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Abstracts

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Cross-Reactions of K^k *and* D^k *Shown by H-2 Restricted Cytotoxic Responses to the Male Specific Antigen, H-Y.* Elizabeth Simpson. CRC, Northwick Park Hospital, Watford Road, Harrow Middlesex, England.

It was an unexpected finding that when either (B10.A(5R) × B10.A)F_1 or (HT1 × B10.A)F_1 females were primed and boosted with B10.A male cells, they gave an H-Y specific *H-2* restricted response in which effector cells killed not only B10.A male target cells (K^kD^d) but also those of C3H.OH (K^kD^k) just as effectively.Since H-2^d male target cells (K^dD^d) were not killed, this finding implies that the private specificity of K^k seen in conjunction with H-Y by cytotoxic T cells cross-reacts with the private specificity of D^k (or L^k).

The question of whether K^k or D^k antigens were also cross-reactive at the level of their being recognised as alloantigens was explored by examining the target cell specificity of cytotoxic responses generated between BALB/c and B10.A on the one hand (anti K^k) and between BALB/c and C3H.OH on the other (anti D^k). In these experiments, a minimal amount of cross-reactivity was seen suggesting that the determinants seen as alloantigens on K^k and D^k were not the same. Similarly, experiments performed generating anti-self TNP responses with B10.A and C3H.OH spleen cells did not reveal K^k/D^k cross-reactivity, perhaps because the anti-TNP response is really directed at altered (haptenated) *H-2*, and this is more like an allogeneic response.

Concanavalin A Can Mimic H-2 Antigens in Boosting Memory Cells into H-2-Specific Killer Cells. T.X. Reme. Laboratoire d'Immunopharmocologie des Tumeurs, Centre Paul Lamarque, Hospital St.-Eloi, 34033 Montpellier, France.

The renewing by Con A of the cytolytic activity evaluated in a short-term chromium release assay in a population of memory cells obtained in a long-term mixed lymphocyte culture is shown to be largely dependent upon the dose of Con A used; a three staged phenomenon in terms of dose response and kinetics is analysed and suggests that, at least for a low concentration of Con A etc. (≤ 0.5 µg/ml), the lectin acts on the same subpopulation and through the same mechanism as the specific antigen, as shown by DNA synthesis inhibition experiments. Preincubation with Con A at doses giving the best secondary-like response strongly inhibits further response to the primary alloantigen. Experiments using mixtures of Con A and alloantigens as stimulators show that both agents can compete in differentiating memory cells into killer cells. All these data suggest an important overlap of the structures on memory cells which are triggered by Con A or specific antigen.

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Preliminary Evidence for Different D End H-2 Molecules Acting As Restriction Antigens with Influenza and Alpha Viruses. R. Blanden and A. Mullbacher. Dept. Microbiology, John Curtin School of Medical Research, Australian National University, Canberra City, Australia.

Two different BALB/c anti-CBA(H-2^k) monoclonal antibodies that bind to K^k and D^k antigens blocked Tc cell-mediated lysis of L929 (K^k, D^k) target cells, but with quite different specificity. One antibody (30R3) powerfully blocked K^k-specific lysis mediated by alloreactive or by K^k-restricted Tc cells immune to ectromelia, Sendai or influenza viruses. The other antibody (27R9) blocked these anti-K^k Tc cells much less than 30R3, but in contrast, 27R9 blocked anti-D^k lysis much more than 30R3. Most importantly, 27R9 strongly blocked D^k-restriced anti-influenza Tc cells, but did not significantly block D^k-restricted anti-Bebaru lysis. This result indicated that different H-2 determinants coded in the *D*-end of *H-2^k* were recognized by influenza- and Bebaru-immune Tc cells. These determinants may be carried on two different molecules coded by the *H-2D* and *H-2L* loci, but other possibilities are not yet excluded.

In Situ Immune Responses in Ascitic Tumors Progressively Growing in Their Host Origin. Lionel A. Manson. The Wistar Institute, 36th Street at Spruce, Philadelphia, Pennsylvania, 19104.

Serveral parallel lines of investigation being carried out in our laboratory have led us to conclude that tumour-associated transplantation antigen(s) (TATA) of several ascitic tumours such as P815Y, L-5178Y and EL-4 is an altered surface-membrane *H-2* antigen complex. We reported several years ago that we could isolate T-cell killers from P815Y ascitic tumour mass growing in its host origin DBA/2 (Biddison, W. E. and Palmer, J. C.: *Proc. Natl. Acad. Sci. 74*:329, W. E. Biddison et al.: *J. Immunology 118*:2243, 1977). Two interesting observations were made at that time, (a) the killer cells were non-*H-2* restricted, i. e., they were cytolytic for EL-4 (H-2^b) with equal efficiency as for P815Y and L-5178Y (both H-2^d), and (b) the P815Y cells isolated from the ascitic mass on day 10 after inoculation were sensitive to these T-cell killers, but the tumour cells isolated from the day 16 ascitic mass were resistant. At that time, this antigenic modulation was proposed as one mechanism by which tumours have evolved to escape immune surveillance. In parallel to these studies, we have been investigating the nature of the *H-2* antigen complex in the membranes of L-5178Y. Standard anti-H-2^d antisera immune precipitate a 45 000 dalton glycoprotein peak and a 12 000 β_2-microglobulin peak from detergent lysates (NP-40) of labelled DBA/2 spleen cells. Immunoprecipitates made with these same sera and lysates of L-5178Y show on SDS-PAGE analysis additional glycoprotein peaks with mobilities of 8 000, 33 000 and 52 000 daltons. When the sera were absorbed with DBA/2 red cells, all the peaks disappeared from the immunoprecipitates together. We have concluded that the *H-2* antigen complex of L-5178Y contains these additional glycoproteins, perhaps in hydrophobic association. Only very preliminary studies have been carried out with EL-4 and P815Y, but additional peaks have been seen on SDS-PAGE.

The third line of study has revealed that an immunoglobulin-like molecule is found attached to all three tumour lines during in vivo growth, either as solid tumours or as ascities. More of this material is bound to the tumour cells after 2 weeks in vivo as compared to 1 week in vivo. This molecule is detected by an ^{125}I-anti-Fab that is routinely used in our laboratory in RIA for anti-H-2 antibodies. Tumour cells that have large amounts of this bound material have lost their capacity to bind anti-H-2^d antibody from standard anti-H-2 antisera. In some experiments, the ascitic tumour cells were tested and were resistant to the ascitic T-killer cells isolated from the same ascitic mass.

An explanation that would account for all the observations is that these tumours have a TATA that is an altered *H-2* antigen complex. When the tumours are inoculated into mice, a primary cell-mediated immune response develops to this altered H-2 antigen ("allogeneic" complex) which would be expected to be non-*H-2* restricted. In parallel, a primary humoral immune response also forms against the TATA, and by the 2nd week is strong enough to block the TATA of the multiplying tumour cells to attack by the T killer cells. In this way the tumour escapes an ongoing immune response. Further experiments are being carried out to validate this hypothesis.

Major Histocompatibility Complex (MHC) Control of Sensitivity to Moloney Leukemia in Mice. Patrice Debre, Sylvie Gisselbrecht, and Jean Paul Levy. INSERUM U152, Laboratoire Immunologie et Virologie des Tumeurs, Hopital Cochin, 75674 Paris, France.

The role of *H-2*-linked genes in the control of C type virus induced malignancies has been well documented for various systems including the leukemias caused by the Gross, Tennant, AKR, RadLV and Friend viruses. Most of these experiments have, however, been concerned with the final appearance of the disease, whereas relatively little is known about the intermediate steps in host-virus relationships.

We have studied the level of viremia and the appearance of leukemias in different *H-2* congenic strains of mice inoculated with the Moloney leukemia virus (M-Mulv). The viremia was regularly measured in individual mice using a radioimmunoassay of the major internal virion component P30. Differences in the mean amount of circulating P30 were observed between mice of various haplotypes on BALB or B10 background demonstrating the MHC control of viremia: BALB/c (H-2^d), BALB/K (H-2^k) or B10.M(H-2^f), B10.A(H-2^a), B10.D2(H-2^d) mice appeared highly susceptible to M-Mulv infection, whereas BALB/B(H-2^b) or B10(H-2^b), B10.S(H-2^s), B10.RIII(H-2^r), B10.BS(H-2^j) mice were resistant. However, non-*H-2* genes must also be involved in the control of viremia since differences exist between mice of the same haplotype, such as BALB/B and B10(H-2^b) or BALB/c and B10.D2(H-2^d). *H-2* recombinants obtained from congenic strains on A or B10 background have allowed the mapping of three different sets of genes within the MHC. Two of them designated *Rmv1* and *Rmv2* appeared to be located in the *I* region in *I-C*, *S* or *G* region, respectively. The third gene *Rmv3* was mapped to the *D* end of the complex in the *D* or *T* region. Crosses between resistant and sensitive strains, involving B10 and B10.A(4R) mice for *Rmv1*, B10.A(4R) and B10.A(2R) for *Rmv2*, B10.A and B10.A(2R) for *Rmv3*, demonstrated that the *H-2*-associated resistance was inherited as a dominant or semi-dominant trait. *Rmv1*, *Rmv2* and *Rmv3* were shown to complement for resistance in trans when the hybrid between B10.A(4R), B10.M and B10.A sensitive strains were examined.

A good correlation was found between viremia and the appearance of leukemias, the most viremic strains having also the most leukemias. Nevertheless, additional non-*H-2* genes must also control the appearance of leukemia as, despite high levels of viremia, some sensitive strains do not become leukemic.

The Serology and Polymorphism of H-2L-Locus Encoded Antigens. Chun-Ming Huang, Huei-Jen S. Huang, and Jan Klein. Abteilung Immungenetik, Max-Planck-Institut für Biologie, 7400 Tübingen, Federal Republic of Germany and Department of Microbiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

To determine the extent of *H-2L* polymorphism, we tested serologically, by the complement mediated two-stage microcytotoxicity test and by in vitro absorption, 30 B10.W congenic lines, 10 inbred strains carrying *H-2* haplotypes of independent origin, and the mutant strain B10.D2-H-2^{dm1}. Three antisera were used in this study. K-654 was generated in mutant BALB/c-H-2^{dm2} anti-parental H-2^d, and presumably only detects H-2L molecules. K-664 was generated in a $(dm1/dm2)_{F1}$ anti-*d* combination. CH-24 was produced in a $(r/k)_{F1}$ anti-*h2* combination. CH-24 has anti D^bL^b activity, and can be rendered more specific to H-2L molecules by absorption with mutant BALB/c-H-2^{dm2}. K-654 reacted with haplotypes *b*, *d*, *ja*, *q*, *u*, and *dm1*. Checkerboard absorption analysis revealed that K-654 contained at least two H-2L antibodies detecting specificities H-2.64 and H-2.65. K-664 reacted strongly with haplotypes *d*, *f*, and *q*, and weakly with haplotypes *b* and *ja*. The demonstration of serological activities in $(dm1/dm2)_{F1}$ anti-*d* antiserum is consistent with the previously reported skin-graft complementation data. The activities of CH-24, after absorption by H-2^{dm2} paralleled those of K-654 with one exception. Both K-654 and CH-24 antisera did react with mutant H-2^{dm1}, indicating the presence of H-2L molecules in mutant *dm1*. However, H-2^{dm1} behaved differently from its standard *d* haplotype in that it failed to react with B10 or B10.WB absorbed K-654. This implies that the H-2L molecules of H-2^{dm1} may be somewhat altered. Analysis of 30 B10.W lines led to the definition of three new specificities of the H-2L molecules, namely 73, 74, and 75. In general, B10.W lines and inbred haplotypes of independent origin can be divided into at least six different phenogroups according to their H-2 specificities detected by *dm2* anti-*d* antiserum. The BALB/c group contains all the specificities which can be detected by this antiserum, namely, 64, 65, 73, 74, and 75. B10.BUA1 group lacks specificity 65 and contains specificities 64, 73, 74, and 75. B10.GAA37 group contains 74 and 64; B10.BUA16 group contains 75 and 64. Specificity 64 is also present in B10.KPB128 group and H-2^{dm1}. It is not clear whether these lines also contain specificity 75.

