray, R. Mage, G. O. Young-Cooper, A. Gilman-Sachs, B. S. Kim, C. Alexander, K. L. Knight, E. A. Lichter and W. C. Hanly.

[b]These roman numbers are arbitrarily chosen to aid in discussion of the groups listed.

[c]The n81 and n82 allotypes have not yet been localized to the constant region of μ chains.

[d]It is not known whether the f^-, and $g^{74'}$ specificities associated with allogroups IV, X, and XI are identical. Rabbits with $g^{74'}$ have IgA that crossreacts with g^{74}, but there is probably more than one type of f^- and $g^{74'}$.

[e]Allogroup XI was derived by apparent recombination between allogroup IV and allogroup X (Mage *et al.*, Nature New Biol. (London) 230, 63, 1971). If the f and g locus markers associated with allogroups IV and X can be distinguished, typing of homozygous animals with the XI allogroup could help substantiate the recombination.

Structural Studies on Human IgM Antigamma Globulins with Cross Idiotypic Specificity. J. Donald Capra and J. Michael Kehoe. Mt. Sinai School of Medicine, New York, N. Y. 10029.

The human IgM antigamma globulins can be divided into three cross idiotypic groups on the basis of their reactivity with absorbed rabbit antisera (H. G. Kunkel *et al. J. Exp. Med. 137*:331, 1973). The heavy chains of two members of the Po group have been subjected to amino acid sequence analysis. Of the 124 positions in the variable region, there are only eight differences. This compares to an average difference of 38 residues in randomly chosen V_HIII myeloma proteins. Five of the eight differences are outside hypervariable regions, and as such, these proteins do not differ any more or less than randomly chosen proteins. However, with only three amino acid differences in the 37 hypervariable positions, and with two entire hypervariable regions being identical, it is likely that the hypervariable regions comprise the major antigenic determinants of the idiotypic determinants.

Relationship between Allotypic and Idiotypic Determinants on Rabbit Antibodies. T. J. Kindt and M. Mudgett. The Rockefeller University, New York, New York.

Allotypic and idiotypic markers of homogeneous rabbit antibodies to streptococcal carbohydrates have been employed to study genes controlling the biosynthesis of antibody variable regions. Hapten prepared from group C streptococcal carbohydrate inhibited binding of the antibodies to idiotypic antisera but not to group a allotypic antisera. Examination of the idiotypic determinants of homogeneous antibodies showed the presence of antibodies with identical or cross-reactive idiotypes but with differences in other V region markers including group a allotype and V_L region structure. In one instance, two anti-group C carbohydrate antibodies produced in the same rabbit differed in group a allotype (a3 and a-) but possessed identical idiotypes and L-chain subclass (V_KI). A study of x and y allotypes of these antibodies carried out by Dr. S. Dray suggested that both the a3 and a- H chains were synthesized from genes in the *CIS* configuration. (Supported by NIH grant AI-11995-01 and AHA Grant-in-Aid 72 1010. TJK is an established investigator of the AHA).

Mutations in Immunoglobulin Producing Cells. B. K. Birshtein, J. L. Preud'homme, and M. D. Scharff. Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461.

Cultured IgG producing mouse cells derived from the MPC-11 and P3,(MOPC - 21) myeloma cell lines have been examined for variants that have undergone mutation in immunoglobulin synthesis, assembly, secretion, or structure. Variants that have lost the ability to synthesize heavy and/or light chains arise spontaneously at a high incidence of 10^{-3}/cell/generation. Mutagenesis with the acridine half mustard ICR 191 or with Melphalan, a phenylalanine mustard, results in a significant increase in variants so that at doses which kill 40%-60% of the cells 1%-2% of the surviving clones are variants. One-third to one-half of these variants continue to synthesize heavy chains which differ from the parent serologically and by peptide maps. Some of these appear to represent deletions while others may represent recombination between IgG_{2b} and IgG_{2a} constant region genes. The most surprising aspect of these studies is that the changes we are observing at such high frequencies are reflected in the constant region of the immunoglobulin polypeptide chain. (Supported by Grants from NIH, NSF, and ACS).