The Genetic Fine Structure of H-2D End. Chella S. David. Mayo Clinic and Medical School, Rochester, Minnesota 55901.

The chromosomal segment to the right of the *S* region is normally designated as the *D* end. This includes the *H-2G* region which was originally defined by erythrocyte antigen H-2.7. Recent studies on the tissue distribution and mapping of the H-2.7 specificity suggests that it might actually map in the *S* region negating the requirement of a separate region which was designated *H-2G*. Two new H-2D markers have been identified recently using new recombinants. They are H-2.10, a private specificity of

H-2D^r locus, and H-2.14, a marker for the *H-2D^v* locus. Studies on the immune response to human hemoglobin in mice suggest that the genes in the *H-2D* region might recognize determinants on the beta chain and some haplotypes such as *H-2^s* deliver a nonresponsive signal making the immune response to the whole hemoglobin low, even though immune response to the alpha chain is mediated by genes in the *K* end. Similar results were also found in studies on autoimmune thyroiditis where genes mapping in the *H-2D* region seem to induce suppression, especially when lymphocyte infiltration of the thyroid gland was involved. These results suggest an important role for the *H-2D* region in immune regulation.

Detection of Foreign H-2 Specificities on Tumour Cells Using an H-2 Restricted Cytostasis System. A. Matossian-Rogers, L. de Giorgi and H. Festenstein. Dept. Immunology, London Hospital Medical College, Turner Street, London E1 2AD, England.

Immunisation of B10.D2 mice with BALB/c spleen cells generated the development of effector lymphocytes which were cytostatic in vitro against macrophage and tumour targets in an *H-2*-restricted fashion. The metabolism of radioactive uridine by the specific macrophage targets from BALB/c but not from BALB.K or BALB.B was inhibited; also, both cytostasis against BALB/c macrophages and the H-2^d tumour LSTRA could be abrogated by the addition of antisera against private specificities H-2.4 and H-2.31 of the *H-2^d* haplotype. This indicated that B10.D2 anti-BALB/c immune effector cells recognised non-H-2 targets on BALB/c in association with H-2^d antigens. Antisera against public specificities and against non-H-2 antigens of BALB/c had no effect on cytostasis.

It was thus an unexpected finding that B10.D2 anti-BALB/c immune lymphocytes which were not cytostatic for the H-2^b tumour Gil 4 did inhibit the proliferation of the H-2^k tumour Gardner. The question of whether tumour specific antigens of Gardner cross-reactive with the BALB/c background were recognised by an H-2-restricted mechanism via extra H-2^d-like specificities on Gardner tumour was investigated. It was demonstrated that antisera against private H-2^d specificities H-2.4 and H-2.31 added separately to lymphocyte-tumour cultures did not affect cytostasis of Gardner, but when the two antisera were added in combination cytostasis was abrogated. This suggested that cytostasis against Gardner tumour was *H-2*-restricted via extra H-2^d-like antigens at either the *K* end or the *D* end. Absorption of a mixture of anti-H-2.4 and anti-H-2.31 with B10.D2 lymphocytes removed the inhibitory effect of these sera on cytostasis of Gardner, while nonspecific absorption with C57BL/10 lymphocytes had no effect. Combinations of antisera against public specificities H-2.3 and H-2.8 common to both the *H-2^d* and *H-2^k* haplotypes and also of antisera against public specificities H-2.28 and H-2.35 present on H-2^d but not on H-2^k lymphocytes had no significant effect on cytostasis of Gardner.

Other antisera tested for their effect on cytostasis of Gardner were against private specificities of the *H-2^k* haplotype; anti-H-2.23 did not effect the level of cytostasis compared with that in the presence of normal serum, while anti-H-2.32 significantly enhanced cytostasis probably by adding an ADCC component to the cell-mediated function. The fact that anti-H-2.23 did not behave as anti-H-2.32 is indicative of the poor representation of the former antigen on the tumour cell surface. This is in agreement with previous reports on the absence of H-2.23 from some H-2^k tumours.

Complexities of H-2 Specificities as Revealed by Studies of H-2 Mutants. I. F. C. McKenzie, G. M. Morgan, H. Dellos, and R. W. Melvold. Department of Medicine, Austin Hospital, Heidelberg, Victoria, Australia and Department of Radiation Biology, Shields Warren Radiation Laboratory, Harvard Medical School, New England Deaconess Hospital, 50 Binney Street, Boston, Massachusetts.

We have continued our serological studies of spontaneous *H-2* mutants and new information on these mutants.

1. Serological studies of mutants *bi*, *bj*, and *bk*, which all arose spontaneously as gain and loss mutations in *H-2K^D*. We have demonstrated that these mutants carry all of the H-2K, D and Ia specificities of the parental *H-2^b* haplotype. However, absorption studies revealed that *bk* and *bj* mutants carried considerably less H-2.33 than *bi*, which was the same as the parental B6 line. These two mutants, *bj*, *bk*, therefore, belong to a group that includes *bd* and *bf*, which have clearly very low expression of the H-2.33 specificity, and it can be separated from another group which has no detectable difference whatsoever, e. g. *bi*, *bg1*, *bg2*, *bh*.

2. One of the unique features of the *H-2* mutants has been the inability to make antibody by immunising directly between the mutants and this was confirmed with the three mutants mentioned above. However, by immunising (C57BL/6 × LP.RIII)F_1 with *bj* a cytotoxic antibody was produced which reacted with *bj* and *k* haplotypes, but with no others; moreover, the activity with *k* could be

removed by absorption leaving activity for *bj*, indicating the presence of two H-2 specificities. This could be precipitated and run on SDS-PAGE to give a molecule of 45 000 molecular weight. A reciprocal immunisation, by anti-A.BY, when tested on C57BL/6 congenic mice of different haplotypes, revealed a further two H-2 specificities.

3. The *bm* mutant is a gain and loss mutation which occurred in the *K* or *Ia* subregion and is distinctive from the K^b mutants mentioned above. However, on absorption testing there appears to be a difference in H-2.33 in this mutant compared with parental B6 lines. Therefore, at least two genes appear to effect the structure of the K^b molecule – that in which the K^b mutants occur, and the second mutant, *bm*, distinctive from the others, which also effects the expression of the H-2.33 specificity.

4. D^b mutants *bo* and *bm*. These gain and loss mutants were mapped to the D^d gene and in direct and absorption studies were both found to suffer a decreased amount of the H-2.2 specificity on their surface.

5. Mutation *dm4* (BALB/c-H-2^{dd}). This is a gain and loss mutation which has been mapped to the K^d gene although under direct testing and by absorption there is no detected difference between the serologically specified antigens on the surface of this strain and the parent BALB/c.

6. *Hh-1* gene testing in *dml* and *dm2* mutants revealed that the *L* locus appeared to be involved in both allogeneic bone marrow responses and in hybrid histocompatibility.

The *H-2* mutants, therefore, continue to provide extremely interesting serological and functional studies involving the structure of the *H-2* complex. (Supported by funds obtained from the N. H. & M. R. C., Australia.)

H-2-Like Cytotoxic Target 'Allodeterminants' on Syngeneic Tumours. H. Festenstein, C. Testorelli, O. Morelli, and W. Schmidt. Dept. Immunology, London Hospital Medical College, Turner Street, London E1 2AD, England.

Cell mediated cytotoxicity experiments following *H-2* congenic allosensitisation and testing of syngeneic tumour targets suggest that the latter unexpectedly shared H-2-like alloantigens with the stimulating cell. We considered several explanations for this finding but had first to exclude the possibility that the target determinants could be normal cross-reacting alloantigens, e. g. Tl, Qa, non-H-2, previously undetected H-2 public specificities, which are more readily detectable on tumours than on normal cells.

To rule out these posibilities, we used F_1 hybrid mice, in which one parent was of the strain of origin of the tumour (K36) and the other parent of the B10 congenic series, i. e. (AKR × B10)F_1. These cells were stimulated by lymphoid cells from other B10 congenic strains B10.A and B10.D2 and tested against the test tumour K36 and several PHA blast controls. Several K36 sublines as well as a clone line of K36 (K36.16) were used and significant cytotoxicity against an H-2^d-like target on these tumour cells was obtained. These data exclude the possibility of a cross-reactive alloantigen, e. g. undetected H-2 public specificity, or differentiation antigens. These results with the K36 tumour were consistent with our immunochemical studies (see abstract Schmidt and Festenstein) and were confirmed and extended by cold target inhibition experiments. In these experiments, B10.BR cells were sensitised by B10.D2 lymphoid cells and tested against B10.D2 (^{51}chromium labelled PHA blasts). Two kinds of normal unlabelled lymph node suspensions as well as the K36.16 tumour cell suspension were used. Significant specific inhibition of between 19% and 40% was obtained using K36 and between 23% and 37% using B10.D2 (positive control). AKR cells (negative control) in contrast were unable to reduce the percentage of specific cytotoxicity.

Since it was already known that the H-$2K^k$ gene product/s are missing from this tumour (W. Schmidt et al.: *Immunogenetics* 8:311–321, 1979) it was of interest to test whether cytotoxic effectors directed against the H-$2K^k$ gene products were able to kill the K36 tumour. Accordingly, B10.D2 lymphoid cells were sensitised to B10.BR (3H.OH) and B10.A targets, respectively and tested against K36 and appropriate controls. Only weak killing was observed when sensitisation was effected against the *K* end of the H-2^k haplotype (i. e. using B10.A as the sensitising cell) but strong and significant cytolysis was found when the sensitisation was against the whole H-2^k haplotype or against the H-$2D^k$ gene product. These results were confirmed by cold target inhibition studies.

These experiments provide further indications for the H-2^d-like characteristics of these allodeterminants. We have already excluded some of the possible explanations for these findings (i. e. cross-reactions with H-2 and non-H-2 normal specificities). The cold target inhibition experiments rule out nonspecific viral effects. We thus favour an alteration in regulatory genes leading to repression of the H-$2K^k$ product and derepression of the H-$2D^d$ product, but cannot formally rule out highly cross-reactive

H-2 like viral determinants. This work was supported by the Cancer Research Campaign of Great Britain, the Deutsche Forschungsgemeinschaft and the European Molecular Biology Organisation.)

Immunochemical Studies on Gains and Losses on K36. Wilhelm Schmidt and Hilliard Festenstein. Department of Immunology, London Hospital Medical College, Turner Street, London E1 2AD, England.

K36, a spontaneous leukemia originating in AKR (H-2^k) mice, was studied for expression of H-2 antigenic specificities by serology and by immunochemistry. Two ascites lines of the tumour as well as tissue culture adjusted and cloned tumour lines were used in these studies with similar results being obtained.

K36 does not express K^k-region encoded H-2 antigens on the cell surface. (1) Alloantisera directed against H-2.23 (private specificity of K^k) and H-2.8, 11 and 25 (public specificities of K^k not present on D^k) are not cytotoxic for the tumour, nor did the tumour absorb out alloantibody from these sera; (2) immunisation of H-2^d and H-2^b mice with K36 does not raise antibody against the K^k-region specificities 23, 8, 11 and 25; (3) from radiolabelled K36 NP40 lysates anti H-2.23 sera precipitated glycoproteins of 45000D and 70000D; however, absorption not only by B10.A but also by unrelated cells (C57BL/6, E♂G2 tumour cells – both H-2^b) inhibits precipitation of the 45000D glycoprotein. This is therefore unlikely to be *H-2*.

K36 tumour cells express D-region encoded H-2 antigens. (1) Anti H-2.32 sera are cytotoxic for and absorbed by the tumour; (2) H-2.D32 precipitated from radiolabelled and detergent solubilised K36 is identical to H-$2D^k$ from B10.BR, as shown by tryptic and peptide analysis.

H-2-like molecules carrying specificities not normally expressed by AKR lymphocytes are present on the K36 cell surface. (1) K36 absorbs alloantibody from H-2.4, 28 and 35 antisera (directed against private and public specificities of H-$2D^d$); (2) a 45000D peak in addition to 70000 and 12000 is precipitated from detergent solubilised 3H arginine radiolabelled K36.16 glycoprotein by anti H-2.4 and anti H-2.28 sera. Absorption of the anti H-2.4 sera with BALB/c (H-2.4 positive) as well as with E♂G2 (H-2.4 negative, GCSA positive) partly reduced the 45000D peak, indicating that viral protein, presumably GCSA, is present in addition to *H-2*; (3) in tryptic peptide maps of the 45000D glycoprotein precipitated by anti H-2.4 sera from radiolabelled K36 all H-2.4 specific peptides were present, both qualitatively and quantitatively. However, additional peptides were present, which could be due to the viral protein/s present.

The changes in H-2 profile on the K36 cell surface are most easily explained by an alteration in a postulated regulator system, leading to repression of the H-$2K^k$ gene product and to the derepression of the H-$2D^d$ product. (This work was supported by the Cancer Research Campaign of Great Britain and the Deutsche Forschungsgemeinschaft.)

H-2D and H-2L Molecules in the Products of H-2.21 Positive Allele D^{dx}. M. Snoek, D. Iványi, R. Nusse, P. Démant. Antoni van Leeuwenhoekhuis, 1066 CX Amsterdam, The Netherlands.

The inbred strains GRS/A and LIS/A carry the haplotype *H-2^{dx}* which was earlier shown to have K^d, I^f, S^f and G^f alleles and a previously unknown *D*-region allele.

Analysis of two sera (B10.D2 anti GRS/A and B10.D2 × ACA anti GRS/A) using a panel of inbred strains and absorption studies, showed that the D^{dx} region products are characterized by a private specificity H-2.63 and a public specificity belonging to the H-2.1 family of specificities. The H-2.1 positivity of the D^{dx}-region products was confirmed by the reactivity of GRS/A target cells with the monoclonal antibodies 11-4.1 (anti K^k). Further investigations of several H-2.1 positive sera to test the presence of other specificities from the 23 inclusion group of the H-2.1 family of antigens showed that only the H-2.1 antigen could be detected in the D^{dx} products. The D^{dx} products were found to be H-2.28 negative and to contain at least two other public specificities. H-2.70 detected by a B10.AKM × C3H.B10 anti B10.A serum and a specificity detected by C3H × BALB/c – H-2^{dm2} anti BALB/c serum. Capping studies showed a molecule distinguishable from the K and D molecules on GRS/A target cells, this third molecule could be detected with H-2.1-like antibodies. Immunochemical studies confirmed the existence of at least two polypeptide chains in the D^{dx}-region products.

A Syngeneic Humoral Immune Response Against a Mouse Sarcoma (MCG 4) That Recognizes a Complex H-2 Alloantigen. Federico Garrido. Tumour Immunology Unit, Facultad de Medicina, Granada, Spain.

MCG 4 is a BALB/c (H-2^d) sarcoma, induced as a solid tumour with 0–2 mg, of methylcholantrene. An ascites form of the tumour was obtained by injecting solid tumour pieces into the peritoneal cavity of syngeneic BALB/c mice.

The pattern of expression of H-2 antigenic specificities in MCG 4 was studied in a complement dependent microradioassay that involved antibody-complement treatment of tumour target cells, followed by the ^{14}C-thymidine uptake of the surviving tumour cells. H-2 alloantisera of restricted specificity defining private and public H-2 antigens were used.

MCG 4 did not express H-2D.4, H-2.3, H-2.8 and H-2.13. These results were confirmed by quantitative absorption studies using MCG 4 and, as controls, positive-negative normal lymphoid cells for a particular H-2 specificity. In addition it was also confirmed that MCG 4 expressed a foreign H-2-like public specificity (H-2.5).

MCG 4 was tested for antibody production in syngeneic BALB/c mice. An alloantiserum anti MCG 4 was obtained with titres 1/2000 – 1/5000. Surprisingly, BALB/c anti MCG 4 syngeneic anti tumour serum killed not only MCG 4 (H-2^d), but also a panel of H-2^b, H-2^k and H-2^a tumours of different etiology and origin. None of the other H-2^d tumours tested were killed by the alloantiserum. So, the cytotoxicity was not related to tumour inducing agents (Moloney virus, Graffi virus, Gross virus, chemicals, etc.), but to the *H-2* haplotype of tumour origin. In this context, the only H-2 public specificity shared by H-2^k, H-2^b and H-2^a haplotypes was H-2.5 which was previously detected in MCG 4.

The isoantiserum anti MCG 4 was absorbed with normal allogeneic cells from B10.BR (H-2^k), CBA/H (H-2^k), B10.A (H-2^a), A/Jax (H-2^a), C57BL/6 (H-2^b), C57BL/10 (B10) (H-2^b), BALB/c (H-2^d), B10.D2 (H-2^d) and tested for cytotoxicity against MCG 4. The activity was completely removed with H-2^k and H-2^a normal cells, but not with H-2^b and H-2^d.

Furthermore, the alloantiserum was tested for cytotoxicity in a wide panel of normal lymphoid cells using the conventional microcytotoxicity test. The results showed that the alloantiserum was positive with the following haplotypes:

H-$2K^k$, H-$2K^b$, H-2^r, H-2^p, H-2^q, H-2^s

and negative with:

H-$2D^k$, H-$2D^b$, H-2^f and H-2^d.

We have favoured the derepression hypothesis to explain these findings, that implies the presence of regulatory genes controlling the expression of H-2 determinants and that the foreign H-2 specificities could function as tumour specific transplantation antigens.

Markers Coded by Chromosome 6 on Human Malignant Cells. S. Ferrone. Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037.

Malignant transformation of human cells may be associated with changes in the expression of structures coded by the *MHC* region or genes linked to it. Thus(1) human tumor cells may absorb (or not absorb) HLA-A and B alloantibodies although their normal counterpart do not (or do); (2) SV_{40} transformation of fibroblasts induces the expression of receptors for monkey (macaca speciosa) red blood cells; and (3) melanoma cells express DR antigens although melanocytes lack them. In spite of these changes in the expression of structures coded by chromosome 6, this chromosome does not appear to be involved in the genetic control of serologically detectable tumor associated antigens in the melanoma system and these antigens have a molecular structure different from that of HLA antigens.

Surface Mapping of the Mouse Thymocyte. L. Flaherty. State of New York Department of Health, Division of Laboratories and Research, Tower Building, Empire State Plaza, Albany, New York 12201.

Relatively little is known on the topographical arrangement of alloantigens on the cell surface. In 1968 by use of the dual blocking assay, Boyse and co-workers found that certain alloantigens on the thymocyte cell surface occupy positions close to one another; namely, Lyt-1 to H-2K, Lyt-2 to H-2D, and H-2D to TL. Presumably in this assay if two alloantigens were sufficiently close to one another, attachment of antibody to one will impede attachment of antibody to the other.

In our studies, we have reexamined these topographical relationships between H-2D, H-2K, TL, Lyt-1, and Lyt-2 by use of the same blocking assay with one modification: Prior to blocking we fixed the thymocytes with paraformaldehyde to prevent movement of cell surface components during the assay.

The results of these studies confirm the original observations of Boyse and co-workers with one notable exception. Whereas on unfixed cells or cells fixed after incubation with blocking antibody, H-2D and TL appeared sufficiently adjacent to produce mutual interference in the attachment of anti-H-2D and anti-TL antibodies, this was not true when cells were fixed with paraformaldehyde before incubation with antibody, TL and H-2D in this situation appearing sufficiently separated from one another to obviate interference in the attachment of anti-TL and anti-H-2D antibodies. These results imply that the positions of TL and H-2D relative to one another are altered by the attachment of specific antibody and that this movement is prevented by paraformaldehyde fixation.

We presume that initially TL and H-2D are separated on the cell surface and that specific antibody triggers a response that brings these two components together. This specific repatterning may exemplify a mechanism whereby cells can specifically change their surface phenotypes without addition or substraction of molecules.

In more recent studies, we have examined the positions of other molecules on the cell surface, H-Y, Qa-2, and H-2L. Our preliminary data on paraformaldehyde-fixed thymocytes indicate that H-Y is sufficiently adjacent to TL such that the attachment of anti-H-Y impedes the attachment of anti-TL and that Qa-2 and H-2L are sufficiently far from H-2D, H-2K, and TL such that the attachment of anti-H-2L or anti-Qa-2 does not interfere with the attachments of anti-H-2D, anti-H-2K, or anti-TL.

Preference of MHC(HLA) Antigens for Altered B_2-Microglobulin. A. R. Sanderson and P. J. Ward. Blond Laboratories, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ, England.

There is a moderately strong dimeric association between the extremely polymorphic major histocompatibility complex (MHC) molecules (e.g. HLA) and nonpolymorphic B_2-Microglobulin (B2M). Following rupture of the two chains, however, the allogeneic determinant can not usually be detected on the larger chain. We seek to understand the nature and significance of this B2M-MHC alloantigen association. In addition, we are exploring the potent immunogenicity of the dimer by coupling it covalently to other unrelated antigens and determinants.

B2M was labelled either with ^{125}I(protein:^{125}I ratio in recovered product = 10:1) or with [^{3}H]-dinitrophenyl ([^{3}H]-DNP) residues (ratio = 2:1). Both products were stable at room temperature for 2 weeks in phosphate-buffered saline (PBS) containing 0.02% sodium azide. Mixing of purified papain-solubilised HLA antigens with iodinated or dinitrophenylated B2M (^{125}I-B2M, [^{3}H] DNP-B2M) in equimolar amounts (about 10^{-7} – 10^{-8}M) at room temperature in PBS, resulted in an association, presumably by an exchange reaction, of labelled B2M with the alloantigenic protein. A more efficient exchange was achieved when the molar proportion of labelled B2M was increased relative to the HLA antigen. Conditions could thus be achieved whereby a majority of the alloantigen chains were associated with labelled B2M. Such dimers were isolated by Sephadex G200 chromatography. When they were then reincubated in the absence of native B2M a slow dissociation into the component chains occurred at room temparature. This process was accelerated when a large molar excess of native B2M was incorporated into the incubation mixture. Under the latter conditions free labelled B2M was released and separated by gel chromatography. By varying the concentration of reactants the dissociation and association rate constants could be calculated together with an overall equilibrium constant for the reaction.

From these measurements it was concluded that the HLA alloantigen chain showed a preferential association for [^{3}H] DNP-B2M and even more so for ^{125}I-B2M. In addition, the half-life of HLA-labelled B2M molecules clearly indicated a stronger association of the two chains than occurs in the native HLA-B2M dimer and in agreement with the order of equilibrium constants calculated.

This preference can also be inferred from the results of others, where human ^{125}I-B2M bound much more strongly to normal mouse lymphocytes than to human peripheral lymphocytes. The additional differences which human B2M will present to H2K and H2D molecules compared to native mouse B2M will superimpose on the iodination label and result in a substantial preference for the xenogeneic association, as was found.

Studies on Structure of H-2 MHC Antigens from Standard and Mutant Strains: Implication for Biologic Specificity.

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In collaboration with B. Ewenstein, T. Nisizawa, H. Uehara, J. L. Brown, R. Nairn, J. E. Coligan and T. J. Kindt, isolation, biochemical characterization, and preliminary alignment of the CNBr fragments of

the H-$2K^b$ glycoprotein has been accomplished. The five major fragments were characterized comprising the NH_2-terminal 80% of the intact K^b glycoprotein.

Complete sequence analysis has been carried out on the first 170 residues and partial sequence analysis has been completed for the 170 to 270 positions. This represents the most extensive sequence data on a mouse MHC molecule. Two carbohydrate moieties are present, the first at position 86 and the second, tentatively, at position 176. Two cysteine linked disulfide loops are present. The first loop is from position 101 to 164, the second loop from position 203 to 258. The papain cleavage site is approximately between positions 270 and 280. Comparative analysis between H-$2K^b$, H-$2K^k$ and H-$2K^q$ in the first 30 residues showed an 85% homology. Similar homologies have been reported between HLA-A and HLA-B glycoproteins.