Regulation of Variable Gene Expression by Antireceptor Antibody. Heinz Köhler. The University of Chicago, Chicago, Illinois.

The antibodies produced in BALB/c mice to phosphorylcholine (PC) are of restricted heterogeneity and idiotypically cross-reacting with the PC-binding BALB/c myeloma TEPC-15 (H. Consenza and H. Köhler: *Science 176*:1027, 1972; W. Lee *et al.*: *Nature 247*:55, 1974). Homologous and heterologous anti-idiotypic sera against TEPC-15 (T15) protein bind to the Ig receptor for PC on antibody precursor cells (B cells). This binding induces specific suppression of the response to PC (H. Consenza and H. Köhler: *3rd Int. Conv. Immunol.*, p. 330, 1972). Thus anti-T15 antibody is operationally antireceptor antibody (ARA) (H. Consenza and H. Köhler: *Proc. Nat. Acad. Sci. USA 69*:2701, 1972). The presence of ARA in experimental animals during different times of ontogeny regulates the expression of the T15 variable gene as measured by the antibody response to PC. a) ARA present in adult mice induces short-term suppression to PC. b) ARA present during the neonatal period of mice induces chronic suppression indistinguishable from tolerance. Short-term suppression to PC can be achieved by a) passive immunization with homologous and heterologous ARA. The suppression lasts not longer than 4 weeks. Cells taken from adult suppressed animals respond normally in vitro and after adoptive transfer. b) Mice actively immunized with T15 and producing ARA cannot respond to PC. c) Mice repeatedly immunized with PC are producing ARA and are partially suppressed to PC. The 'auto-ARA' in the serum of these mice can be demonstrated by passive hemagglutination of T15-SRBC. Spleen cells from such hyperimmunized mice have plaque forming cells against T15-SRBC. Furthermore, serum from animals producing 'auto-ARA' can suppress specifically the *in vitro* response to PC of normal spleen cells.

In contrast, neonatal animals treated with ARA are unresponsive for more than 8 months. Their spleen cells remain unresponsive in culture and after adoptive transfer. Mixing of normal cells and unresponsive cells at different ratios does not impair the response of normal cells to PC in vitro and after adoptive transfer. Indirect surface fluorescence induced by heterologous ARA shows that antibody-precursor cells are absent in spleens of neonatally suppressed animals while such cells can be detected in adult suppressed animals after shedding of the receptor-ARA complex in culture. These findings suggest that ARA can regulate the expression of the variable region genes coding for anti-PC antibody by two different mechanisms: a) reversible blockade of the receptor for PC on the antibody presursor cells in adult animals, and b) elimination of antibody precursor cells for PC in the neonate. (Supported by NIH grants AI 11080 and AI 9268. H. K. is the recipient of Research Career Development Award AI 70559).

Functional Specificity of the T lymphocyte in Guinea Pigs. C. A. Janeway, Jr. and B. E. Cohen. Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland 20014.