Analysis of the H-$2K^b$ MHC mutant series was also reported. In earlier studies on the *H-$2K^b$* mutants, tryptic peptide chromatographic analysis of the mutants M505 (bm3) and HZ1 (bm1) showed small but discrete peptide differences between the mutant H-$2K^b$ glycoproteins and the parent H-$2K^b$ glycoprotein. In further studies, each cyanogen bromide fragment was isolated separately and subjected to tryptic peptide analysis. Peptide differences between the K glycoprotein of bm3 and that from the standard strain were localized in CNB fragment Ib and differences between the K molecule of bm1 and that of the standard strain in CNBr fragment Ia.

Initial studies suggest that two different amino acids have been altered in the Ib fragment of bm3 and that both changes are in the region of the carbohydrate binding site. The alterations appear to be at position 76 and position 89. The alteration in the bm1 mutant appears to be a single amino acid exchange. It has been localized to position 155 in the K bm1 molecule. The K glycoprotein of a third mutant, bm11, has been found to be similar but not identical to that of bm3 using peptide maps. These results and additional preliminary data on the H-$2K^b$ mutant series suggests that, at least for the K^b mutants examined so far, single point mutations might be the predominant alteration in the mutant glycoprotein molecule. The changes so far localized occur in the first 150 amino acids from the amino terminal end. These studies suggest that this part of the molecule may be particularly important for T-cell recognition, and further that relatively small discrete changes in amino acid sequence of the H-2 glycoprotein appear to have far-reaching effects in their biological consequences for cellular interaction.

Variation in Expression and Immunogenicity of an H-2K-Coded Alloantigen on Murine Tumours. W. J. Martin and M. Imamura. Division of Virology, Bureau of Biologics, Food and Drug Administration; Immunology and Experimental Pathology Branches, National Cancer Institute, Bethesda, Maryland 20014.

Two distinct H-2K-region coded alloantigens have been identified in mice of *H-$2K^k$* haplotype. One of these alloantigens, designated k-common, is expressed by mice of the C3HfB/HeN strain (C3Hf). The other alloantigen, designated K-unique, is not expressed by C3Hf mice. The *H-2K* haplotype of C3Hf mice has been classified kvl, and the variant antigen distinguishing this strain from mice of *H-$2K^k$* haplotype has been designated kvl-unique. Several transplacentally induced lung tumours of C3Hf mice express the k-unique rather than the expected kvl-unique antigen. Consequently, these tumours grow preferentially in C3H compared to C3Hf recipients and specific radioresistant immunity against these tumours can be induced in C3Hf mice by immunization with normal tissue of mice of *H-$2K^k$* haplotype. The unique antigens distinguishing C3H and C3Hf mice are (1) present on all normal tissues tested including foetal tissue, brain and sperm; (2) capable of stimulating a mixed lymphocyte reaction and the generation of unique-antigen specific cytotoxic T lymphocytes and (3) provide an associative recognition function for *H-2K*-restricted T-cell mediated lysis of viral infected cells. This function is also provided by the k-common antigen. The relative contribution of the unique and common antigens in associative recognition varies with the target cell, strain of mouse immunized, and the specific viral antigen. Mixed lymphocyte reaction derived cytotoxic T lymphocytes specific for the k-unique antigen are capable of suppressing the growth of the C3Hf derived lung tumour 85 in syngeneic recipients. The immunogenicity of the k-unique antigen on C3Hf derived lung tumours and on tumours derived from C3H and A strain mice (*H-$2K^k$* haplotype) varies with different tumours. In particular, the capacity of the k-unique antigen to induce radioresistant immunity in C3Hf mice appears to be lost on several of the tumours even though these tumours are susceptible to in vivo immune responses directed against the k-unique antigen. Alteration in expression and immunogenicity of unique H-2-coded alloantigens may dictate the nature and efficacy of immune surveillance of autochthanous tumours.

Alterations in Expression of Glycoproteins by a Murine Fibrosarcoma Detected with Staphylococcus Aureus. G. N. Callahan, M. A. Pellegrino and L. E. Walker. Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037.

In the process of attempting to characterize the H-2 antigens on the murine fibrosarcoma TP1422, we found an unusual interaction between some of the glycoproteins of these cells and *Staphylococcus aureus.* When radiolabeled TP1422 glycoprotein purified on lentil lectin sepharose from extracts of TP1422 cells was reacted with SACI and the bound glycoproteins eluted with SDS and analyzed by SDS-PAGE we found that many of the glycoproteins were bound by the SACI. This was in marked contrast to what we had seen with normal syngeneic C3H splenocytes. This binding was not via protein A since prereaction of the SACI with serum did not influence the reaction nor did substitution of a strain of *Staphylococcus aureus* which does not bear protein A. Neither did the binding occur as a result of interaction between the TP1422 glycoproteins and the reaction vessel since, when the SACI were omitted from the reaction mixture, no radiolabeled proteins were observed in the SDS-PAGE electropherogram. Rather, it appeared that the binding was the result of widespread alterations in the glycoproteins of the TP1422 cells. This phenomenon was not restricted to the TP1422 fibrosarcoma since we obtained similar results when we reacted P815 cells, a DBA/2 mastocytoma, with SACI. However, we also found that this was not a property common to all murine tumor cells since glycoproteins isolated from 6C3HED, a lymphoma of C3H mice or F9, a teratocarcinoma of strain 129 mice, showed little or no reactivity with the SACI.

Although several glycoproteins were clearly present, one of the major species had M_r of approximately 45 000. Since H-2 antigens also have a molecular weight of approximately 45 000 we attempted to determine whether H-2 antigens might represent a portion of the altered glycoprotein on the TP1422 cells and as a result bind to SACI. Therefore, we reacted a radiolabeled TP1422 glycoprotein preparation sequentially with SACI until all material which reacted with SACI had been removed. This depleted glycoprotein preparation was then reacted with an anti-H-$2K^k$, Ia^k alloantiserum. The results clearly showed that H-2 and Ia antigens were undetectable in this depleted extract. When similar experiments were performed using glycoproteins purified from extracts of normal C3H splenocytes, H-2 and Ia antigens were demonstrable in SACI depleted extracts. Since we had previously demonstrated that these tumor cells expressed H-2 but not Ia antigens by both microcytotoxicity and indirect rosetting assays with monoclonal anti-H-$2K^k$ antibody, these results suggested that H-2 antigens might well be involved in the observed modifications to the glycoproteins of the TP1422 fibrosarcoma.

Immunochemical Properties of H-2 Antigens on SL2 Lymphoma Cells. R. Robinson and V. Schirrmacher. German Cancer Centre, DKFZ, D-6900 Heidelberg, Federal Republic of Germany.

A number of observations have suggested that the expression of mouse histocompatibility antigens may differ between tumour cells and lymphocytes from the strain of origin. Such differences have been detected by direct cytotoxicity and absorption tests using anti H-2 sera (Garrido, F., Festenstein, H., and Schirrmacher, V.: *Nature 261*:705, 1976, Meschini, A., Invernizzi, G., and Parmiani, G.: *Int. J. Cancer 20*:271, 1977) and have indicated that tumours may gain or lose H-2 determinants relative to the corresponding lymphocytes.

SL2, an established lymphoma cell line of DBA/2 (H-2^d) mice reacted in cytotoxicity tests with some antisera directed against H-2 determinants of other haplotypes. Some of these sera were tested on SL2 using an immunoprecipitation assay and SDS polyacrylamide gel electrophoresis and found to precipitate molecules with a molecular weight similar to that of H-2 heavy chains. One of these sera, D15, produced in strain combination B10A(2R) × A.CA anti B10WB, reacted strongly with SL2 cells in immunofluorescence and cytotoxic assays but did not react significantly with DBA/2 lymphocytes under similar conditions. This antiserum also precipitated components of molecular weight 45 000 and 12 000 daltons from SL2 cell extracts.

DBA/2 spleen cells, solubilised in Triton X-100 detergent, were able to block the reaction of serum D15 with SL2 antigens indicating that DBA/2 carries the same or a similar antigen. Complete blocking was also achieved with extracts of B10A (H-2^a) and B10WB (H-2^{ja}) cells, but not with B10BR, indicating that the antiserum reacts with antigens on SL2 coded for by the *D* region of the *H-2^d* haplotype. This appears to be the result of an antigen shared between H-2^{ja} and H-$2D^d$. Similar results were obtained using a sequential immunoprecipitation technique in which the D, K, and L region molecules of the *H-2^d* haplotype were removed prior to treatment with D15 antiserum. This experiment confirmed that the antiserum reacts principally with the H-$2D^d$ molecule.

Cytotoxic data suggested a diminished expression of at least one H-2 determinant on the surface of DBA/2 lymphocytes. This determinant could be rendered accessible to antibodies by destruction of the cell membrane with detergent. We therefore compared the absorption capacity of intact and solubilised lymphocytes and tumour cells. D15 antiserum was pretreated with either intact or Triton X-100 solubilised DBA/2 or SL2 cells and the remaining antibodies tested for their capacity to preciptitate ^{125}I-labelled H-2 antigens from SL2 cells. The amount of radioactive material of molecular weight 45000 daltons was measured. The results show that solubilised DBA/2 lymphocytes absorbed D15 antiserum about ten times as efficiently as intact cells. Solubilised SL2 cells absorbed the activity about twice as effectively as intact cells. There was an overall increase in the amount of H-2 antigen on SL2 cells compared with DBA/2 lymphocytes. This increase can be attributed to the difference in size between SL2 cells and spleen lymphocytes.

Pulse-chase experiments have shown that both the H-2D^{d} and H-2K^{d} antigens of SL2 lymphoma cells are processed during intracellular transport, and that the antigens which appear at the cell surface are heterogeneous with respect to molecular weight. This difference is probably due to heterogeneity in the carbohydrate side chains. Although it is generally accepted that carbohydrate per se is not part of H-2 antigenic determinants it is possible that its presence may impose a steric restraint on the binding of anti H-2 antibodies and account for the variation in expression of certain H-2 determinants. Studies are in progress to determine whether these or other post-translational modifications can give rise to unexpected changes in the expression of H-2 antigens.

Heterogeneity of H-2D Region Molecules, Recognized by Anti H-2.28 Sera. D. Iványi and P. Démant. Division of Genetics, Antoni van Leeuwenhoekhuis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

By the surface antigen redistribution method (double labeling immunofluorescence, "capping") we have studied the molecular relationship between private and public H-2 specificities of the *D* region of *b*, *f* and *s* alleles. In each allele, we identified two distinct molecules, H-2D, which reacts with sera against D-region private specificity (anti H-2.2, H-2.9 and H-2.12, respectively) and H-2L, which does not react with anti-private sera but with sera directed against H-2.28 family of specificities.

Independence of H-2D and H-2L molecules on the cell surface was tested on H-2^{a} (K^{k}D^{d}L^{d}) cells. This independence has not been shown yet, because the sera used for redistribution of H-2L^{d} molecules possessed besides anti H-2L28 antibodies also anti H-2D28 antibodies and thus redistribution of H-2D molecules was achieved together with redistribution of H-2L molecules, while anti D^{d} private sera (anti H-2.4) were able to distinguish between H-2D^{d} (H-2.4 positive) and H-2L^{d} (H-2.4 negative) molecules (Lemonnier et al.: *Immunogenetics 2*:517–529, 1975). However, when anti-H-2L28 sera (e. g., *k* anti *b* are absorbed with dm2 mutant cells, only H-2L^{d} molecules are capped, while H-2D^{d} molecules remain outside the caps.

The products of the *D* region were further studied in mutant mouse strain B10.D2 (M504), (H-2^{dm1}, previous designation H-2da). Serological and capping experiments have shown that also mutant haplotype *dm1* has two molecules in the products of the *D* region, H-2D^{dm1}, detectable by anti H-2.4 sera and H-2L^{dm1}, detectable by anti H-2.28 sera. However, both H-2D^{dm1} and H-2L^{dm1} molecules differ serologically from the original nonmutant B10.D2 strain.

Anti H-2.28 sera produced in different combinations besides anti-L28, could also possess anti-D28 or anti-K28 activity which could be tested serologically and by absorption experiments on *d* (K28+, D28+, L28+), *dm2* (K28+, D28+, L28−) and *0* (K28+, D28−, L28−) haplotypes. By anti H-2.28 serum possessing both anti D28 and L28 activity both D and L molecules should be redistributed. By using such a serum (*r*/*k* anti *h2*) we could cap all H-2.4 positive (D^{d}) molecules in the *dm1* mutant haplotype. However, the same antiserum could cap only a part (30–40%) of H-2.4 positive molecules in *dm2* and *d* haplotypes, while by anti H-2.4 serum we could cap for anti D28 serum in *dm2* mutant. When another anti H-2.28 serum (*k* anti *b*) also possessing anti H-2.35 and anti H-2.36 antibodies was used for capping, all H-2.4 positive molecules were capped in *dm1* as well as *d* and *dm2* haplotypes. These experiments indicate that the D^{d} region produces, besides the H-2.4 negative H-2L^{d} molecule, two other molecules, H-2D and H-2M. These can both be capped by anti H-2.4 serum but only one of them is capped by some anti H-2.28 sera.

Wether the "new" H-2M molecule is a product of a "new" locus (*H-2M*) or whether H-2.4 positive molecules coded by *H-2D* locus are heterogenous with respect to the expression of some public specificities, is not known. The similar phenomenon of heterogeneity of molecules bearing D private and also K private specificities is indicated by our recent experiments which also include other haplotypes.

Isolation and Characterisation of H-2 variants with the Fluorescence Activated Cell Sorter. B. Holtkamp, K. F. Lindahl, M. Segall, and K. Rajewsky. Institute for Genetics, University of Cologne, Cologne, Federal Republic of Germany.

We are interested in the regulation of *H-2* expression and isolate for this purpose variants of *H-2* expression with the help of the Fluorescence Activated Cell Sorter (FACS). H-2 negative variants were selected from the heterozygous lymphoma cell line LDHB ((C3H × DBA/2)F_1, H-2$K^{k/d}D^{k/d}$) which generates H-2K^k negative variants in high frequency when growing in culture. The analysis of the cloned sublines by serological methods and cell mediated cytotoxicity showed that in the clones the expression of antigens encoded by the *H-2* regions K^k, D^k and K^d was uncorrelated, while the expression of H-2K^k antigens detectable in fluorescence analysis correlated well with the susceptibility to cell-mediated cytotoxicity by H-2K^k specific cytotoxic T cells. In part of the negative variants, *H-2* expression could be stimulated by growth in vivo or by interferon (IF) preparations, indicating that at least these variants were regulatory. Even a totally H-2K^k negative clone which could not be stimulated to reexpress H-2K^k antigens, expressed antigens of the *H-2* regions D^k and K^d. The loss of H-2 antigen was not limited to the *H-2*K^k region. We have also isolated an H-2D^k negative clone which still expressed K^k and K^d antigens. In contrast to the K^k negative clone, the D^k negative cells expressed the D^k antigen when grown in vivo or upon treatment with IF preparations. In contrast to cells growing in vivo, cells stimulated for expression of H-2 antigens by IF were less susceptible to lysis by H-2K^k specific cytotoxic T cells than would have been expected from the amounts of H-2K^k antigens detectable on their surface in fluorescence analysis. In fact, IF treated cells were even less susceptible than cells of the same clone growing in culture without IF. This phenomenon does not seem to be due to a resistance of the IF treated cells to the lytic mechanism of the cytotoxic T cells because IF treated cells could be lysed as effectively as untreated cells or cells grown in vivo when they were unspecifically bound to the cytotoxic T cells by phytohemagglutinin.

Attempts to isolate spontaneous variants of myeloma cells which express "wrong" H-2 antigens (H-2D^d on H-$2^{k/k}$ cells) have entirely failed. The results indicate that if such variants occur in this system their frequency is either below 1×10^6 or they are at a growth disadvantage as compared to the wild type cells.

Biological and Biochemical Characterization of Alien H-2 Antigens of a Chemically Induced Sarcoma. Giorgio Parmiani. Division of Experimental Oncology A, Instituto Nazionale per lo Studio e la Cura dei Tumori, Via G. Venezian 1, 20133 Milano, Italy.

The presence of alien H-2 antigens has been studied on a methylcholanthrene-induced sarcoma of the BALB/c (H-2^d) strain. A serological approach was first undertaken and a BALB/c (H-2^d) sarcoma was shown to possess all the private and public antigens of *H*-2^d haplotype (H-2.31, 4, 3, 8, 35, 28) by its capacity to specifically absorb the C'-dependent cytotoxicity of monospecific anti-H-2 alloantisera on normal ^{51}Cr-labeled BALB/c lymphoid cells. In a similar assay, this tumor (C-1) was also able to absorb sera directed to the H-2K^k specificities 23, 1, 5, 11 and 25, although other similarly induced BALB/c sarcomas were not. A syngeneic serum raised in BALB/c mice to C-1 sarcoma cells was studied in a C'-dependent cytotoxicity test on normal lymphoid cells and by absorption and found to contain anti-H-2^k-like antibodies. Histogenetic experiments were carried out in which it could be shown that BALB/c mice immune to BALB.K (H-2^k) congenic mice but not to BALB.B (H-2^b) normal tissues develop resistance to the subsequent challenge of C-1 sarcoma. Immunochemical studies also showed that partially purified H-2^k-like antigens of C-1 were distinct from molecule expressing normal H-2^d antigens by the sequencial RIP assays. Like normal H-2 antigens, alien H-2^k-like determinants have a m.w. of 48000, are glycoproteins and are noncovalently bound to B_2-microglobulin. The alien H-2^k antigens appear to differ from H-2^d antigens in their susceptibility to papain digestion and overall stability.

Altogether, the data suggest that the alien antigens may have appeared through a derepression of silent genes caused by the neoplastic transformation.

Autoreactive Cytotoxic T Lymphocytes Specific for H-2D Antigens. Gustavo Cudkowicz, Keiichiro Nakano, and Ichiro Nakamura. Departments of Pathology and Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214.

Responder spleen cells of B6D2F_1 hybrid mice (*H*-$2^{b/d}$) cultured in vitro with irradiated parental B6 spleen cells (*H*-$2^{b/b}$) generate cytotoxic T lymphocytes (CTL) specific for target cells sharing genes of the *H*-2^b haplotype with parental stimulators. The F_1 anti-parent cell-mediated lympholysis (CML) response was developed as an in vitro correlate of natural resistance of lethally irradiated mice to the

growth of transplanted bone marrow cells of parental or allogeneic donors. Anti-parent cytotoxicity can be induced in spleen cell cultures of several other hybrid strains, not only against *H-2^b^* but also against *H-2^d^* or *H-2^k^* homozygous cells. Sensitive targets for such F_1 anti-parent CTL are mitogen-stimulated splenic lymphoblasts, thioglycollate-induced peritoneal exudate cells (PEC), or lymphoma cells.

Two major topics related to the in vitro development of F_1 anti-parent CML will be addressed: (1) the identification of the *H-2* region coding for, or regulating the expression of target determinants in $H\text{-}2^b$-homozygous cells; and (2) the recognition of such determinants by F_1 effector cells on $H\text{-}2^b$-heterozygous targets, a manifestation of autoreactivity. Cytotoxic effectors were generated in 5 day $B6D2F_1$ anti-B6 mixed spleen cell cultures and then tested on cells labeled with radioactive chromium. Specific target lysis was measured in 4 chromium release assays; PEC were used as targets in direct cytolysis and as cold inhibitors in competitive inhibition of specific lysis.

PEC from several unrelated $H\text{-}2^b$ mouse strains were lysed to about the same extent by F_1 anti-B6 effectors in direct cytotoxic assays, irrespective of polymorphism at a multiplicity of loci other than *H-2*. Specificity was demonstrated by the lack of significant lysis of $H\text{-}2^a$, $H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^s$ targets. Positivity for $H\text{-}2D^b$ was the necessary and sufficient condition for target cell lysis, irrespective of the alleles present at other *H-2* regions. Critical targets for this conclusion were not only those of $H\text{-}2^b$-positive strains with recombinant $H\text{-}2^b$ haplotypes, but also those of $H\text{-}2D^b$-negative $H\text{-}2K^b$-positive mice. The involvement of genes in the *T* region was excluded by results obtained with PEC of two recombinant strains between *D* and *T*. Heterozygous F_1 hybrid PEC were not significantly lysed even though such cells possess one copy of $H\text{-}2D^b$ and associated *D*-region genes. The mapping of target determinants to the *D* region was confirmed by competitive inhibition experiments.

PEC syngeneic with $B6D2F_1$ anti-B6 CTL – or, in general, $H\text{-}2D^b$-heterozygous PEC – were not lysed under standard conditions. Moreover, F_1 spleen cells did not induce cytotoxicity when used as irradiated stimulators. No evidence for autoreactivity was thus obtained from the direct analysis of the inductive and effector phase of CML. F_1 PEC were capable, however, of competitively inhibiting specific anti-parental B6 target cell lysis. The inhibition by F_1 PEC was less effective, for given numbers of cells, than that afforded by $H\text{-}2^b$-homozygous PEC. For inhibition of cytotoxicity to occur, PEC from heterozygotes had to possess one copy of the $H\text{-}2D^b$ allele, irrespective of the alleles at other *H-2* regions. The critical F_1 hybrids were derived from parental mice bearing recombinant $H\text{-}2^b$ haplotypes. F_1 embryonic fibroblasts replaced F_1 PEC in inhibition assays; this observation favors the competitive as opposed to the blocking mechanism of inhibition.

The existence of lysis-negative, inhibition-positive F_1 hybrid target cells in F_1 anti-parent CML is an indication of autorecognition of MHC products not leading to target cell death. If operative in vivo, such autorecognition may represent a mechanism for the control of normal and transformed hemopoietic cells.

Target Determinants for F_1 Anti-Parent and Allogeneic Anti-$H\text{-}2^d$ Cytotoxic T Cells on Cells of $H\text{-}2^{dm1}$ and $H\text{-}2^{dm2}$ Type Ichiro Nakamura, Keiichiro Nakano, and Gustavo Cudkowicz. Departments of Pathology and Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214.

Primary $B6D2F_1$ anti-parental DBA/2 mixed spleen cell cultures generate cytotoxic effectors specific for $H\text{-}2^d$ targets including peritoneal exudate cells (PEC) used in this study. Target determinants are controlled by the *D* end of the *H-2* haplotype according to the results of recombinant analysis, i. e., the substitution of DBA/2 targets with PEC bearing the products of $H\text{-}2^d$ recombinant haplotypes. Two *D*-end mutations of $H\text{-}2^d$ – the loss-gain $H\text{-}2^{dm1}$ mutation and the *H-2L* gene deletion referred to as $H\text{-}2^{dm2}$ – were investigated to determine (1) whether the products of both the *H-2D* and *H-2L* loci contribute to F_1 anti-parent cytotoxicity; and (2) whether the cross-lysis of $H\text{-}2^d$ and $H\text{-}2^{dm1}$ targets by allogeneic $H\text{-}2D^b$ anti-$H\text{-}2D^d$ cytotoxic T lymphocytes (CTL) also occurs when F_1 anti-parent CTL are tested.