Peritoneal exudate lymphocytes (PELs) of guinea pigs immunized with the hapten 2,4-dinitrophenyl (DNP) coupled directly to *Mycobacterium tuberculosis* are stimulated to synthesize DNA in vitro by DNP conjugated to a variety of carrier proteins. Unconjugated proteins do not stimulate such cells, nor can PELs from guinea pigs immunized with *M. tuberculosis* be stimulated by DNP proteins. The responding cells are thought to be thymus derived, as such cells comprise the majority of PELs. Their specificity correlates with delayed hypersensitivity and MIF production. Depletion of the few immunoglobulin bearing cells on nylon wool columns does not affect the proliferative response. The para-nitro group is essential for this response, while the ortho-nitro group is unimportant. Anti-DNP antibody from these guinea pigs shows the same specificity. On the other hand, linkage to carrier and the nature of the carrier are important for stimulation of cells, as DNP coupled to protein via a tripeptide spacer or coupled to a copolymer of L-glutamic acid and L-lysine (L-GL) will not stimulate the cells but inhibits anti-DNP antibody as well as stimulatory compounds. By using synthetic carriers containing tyrosine, it has been possible to show that a large portion of the specificity of these cells is directed at DNP coupled to the hydroxy group of tyrosine. These DNP groups can be selectively removed from the carriers with 2-mercaptoethanol, leaving DNP coupled to lysine intact. Such treatment almost entirely abolishes stimulation by these compounds. Thus far, it has not been possible to demonstrate antibody with specificity for DNP protein which is not fully inhibited by DNP-lysine and DNP-L-GL. Thus, no serological correlate to the specificity of these T cells has been shown. The T cell receptor for the hapten DNP appears to have a very high degree of specificity for hapten, but requires also the correct local environment on the carrier; a part of the local environment apparently involves tyrosine. It should be added that transfer of DNP groups from tyrosyl residues to other proteins or to cells during culture has not been ruled out as an explanation of these findings. High levels of mono-O-DNP-tyrosine do not give stimulation. Further studies of this point are in progress.

A Direct Approach to Characterize T Cell v-and (or) Ir-genes. H. Vachek and E. Kölsch. Heinrich-Pette Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Germany,

The T cell-mediated cytotoxic immune response (^{51}Cr-release) to DBA/2 mastocytoma cells P 815-X2 in different inbred strains of mice is under the control of hitherto unknown genes. C57BL/6 mice respond well to the P 815-X2 cells at a broad dose range. The total lack of responsiveness in DBA/1 (*H-2^q*) and the partial lack in SJL (*H-2^s*) mice cannot be explained by tolerance to the H-2 specificities of DBA/2 (*H-2^d*) mice. Low responsiveness of DBA/1 mice to P 815-X2 is inherited as a single recessive trait, showing mendelian segregation in the backcross (C57/BL × DBA/1) × DBA/1. It is restricted to a limited number of membrane determinants, since the cytotoxic response of DBA/1J mice against the C57BL/6 lymphoma EL 4 is normal. The responsiveness of SJL mice to the DBA/2 mastocytoma shows a strong dependency on the antigen dose. SJL respond equally well as C57BL/6 mice at high antigen dose (10^7 cells) but behave as low responders if the antigen dose is reduced by a factor of 10. Similar types of low responsiveness as described here for T cell-mediated cytotoxicity are already known for humoral responses. The specific restrictions in T cell cytotoxicity suggest similar limitations in the reactivity of both cytotoxic T cells and helper T cells. The direct measurement of the genetic control of the specificity of T cell-mediated cellular cytotoxicity as described in these experiments might be a tool for deciding, whether the T cell recognition unit involved is coded for by variable region - or *Ir*-genes. (Supported by the Deutsche Forschungsgemeinschaft).

Alteration of Ig Expression in Congenic Mouse Strains. M. Bosma and G. Bosma. The Institute for Cancer Research, Philadelphia, Pennsylvania.

BALB/c mice that were specially bred to carry and express only immunoglobulin (*Ig*) genes of the C57BL allotype (Ig C_H G^b) would seem to consitute a model system for studying the inheritance and expression of *Ig* genes. A different number of backcrosses preceded the derivation of several such congenic mouse strains (CB-17, CB-23, and CB-26). This means that the chromosome (CB) containing the *Ig* C_H G^b marker was paired repeatedly with its BALB/c homologue for a different number of generations prior to deriving mice that were homozygous for the CB chromosome. As a result of genetic recombinations, we would expect the CB chromosome to acquire many BALB/c genes including some BALB/c *Ig* genes. The postulates that we are testing are as follows: a) that continued backcrossing results in a diminishing number of C57BL *Ig* genes on the CB chromosome partly at the expense of an increasing number of nontranscribed, BALB/c recombinant