H-2L gene products need not be present on homozygous stimulators to evoke $B6D2F_1$ anti-$H\text{-}2^d$ cytotoxicity: $H\text{-}2^{dm2}$ cells induce $B6D2F_1$ effectors that lyse equally well $H\text{-}2^d$ and $H\text{-}2^{dm2}$ targets. Furthermore, the lysis of $H\text{-}2^d$ or $H\text{-}2^{dm2}$ targets by such effectors is inhibited by cold $H\text{-}2^d$ as well as by $H\text{-}2^{dm2}$ PEC, presumably because the F_1 effectors are specific for H-2D antigens. When H-2D and H-2L gene products are both present on stimulators (e. g., DBA/2 or BALB/c cells), cytotoxic activity of F_1 effectors is directed against determinants controlled by both *H-2D* and *H-2L*. The direct lysis of $H\text{-}2^d$ targets ($D^d + L^d$) is significantly greater than that of $H\text{-}2^{dm2}$ targets (D^d only), and $H\text{-}2^{dm2}$ cells are less effective than $H\text{-}2^d$ cells in inhibiting F_1 anti-$H\text{-}2^d$ CTL assayed on $H\text{-}2^d$ targets. However, $H\text{-}2^{dm2}$ and H-

2^d cells are equally effective in inhibiting cytotoxicity directed exclusively against $H\text{-}2D^d$ gene products, as in F_1 anti-DBA/2 CTL assayed on $H\text{-}2^{dm2}$ targets. Hence, target antigens for $B6D2F_1$ anti-$H\text{-}2^d$ CTL are multiple (i.e., a minimum of two) and genetically controlled by structural or regulatory genes associated with *H-2D* and *H-2L*. The response to *H-2D* is independent of that to *H-2L* for both the inductive and effector arms.

Target determinants lost as a consequence of the $H\text{-}2^{dm1}$ mutation map to both the *H-2D* and *H-2L* loci according to an analysis with allogeneic effectors. The existence of gain-type determinants controlled by the *D* end was confirmed, but such determinants could not be assigned to the *H-2D* or *H-2L* genes. Despite these alterations of D-end gene products, $H\text{-}2^{dml}$ cells retain surface structures cross-reactive with antigens controlled by the unaltered $H\text{-}2D^d$ and $H\text{-}2L^d$ genes. Results of direct lytic assays and cold inhibition tests are consistent in this regard. Moreover, $B6D2F_1$ anti-parental $H\text{-}2^d$ CTL extensively cross-lyse $H\text{-}2^{dml}$ targets. Hence, the effect of the $H\text{-}2^{dm1}$ mutation on target structures for F_1 anti-parent and for allogeneic CTL is qualitatively indistinguishable. Cross-lysis of $H\text{-}2^d$ and $H\text{-}2^{dml}$ targets by F_1 antiparent CTL is in sharp contrast to the lack of such cross-lysis by *H-2D*-restricted anti-viral CTL.

It was noted both in F_1 anti-parent and allogeneic responses induced by stimulation with unaltered $H\text{-}2D^d$ or $H\text{-}2L^d$ antigens that the CTL population included clones reacting more strongly with $H\text{-}2D^{dml}$ or $H\text{-}2L^{dml}$ antigens of PEC targets than with the inducing antigens.

Inappropriate Alloantigen-Like Specificities Detected on Reticulum Cell Sarcoma of SJL/J Mice. Benjamin Bonavida and Janet M. Roman. The Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024.

The SJL/J strain of mice was established in 1963 at Jackson Laboratories by Murphy. It exhibits a very high incidence of reticulum cell sarcoma (RCS) which originate in mesenteric lymph nodes and Peyer's patches. Our studies have shown that normal and tumor-bearing mice are immunocompetent and elicit humoral and cell-mediated immune responses. The percentage of successful takes of primary tumors in syngeneic hosts is low, implying an active tumor resistance on the part of the recipient.

All kinds of tumors (spontaneous, transplantable, and cell lines) stimulate a very strong syngeneic response in a mixed lymphocyte tumor interaction as assessed by ^3H thymidine incorporation. The cell lines in vitro served good stimulator and targets in mixed leukocyte cultures, whereas the in vivo passaged lines were not consistently good stimulators or targets for syngeneic killing. However, all in vivo and in vitro lines served as good stimulators and good targets for allogeneic killing. An unpredicted finding was the demonstration that anti-RCS cytotoxic cells also killed allogeneic targets, particularly those bearing $H\text{-}2^d$ or $H\text{-}2^b$ alloantigens. Furthermore, normal SJL/J lymphocytes sensitized to normal allogeneic cells killed syngeneic RCS. Cold target cell competition showed that SJL/J anti-BALB/c cytotoxic cells are competed out by BALB/c and SJL/J reticulum cell sarcoma cells, suggesting that these tumor cells share killer cell target antigens with BALB/c. Other target cells were used as competitors and did not compete with the response. Further studies were investigated to delineate the nature of inappropriate-like specificities present on RCS. Both SJL/J anti-BALB/c and SJL/J anti-C57B1/6 sera were cytotoxic for in vitro RCS lines, and the reactivities were absorbed out with BALB/c and C57B1/6 cells, respectively. Anti-K^d and anti-D^d sera were cytotoxic for RCS in culture, whereas anti-K^b and D^b sera were not. Anti-K^s and D^s were not cytotoxic for RCS cells maintained in culture, although these cells fluoresce with anti-H-2 sera. In contrast to the in vitro lines, freshly explanted RCS in vivo were poorly cytotoxic with anti-$H\text{-}2^d$ and $H\text{-}2^b$ sera, while they were cytotoxic with anti-$H\text{-}2^s$ sera. Most of the in vivo tumor cells, however, fluoresced with anti-$H\text{-}2^d$ and anti-$H\text{-}2^b$ sera. Furthermore, of 33 spontaneous tumors examined, 22 or 67% were positive with either anti-$H\text{-}2^d$ or $H\text{-}2^b$, or both. These results suggested that both in vitro and in vivo RCS lines express $H\text{-}2^d$ and $H\text{-}2^b$ allospecificities as detected by cell-mediated cytotoxicity, cytotoxic antibody, indirect immunofluorescence and quantitative absorption.

The nature of the molecules involved in cross-reactivity between tumor and allogeneic cells was critically examined by the two-dimensional gel electrophoresis techniques originally described by O'Farrell which allows comparison of molecular weight and isoelectric points of molecules. Normal SJL/J or BALB/c lymph node cells and either in vivo or in vitro RCS tumor cells labeled with $^S35$ methionine were lysed with NP-40 in the presence of 0.1% SDS and were precipitated in the presence of various specific antisera or normal mouse sera with *S. aureus* organisms. The precipitates were analyzed by two-dimensional polyacrylamide gel electrophoresis. Antibodies against determinants on allogeneic cells bearing $H\text{-}2^d$ precipitate molecules from normal BALB/c lymph node cells and precipitate very similar molecules from SJL/J in vivo and in vitro RCS tumor cells. Furthermore, normal SJL/J lymph node cells also express a molecule of 45000 molecular weight which is precipitated by anti-$H\text{-}2^d$ sera and

which exhibits a pattern on 2D gels nearly identical to the BALB/c pattern. Thus, it appears that the inappropriate specificities apparent on tumor cells are also detectable on normal cells, but these are cryptic on the surface membrane of normal cells.

The alloantigens on tumor cells are immunogenic in SJL/J since tumor bearing animals have been shown to contain in their serum activities which react with both tumor cells and H-2^d or H-2^b cells but not with normal SJL/J cells. In spite of the in vivo immunogenicity of RCS tumor cells, the tumor grows and kills the animals. We have demonstrated that tumor enhancement may be the result of specific anti-tumor antigen reactive cell opsonization by circulating antigen-antibody complexes and macrophages. (Supported by NIH grants CA 19753 and CA 24314 from the National Cancer Institute.)

Foreign H-2-Like Alloantigens on a Murine Tumor Recognized by Syngeneic Cytolytic T Lymphocytes. V. Schirrmacher and F. Garrido. Institut für Immunologie und Genetik am Deutschen Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany and Tumorimmunology Unit, Facultad Medicina, Granada, Spain.

The question of regulation of expression of major histocompatibility complex (MHC) genes was studied by comparing MHC products on normal cells and tumor cells of the mouse. The rationale for this was the impression that gene expression in tumor cells is less stringently controlled than in normal cells as exemplified by lung tumors producing pituary hormones or by various tumors expressing fetal antigens or endogeneous viral antigens. We also hoped to learn about the high frequency of alloreactivity observed as a result of stimulation of syngeneic lymphoid cells by tumor cells or virus-infected cells.

We previously reported on qualitative and quantitative differences in the expression of H-2 antigens on long term passaged murine lymphomas when compared to normal lymphoid cells. Now we report on our results obtained with a newly induced tumor of BALB/c (H-2^d) origin. This methylcholanthrene induced tumor, MCG4, which could be adapted to the ascites form and to tissue culture, had a high rate of spontaneous regression in syngeneic animals. Serological *H-2* typing revealed a loss of four antigenic determinants expressed by normal H-2^d lymphoid cells and a gain of one public determinant, H-2.5, not expressed by normal H-2^d cells. The only private specificity detectable was H-2.31, which characterizes K^d molecules. No reactivity of MCG4 was observed with anti H-2.4 (D^d), anti H-2.23 (K^k) or anti H-2.32 (D^k) sera. MCG4 induced in BALB/c mice a strong isoantiserum reacting specifically with the tumor and with normal foreign lymphocytes of defined H-2 type but not with normal H-2^d type cells.

Foreign H-2-like antigens on MCG4 were also detected by syngeneic cytolytic T lymphocytes (CTL).

In summary, the following results were obtained:

1. Specific reactivity of MCG4 (BALB/c) with BALB/c anti H-$2K^k$ CTL, BALB/c anti H-$2D^k$ CTL and BALB/c anti H-$2K^b$ CTL.
2. Induction of primary and secondary BALB/c anti MCG4 CTL responses in vitro.
3. Alloreactivity of the syngeneic BALB/c anti MCG4 CTL; reactivity with lymphoblasts of the H-2 type K^k, D^k, K^b, H-2^r, H-2^s and others; no reactivity with H-2^d type lymphoblasts;
4. Specific neutralization of alloreactivity in (BALB/c × H-2^b)F_1 and (BALB/c × H-2^k)F_1 animals; these animals were more susceptible to the tumor and showed a reduced but not completely abrogated CTL response against MCG4.
5. Distinction of three cross-reactive allogeneic determinants on MCG4 by competitive cold target inhibition; one K^k-like one D^k-like and one broadly cross-reactive;
6. K^k-like molecules on MCG4 functioning as restricting element for H-2^k-derived Sendai virus specific CTL.

These and the serological data indicate that the tumor MCG4 expresses alloantigens which fulfill most criteria of true foreign H-2 molecules. They appear similar to K^k and D^k but, since they do not carry the private specificities, their exact identity remains obscure. These molecules could either be products of derepressed genes normally expressed only in foreign (as yet undetermined) haplotypes or they could be mutated or otherwise modified H-2^d gene products. The latter alternative appears less likely in view of the simultaneous expression on the tumor of two H-2^k-like molecules. An immunochemical characterization of the foreign H-2-like molecules is presently being performed.

Association Between H-2 and Vaccinia Virus-Induced Antigens on the Membrane of H-2^d Infected Cells. Anna Senik, Catherine Neauport-Sautes, and Aimé Vazquez. Laboratoire d'Immunologie Cellulaire, I.R.S.C., 94800 Villejuif, France.

Mild infection of K-Balb fibroblastic cells and LSTRA lymphoma cells led to the appearance on the cell membranes of vaccinia virus-induced antigens without alteration of their H-2 antigens as assessed by immunofluorescence titration of anti-H-2 antibodies, and by their susceptibility to lysis by alloreactive CTL's. By the differential redistribution method, we have studied the relationship between H-2 molecules and vaccinia antigens on LSTRA infected cells. Monospecific anti-H-2 sera which induced the complete capping of either H-2K or H-2D, or H-2L molecules, also induced the co-migration of all vaccinia-antigens. This suggests that H-2 molecules cluster round vaccinia antigens. However, only part of H-2 molecules are involved in this association since capping of vaccinia antigens upon addition of anti-vaccinia antibodies did not induce the complete redistribution of H-2 molecules. Moreover, we could still detect independent migrations between nonassociated H-2K and H-2D molecules, on one hand, and between H-2D and H-2L molecules on the other hand.