Ig genes; b) that the quiescence of the latter is due to the absence of specific regulator genes that may be under thymus control; and c) that in the breeding of CB mouse strains, one selects against the acquisition of BALB/c regulator genes. Evidence consistent with the first postulate is the finding that decreasing concentrations of serum IgG^b are found in C57BL (1.2 mg/ml), CB-17 (0.35 mg/ml), and CB-26 mice (0.17 mg/ml). Also, there have been unexplained occasions in which Ig's of the BALB/c allotype appeared in the serum of CB-17 mice (M. Bosma and G. Bosma: *J. Exp. Med. 139*: 512, 1974). In our attempts to induce the synthesis of Ig^a, we injected X-irradiated, thymectomized CB-17 mice (XR CB-17 mice) with 5×10^6 thymus cells from BALB/c donors that were immune to Ig^b. Repeated experiments of this kind result in the expression of Ig^a followed by the suppression of Ig^b synthesis; both of these phenotypic transitions in CB-17 recipients still persist at 43 weeks after cell transfer. Except for the lack of Ig^b suppression, the same results are obtained following the injection of thymus cells of normal BALB/c mice into XR CB-17 mice. The passing of thymus cells though nylon wool columns to remove contaminating B cells before injection into XR CB-17 mice did not alter the above sequence of Ig^a expression and Ig^b suppression. Moreover, the converse experiment of transferring normal CB-17 thymus cells into XR BALB/c mice did not lead to the detection of Ig^b in these recipients. Therefore, B cell contamination in the BALB/c thymus preparations does not appear to be a sufficient variable to account for these findings. Rather, we presume that thymus cells of BALB/c mice serve some regulatory function for "turning-on" hidden Ig^a structural genes in CB-17 mice. (This work was supported by US Public Health Service grants CA-04946, CA-06927 and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.)

The Regulation of Expression of B Cell Clones. Normal R. Klinman and Joan L. Press. Department of Pathology, Medical School, University of Pennsylvania, Philadelphia, Pennsylvania 19174.

The analysis of antibody forming cell clones derived from isolated B cells in fragment cultures has permitted defining the distinguishing characteristics of B cells in spleens of immune as opposed to nonimmune mice and characterizing the extensiveness of the populations of cells responsive to defined antigens. By comparing the increment in antigen binding cells after immunization to the increment of in vitro clones, it has been possible to estimate the efficiency of the cloning procedures and hence the absolute precursor frequency. In spleens of nonimmune BALB/c mice 1/10,000 cells is responsive to the 2,4-Dinitrophenyl (DNP) determinant, 1/10,000 to the 2,4,6-Trinitrophenyl (TNP) determinant and 1/17,000 to the Fluorescein (Fl) determinant. These populations appear to be exquisitely specific since overlap stimulation of DNP specific B cells by TNP is not detectable. The presence of these cell populations is apparently unrelated to previous antigenic stimulation, since B cells in nonimmune mice are clearly distinguishable from those of immunized mice and frequencies are essentially identical in germ free mice. The frequency of 1/10,000 for B cells specific for nitrophenyl determinants combined with a repeat frequency of 1/8000 for clones expressing identical anti-nitrophenyl antibodies (clonotypes), indicates that in the mouse B cell population, there exists $1\text{-}5 \times 10^7$ clonotypes each expressed by 10-50 B cells. Since the half-life of B cells is short, the maintenance of these cell populations may be considered as the product of recurring events 1/10,000 of which gives rise to DNP specific cells, 1/17,000 to F_1 specific cells and so on. Since the adult B cell population is so complex, studies of the B cell population in neonatal mice were inititated so that factors which control such clonotype expression could be analyzed in a more restricted cell population. Several of the intital findings and their possible revelance to regulation of clonotype expression may be listed as follows: a) Neonatal B cells, in the environment of a splenic fragment from a lethally irradiated carrier primed adult mouse, are fully competent to respond to antigenic stimulation by giving rise to a clone of antibody producing cells. The parameters of stimulation of neonatal cells indicates that they are highly specific and clearly different from B cells from immune donors, thus lending support to the notion that normal B cell populations derive independently of antigenic stimulation. b) The frequency of DNP and TNP specific B cells in spleens of 1 to 3-day-old neonates is at least as high as the frequency in adults while F1 specific B cells are very low even into the second week of life. c) Analyses of neonatal monoclonal antibodies by isoelectric focusing indicate that at least three DNP specific clonotypes and three TNP specific clonotypes occur repeatedly in neonatal spleens. This is considered as evidence that the genetic information for such predominant clonotypes is either present in all individuals or represent the frequent product of permutations of this information. d) The frequency and pattern of expression of these predominant clonotypes indicates that they each may be expressed in approximately one-third of BALB/c neonates. This implies that even clonotypes of putatively "germ-line" specificity may be expressed randomly. e) When predominant clonotypes are expressed, they achieve a clone size of 100-200 B cells. Thus clonotype expression appears precommitted prior to antigenic stimulation and is characterized by 6-8 cell divisions. f) In addition to

these predominant clonotypes, other clonotypes occur sporadically even in the first day of neonatal life. These clonotypes have not yet been found to repeat even in a single donor. At days 1-3 of neonatal life sporadic clonotypes represent approximately 10%-20% of DNP and TNP specificities and all F_1 specific clonotypes. By days 9-11 of neonatal life sporadic clonotypes represent a majority of the B cells present. Littermates have a tendency to express the same predominant clonotypes indicating that the maternal environment may influence clonotype expression. These findings indicate that the BALB/c neonatal B cell repertoire may include at least 12,000 predominant clonotypes of which any individual may express 4,000-5,000. In addition to these specificities, information may exist or be generated for a variety of sporadically occurring clonotypes. While the expression of any given clonotype may be the product of a random spontaneous event, evidence in littermates could indicate the potential to control expression at this early stage. If similar events control clonotype expression in adults, given a half-life of B cells of 24-48 hours and an average clonotype population at 10-50 cells, diversity could be viewed as the product of recurrent, periodic, spontaneous expressions of a vast set of $1\text{-}5 \times 10^7$ clonotypes. Control of expression by genetic, suppressive or tolerogenic influences may thus occur at the level of the clonotype precursor cell. (Supported by US Public Health Service Grant AI-08778.)

T-Cell Activation: The Role of Ir Gene Products. Ethan Shevach. Laboratory of Immunology, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.

Alloantisera prepared by reciprocal immunization of strain 2 and strain 13 guinea pigs specifically block the activation of T lymphocytes by antigens, the response to which is controlled by *Ir* genes. To further study the mechanisms by which alloantisera block T-lymphocyte antigen recognition, the 13 anti-2 serum was adsorbed with different populations of lymphoid cells. Cells obtained from a normal animal were as effective as cells obtained from a DNP-GL immune animal in their absorptive capacity. Thus, it is unlikely that the inhibitory activity of the anti-2 serum on T-cell proliferation is due to the presence of antibodies specific for idiotypes of clonally distributed T-lymphocyte receptors. B lymphocytes were much more effective than T lymphocytes in removing either the cytotoxic or inhibitory activity of the anti-2 serum. However, it is not clear from this result whether the inhibitory activity of the alloantisera is due to antibodies specific for the *Ir* genes products or for antibodies specific for linked antigens in the major histocompatibility (*H*) complex. Whether the anti-2 serum could inhibit function of an *Ir* gene, the *GA gene,* which is normally linked to strain 2 *H* genes, when this gene occurs in an outbred animal lacking strain 2 *H* genes was examined. The anti-2 serum was capable of inhibiting the proliferative response of T cells from animals that were GA+ 2+, but the serum had little if any effect on the GA response of T cells from GA+ 2- animals. Furthermore, an antiserum prepared in strain 13 animals against the lymphoid cells of the GA+ 2- outbred animal was devoid of inhibitory activity, while an antiserum prepared in strain 13 animals against the lymphoid cells of a GA+ 2+ outbred was capable of specifically inhibiting the response to GA. It thus appears that inhibition of the GA response by the anti-2 serum is mediated via antibodies directed toward strain 2 H antigen, with resultant steric interference with the GA *Ir* gene product on the cell surface.