To assess the specificity of these cocapping events, two other markers of LSTRA cells were studied: FMR antigens expressed on 100% of the cells and Fcγ receptors allowing 20% to 60% rosetting cells in presence of IgG coated SRBC. Capping of FMR antigens by anti-FMR antibodies or capping of Fcγ receptors by aggregated rabbit IgG still allowed the detection of vaccinia antigens outside the capped FMR antigens or Fcγ receptors. Therefore, the observed co-migration between H-2 and vaccinia antigens is in favor of a specific association between H-2 molecules and vaccinia antigens.

These results were strengthened by antisera blocking experiments. Monospecific alloantisera directed against the private or public H-2 specificities of K-Balb infected cells, exerted potent blocking capacity on the vaccinia-immune T-cell killing. Strikingly each monospecific alloantiserum was able by itself to block the killing almost completely. By contrast, anti-fibronectin serum directed to another membrane component had no inhibitory effect. Concerning H-2L gene products, their ability to induce the co-migration of vaccinia antigens under capping conditions, and the blocking of an anti-vaccinia CMC by an anti-H-2L serum strongly suggest a physical association between H-2L and vaccinia virus encoded molecules. However, H-2^q and H-2^d mice, although bearing serologically identical H-2L molecules, do not produce cross-reactive vaccinia-immune CTL's. This implies, either that H-2L molecules are not target determinants for vaccinia-immune CTL's or that they express private and public determinants.

Whatever the case may be, of particular importance may be the fact that any H-2 product is able to associate with viral products. It may be the minimum requirement for the generation of anti-viral CTL's.

H-2 Public Specificities Act as Target Determinants for Alloreactive Cytotoxic T Lymphocytes. C. Neauport-Sautes, A. Vasquez, and W. H. Fridman. Laboratoire d'Immunologie Cellulaire, I.R.S.C., B.P. No. 8, 94800 Villejuif, France.

The *H-2K*, *H-2D* and *H-2L* loci code for the 45 000 d H-2 molecules which bear the serologically detectable H-2 specificities. Besides controlling the expression of H-2 specificities, these *H-2* loci code for antigens acting as target determinants for alloreactive cytotoxic T lymphocytes (CTL). Whether the determinants on the H-2 molecules recognized by anti-H-2 antibodies are identical to those recognized by CTL is unclear as yet. In the present study, we investigated the specificity of secondary stimulated alloreactive CTL. Their killing capacity was tested on target cells expressing public H-2 specificities which the effector cells should theoretically be able to recognize as target determinants. In view of their wide distribution among haplotypes and of their expression on the H-2K, H-2D and H-2L molecules, the reactivity of CTL towards public specificities of the H-2.28 and H-2.1,5 families was most extensively studied. The cytotoxic activity of cells from H-2.28 or H-2.1,5 negative recipients stimulated with cells from H-2.28 or H-2.1,5 positive mice, respectively, was tested on a panel of 51 chromium labeled target cells. The following results were obtained: (1) 70% to 80% of lysis was observed on target-cells identical to stimulating cells (i.e., sharing the same private and public specificities) and (2) 20% to 40% of lysis was observed on target cells sharing at least the public specificities of the H-2.28 or the H-2.1,5 family with the stimulating cells. In all combinations, cross killing was always observed when the target cells expressed public specificities that the cytotoxic T cells should theoretically recognize. In contrast, using B10Y anti B10 RIII effector-cells, no lysis was found on ACA target cells not expressing public specificities recognized, according to the H-2 chart, by the effector cells. Indeed, the same effector cells could cross kill other target cells where public specificities could theoretically be recognized. Since in some combinations, interference of Ia or Qa antigens could be ruled out for genetic reasons, our data suggest that (1) alloreactive cytotoxic T lymphocytes recognize the public specificities of H-2 molecules, (2) recognition of only public specificity is sufficient to induce killing.

These observations were substantiated by the fact that the serological cross-reactions observed between the K and D region products of the $H\text{-}2^d$ and $H\text{-}2^k$ haplotypes also occured at the CML level. CTL raised against K^d region products killed target cells expressing D^d region products and vice versa. The same observations were made in the $H\text{-}2^k$ haplotype.

Anti-H-2 antisera directed against public specificities recognized by the CTL blocked the lysis as well as antisera directed against any H-2 specificity (private or public) expressed on the same molecule as the public specificity acting as target determinant. In contrast, anti H-2 antisera reacting against the other H-2 molecule did not exert any inhibition. The fact anti H-2 antisera directed against private specificities block the cross killing toward public specificities is probably due to a steric hindrance effect. It is similar to previous serological observations showing that antibodies directed against private specificities impede further fixation of antibodies directed against public specificities on the same molecule.

Variations in H-2 Expression in P815 Cells after Infection with Vesicular Stomatitis Virus. Paul L. Black, Ellen S. Vitetta, James Forman, Chil-Yong Kang, and Jonathan W. Uhr. Department of Microbiology, University of Texas Health Science Center, Dallas, Texas 75235.

We have investigated the molecular nature of the cell surface modifications which render virus-infected cells susceptible to lysis by syngeneic, virus-immune cytotoxic T lymphocytes. Three major observations have emerged from our studies of molecular changes on the surfaces of P815 cells infected with vesicular stomatitis virus (VSV). (1) Glycosylation of *H-2* and/or viral glycoprotein is a prerequisite for *H-2*-restricted cytolysis of VSV-infected cells. The antibiotic Tunicamycin specifically inhibits the addition of sugars to the polypeptides of glycoproteins by preventing transfer of the core sugar N-acetyl glucosamine. Treatment of P815 cells with Tunicamycin inhibited glycosylation of *H-2*. An H-2 peak could not be detected by immunoprecipitation in the glycoprotein pool (GPP), eluted from *Lens culinaris* lectin-Sepharose, from lysates of Tunicamycin-treated cells labeled biosynthetically. Treatment with Tunicamycin before and during VSV infection also inhibited cytolysis of infected P815 cells by syngeneic, virus-immune cytotoxic cells. This inhibition was seen over a wide range of multiplicities of infection (MOI), from 5–500 plaque-forming units (PFU)/cell. Tunicamycin treatment did not inhibit surface expression of VSV antigens, as judged by immunoprecipitation of lysates of surface-labeled, infected cells with rabbit anti-VSV serum. However, we have no direct evidence that the viral glycoprotein (G) is being expressed on the cell surface. In contrast, Tunicamycin treatment had no effect on lysis of P815 cells by alloreactive cytotoxic cells. Thus, the inhibition of *H-2*-restricted lysis does not result from non-expression of *H-2* on the cell surface or from nonspecific inhibition of the lytic process by Tunicamycin. (2) VSV infection caused a signficant decrease in the amount of *H-2* on the surfaces of infected cells. Surface radioiodination of P815 cells 3 h after VSV infection revealed a decrease in *H-2* detectable by immunoprecipitation in the GPP of the cell lysate. The size of the decrease varied from about a third to 100% in different experiments. The decrease probably does not result from alteration in the antigenicity of *H-2* or from loss of surface *H-2* from the cell during VSV maturation. No decrease in the amount of *H-2* in the cells could be detected by immunoprecipitation when cells were surface radiolabeled prior to VSV infection. Thus, the decrease in surface *H-2* after VSV infection results from blocking of surface *H-2* from radioiodination or from displacement and internalization of surface *H-2* during VSV maturation at the cell surface. (3) Surface *H-2* and viral antigens do not form a detergent-stable physical association. P815 cells were surface-labeled, then infected with VSV (MOI of 75 PFU/cell) and lysed. The GPP was treated with rabbit anti-VSV (or control) serum and *S. aureus* in order to deplete VSV antigens. If surface *H-2* and viral antigens form stable associations, then depletion of VSV antigens should also have depleted *H-2*. Depleting VSV antigens by immunoprecipitation had no detectable effect on the subsequent recovery of *H-2*. We estimate that a 15–20% depletion of *H-2* could have been detected. The results, therefore, provide no evidence that H-2 antigens form detergent-stable associations with viral antigens.

Protective and Destructive Effects of H-2 Antibodies on Allografts. R. A. P. Koene, P. J. A. Capel, R. M. W. de Waal, J. H. M. Berden, S. P. M. Lems. Dept. of Medicine, Division of Nephrology, Sint Radboudziekenhuis, University of Nijmegen, Netherlands.

Enhancement of mouse skin allografts can be induced by the administration of appropriate alloantisera at the time of transplantation. The same antisera contain destructive antibodies since administration together with an efficient heterologous complement causes acute destruction of established grafts in animals in which cellular immunity has been temporarily suppressed by treatment with anti lymphocyte serum. This makes it possible to study the relative roles of antibodies directed against the different *H-2* regions in enhancement and destruction.

Antibodies directed against the H-2K, H-2D, and H-2L antigens were all able to cause acute graft destruction if administered together with rabbit complement. Ia-antibodies were nondestructive. In allogeneic combinations of increasing genetic disparity, destruction was facilitated as far as rejection could be evoked by lower concentrations of antibody and with less efficient complement sources, such as guinea pig complement and human complement although in all cases the entire classical pathway was required. The destruction was Fc-dependent, since $F(ab^1)_2$ fragments obtained by pepsin digestion of IgG-antibodies were unable to induce acute rejection and could even specifically block the destruction by whole antiserum and complement.

Enhancement of skin grafts could be induced by Ia-antibodies, and to a lesser extent, by H-2K and H-2D antibodies. Preliminary results suggested that H-2L antibodies also contained enhancing activity. $F(ab^1)_2$ preparations of IgG antibodies that had the same antigen binding capacity as the intact antibodies and from which undigested IgG had been carefully removed, completely lacked enhancing activity in the skin graft model. The same preparation was also inactive in a tumor allograft model, in which intact antibody induced enhanced tumor growth leading to ultimate death of the animals. This shows that enhancement of skin and tumor allografts is Fc-dependent. We propose that enhancement is caused by a specific and continuous elimination of antigen reactive cells by complexes that combine the variable part of the antigenic structure (passenger cells, antigens released from the graft) and the Fc-part of the corresponding antibodies. In this respect, the enhancing activity of antibodies is not essentially different from their destructive activity, since in either case the final result is elimination of appropriate target cells.

Adoptive Immunotherapy of Murine Leukemia Using Cells from Alloimmunized H-2-Compatible Donors. Mortimer M. Bortin, Robert L. Truitt, and Alfred A. Rimm. The May and Sigmund Winter Research Laboratory, Mount Sinai Medical Center, 950 North 12th Street, Milwaukee, Wisconsin 53233 and the Departments of Medicine and Biostatistics/Epidemiology, the Medical College of Wisconsin.

Supralethal chemoradiotherapy in combination with transplantation of bone marrow from allogeneic *HLA*-compatible sibling donors is being used with increasing frequency for patients whose leukemia is refractory to conventional chemotherapy regimens. Despite this approach, leukemia has recurred in about one-third of the patients. Transplantation of bone marrow with antileukemic reactivity in order to kill residual leukemia cells through an adoptive immunotherapeutic effect offers one possible solution to this problem. Unfortunately, both in human and animal acute leukemias, transplanted marrow with graft-vs-leukemia (GVL) reactivity invariably has been associated with moderate, severe, or lethal graft-vs-host (GVH) disease. As a consequence, the lowered risk of leukemia recurrence was offset by an increase in GVH-related mortality.

We investigated alloimmunization of *H-2*-compatible donors as a means to generate immunocompetent cells with reactivity against the T cell acute lymphoblastic leukemia of AKR ($H\text{-}2^k$) mice. CBA, C3H/He and B10.BR ($H\text{-}2^k$) donors were immunized with 2 or 6 weekly injections of lymphoid cells from a variety of healthy *H-2*-compatible and incompatible allogeneic strains of mice. Spleen or bone marrow plus lymph node cells from the alloimmunized donors were then transplanted into immunosuppressed leukemic AKR hosts to measure their GVL reactivity. In most instances, all viable leukemia cells were eliminated from the leukemic mice within 6 days as measured in a bioassay which has been described in detail (Bortin, M. M. et al.: *Science 179*:811–813, 1973). T lymphocytes were essential for the GVL reaction because treatment of donor cells with antitheta serum and complement eliminated the antileukemic effect. Immunocompetent cells from unimmunized or isoimmunized *H-2*-compatible donors had no detectable GVL reactivity.

To measure GVH reactivity, immunocompetent cells from unimmunized and immunized CBA donors were transplanted into lethally irradiated nonleukemic AKR hosts. Lymphoid cells from alloimmunized donors caused no augmentation in the mild GVH reactivity observed following transplantation of cells from unimmunized or isoimmunized donors. In contrast, transplantation of lymphoid cells from CBA donors that were specifically immunized with irradiated AKR leukemia cells resulted in a significant increase in GVH-related mortality.

These findings may have importance for the development of a treatment strategy for acute leukemia incorporating histocompatible marrow transplantation. Alloimmunization induced an effective GVL reaction without intensification of GVH disease and, thus eliminated one of the major problems which has prevented successful application of adoptive immunotherapy. (Supported by USPHS grant CA 20484, and grants from the Leukemia Research Foundation and the Briggs and Stratton Corporation Foundation. RLT is a Scholar of the Leukemia Society of America.)

Dissection of the H-2D Region with Monoclonal Antibodies and Cytotoxic T cells. K. Fischer Lindahl, H. Lemke, and G. J. Hämmerling. Basel Institute for Immunology, Basel, Switzerland and Institute for Genetics, University of Cologne, 5000 Cologne 41, Federal Republic of Germany.

Monoclonal antibodies have been found a useful tool for defining the specificity of cytotoxic T lymphocytes (CTL). Three different monoclonal BALB/c-anti-CBA antibodies that reacted with public specificities of H-2K^k were studied in particular detail. They all inhibited T-cell-mediated cytotoxicity specific for H-2K^k, independent of the responder genotype, but did not interfere with the lysis by effector cells from the same responder directed against H-2K^d, H-2D^b, or H-2D^d on the same target cell, showing that inhibition was not due to nonspecific steric hindrance covering the whole cell surface, but rather to a specific blocking of the molecules recognized by the CTL. The antibodies did not interfere with allogeneic effector cells expressing H-2K^k. All three antibodies inhibited H-2K^k-restricted killing specific for the male-antigen, H-Y, or for minor histocompatibility antigens of the C57BL/10 background as efficiently as allogeneic cytotoxity. At the *K* end, the same molecule thus appears to serve as a target whether it is recognized as an alloantigen or as self (Lindahl, K. F. and Lemke, H.: *Eur. J. Immunol*, in press, 1979).

One of these antibodies, 27R9, was found also to bind strongly to a product of the *H-2D^k* region. Preliminary results show that 27R9 co-caps with H-2.32, the private specificity of H-2D^k, and hence most likely reacts with the H-2D^k molecule (P. Démant, personal communication). We found that 27R9 completely inhibits male-specific cytotoxicity restricted to the *H-2D^k* region, but it does not interfere with anti-male killers restricted to H-2K^d on the same targets. Most likely only H-2D^k, not H-2L^k, serves as a restriction element for H-Y specific CTL.

Under conditions where 27R9 antibodies completely inhibit H-2D^k-restricted male-specific lysis of C3H.H.-2° target cells, they only occasionally, and then only partially, inhibit allogeneic H-2^d-anti-H-2^k and H-2^k-restricted AKR-anti-C3H and B6.*H-2^k*-anti-C3H effector cells. Model experiments using H-2^b target cells and anti-K^b and anti-D^b monoclonal antibodies have shown that if only one out of two target determinants is blocked, inhibition is rarely detected. The results, therefore, suggest that other H-2D^k region molecules (H-2L^k?) in addition to H-2D^k are recognized by allogeneic CTL and serve as restriction elements for CTL specific for C3H minor histocompatibility antigens.

No similar dissociation of restriction specificities was detected in the *H-2D^b* region, where one single monoclonal antibody, B22–249, inhibited H-2D^b-restricted lysis specific for H-Y or for C57BL/6 minor antigens as well as allogeneic killing specific for the *H-2D^b* region.

Qed-1: A Target for Unrestricted T cell Killing Encoded Distal to Qa-2. K. Fischer Lindahl and B. Hausmann. Basel Institute for Immunology, 487, Grenzacherstrasse, Postfach, CH-4005 Basel 5, Switzerland.

Cytotoxic T cell responses elicited between lymphocytes of the two *H-2^k* strains, C3H/HeJ and B10.BR, show *H-2^k*-restricted cytotoxicity against minor H antigens; in addition, they contain subpopulations of cells which will kill, with antithetical specificity, target cells from a large number of mouse strains tested, independent of their *H-2* type. The expression of these target determinants is controlled by a single gene (or cluster of closely linked genes), named *Qed-1*, in the *Tla* region, since C3H/HeJ anti B10.BR reacts with B6.*Tla^a* (this reaction defines Qed-1^a) but not with C57BL/6J, whereas B10.BR anti C3H/HeJ reacts with C57BL/6J (defining Qed-1^b) but not with B6.*Tla^a*.*Qed-1* may be identical to *H-2T*. Other mouse strains can be typed for *Qed-1* by the ability of excess cold target cells prepared from these mice to inhibit one of the above cytotoxic reactions (Lindahl, K. F.:*Immunogenetics* 8:71–76, 1979).

F_1 hybrids between *Qed-1^a* and *Qed-1^b* strains express both sets of antigens. *Qed-1* has been mapped to the right of *Qa-2*, since B6.*Tla^a* is Qued-1^a, whereas B.6*H-2^k* and B6.K2 are Qed-1^b. Out of 55 inbred laboratory strains of mice tested, 52 expressed either Qed-1^a or Qed-1^b. The last three strains, all *H-2^r*, did not inhibit Qed-1^a specific lysis and only partially inhibited Qed-1^b specific lysis; these strains have a third haplotype, *Qed-1^c*, and express only some of the antigens found on *Qed-1^b* strains.

In the C57BL/6J strains, Qed-1^b was found on normal lymphocytes from normal thymus, spleen or lymph nodes as well as from *nu/nu* spleen and lymph nodes and on LPS, PHA and Con A activated lymphoblasts. *Qed-1* did not appear confined to any lymphoid subpopulation. The strain distribution of alleles for *Qed-1* closely parallels that of *Qa-1* and *Tla*, both of which control serologically defined differentiation antigens primarily or exclusively expressed on T cells. It is worth pointing out that if *Qed-1* and *Qa-1* are identical, then the serologically negative Qa-1$^-$ strains must carry a Qa-1 product on their cell surfaces, the allelic differences of which are strongly immunogenic only to T cells, not to B cells. In addition there are allelic differences between the *Qa-1^b* strains, i. e., *Qed-1^b* and *Qed-1^c*.

Cytotoxic responses specific for antigens controlled by the *Tla* region and not restricted by *H-2* could be induced in several different combinations of *H-2* identical strains differing for *Qed-1*; in some combinations, notably NZB-anti-BALB/c and SWR-anti-C3H.Q, the cells even responded directly in culture without previous sensitization in vivo.

Serotyping of MuLV Positive B10.A V+ and Negative B10.A V− substrains. Pavol Iványi, Cornelius J. M. Melief, Peter van Mourik, Arjen Vlug and Paul de Greeve. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical. Immunology, University of Amsterdam, Amsterdam, The Netherlands.

By selection of murine ecotropic leukemia virus positive and virus negative B10.A (H-2^a) mothers and fosternursing B10 (H-2^b) new borns. 4 sublines of C57Bl/10 mice were obtained: B10.A V +, B10.A V −, B10.V +, B10.V − (Melief et al.: *J. Natl. Cancer Inst.* *55*:691, 1975) Milk-transmission of naturally prevalent B-tropic virus leads to persistent infection of all offspring in the V+ sublines in subsequent generations, while the V− sublines remain essentially free of virus infection.

Groups of B10.A. V+ and B10.A V− mice were immunized with B10 V+ and B10 V− lymphoid cells, respectively. Anti-H-2^b antibodies were present in all sera. In the combination B10 V+ donors, B10.A V− recipients, after prolonged immunization, sera were obtained that reacted positively in the routine microlymphocytotoxicity test with B10.A V+ but not with B10.A V− lymph-node or spleen cells. Both B10 V+ and B10 V− cells absorb all cytotoxic antibodies from the B10.A V− anti-B10 V+ sera, including the activity which is directed against B10.A V+ cells. B10.A V− cells did not absorb the anti-B10.A V+ activity. The target structure on the B10.A V+ cells, responsible for the reactivity with anti-B10 V+ sera, developed postnatally after day 15.

Hence, this target structure develops much later than do H-2 antigens which are detectable by cytotoxicity on spleen cells within 1–3 days after birth. The follow-up of pre- and post-natal development of the respective target structure indicates that a few days after birth, the milk transmitted virus, introduces a serologically detectable antigen. This virus induced antigen becomes immunogenic in the late phase of B10.A V− anti-B10 V+ immunization, and the target structure on B10.A V+ cells in the microlymphocytotoxicity test. The observation that antibody against this antigen was only produced after alloimmunization in the combination B10 V+ donor, B10.A V− recipient, as well as the finding that B10 V cells absorbed all antibody activity, suggests that the antigen is intimately related to *H-2* and possibly represents a virus-altered H-2 antigen.

In brief, three conclusions emerge from these observations: (1) Simple "serotyping", with the routine microlymphocytotoxicity test, of the B10.A V+ versus B10.A V− difference became possible. (2) The target structure responsible for the positive reactions of the B10 A V+ cells is presumably a virus-altered H-2 antigen. The virus alteration induced either a structural change or a modified presentation of the H-2 molecule(s). (3) Two groups of B10.A (H-2^a) strain mice were immunized, namely B10.A V+ and B10.A V−. These two groups of genetically identical individuals were immunized with the same immunogen (B10 V+). However, the cross-reactivity pattern of the two groups of sera greatly differ. Hence, the virus status of recipient mice, in an H-2 alloimmunization, exerts an influence on the cross-reactivity pattern of the anti-H-2 antibodies (Ivanyi et al. *Tissue Antigens 12*:32, 1978). (The investigations were supported in part by the Foundation for Medical Research Fungo which is subsidized by the Netherlands Organization for the advance of Pure Research (ZWO) and the Queen Wilhelmina Foundation for the Fight against Cancer.)

Target Specificity of Cytotoxic T Cells Directed Against H-2 L. C. J. M. Melief, L. P. de Waal, M. Y. v. d. Meulen, P. de Greeve, and P. Iványi. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands.

Target antigens for alloimmune cytotoxic T lymphocytes specified by *H-2L* were analysed using the H-2L loss mutant BALB/c-H-2^{dm2} (dm2). Mutant mice were immunized with a single intraperitoneal injection of 100×10^6 spleen cells from the strain of origin (BALB/c-H-2^d). Two to six weeks later, primed dm2 spleen cells were restimulated in mixed lymphocyte reaction (MLR) with irradiated BALB/c spleen cells. The specificity of killer cells generated in these cultures was tested in cell mediated lympholysis (CML) against concanavalin A transformed target lymphocytes of a panel of mouse strains. Below we report the mean results of at least experiments obtained with 200×10^3 effector cells and 100×10^3 ^{51}Cr

labeled target cells (E/T ratio 2:1): (1) H-2^{dm2} target cells were not killed ($\leq 3\%$ lysis in all experiments); (2) strong CML (63–73% lysis) was observed against all targets with H-2^dalleles at the D region (BALB/c, DBA/2, B10.A, B10.A (5R), B10.S (7R), B10.S (9R), A.TFR5.); (3) targets from other strains, positive for public H-2 specificities of the "28" family were never lysed to the same extent. This supports the observation that these "28" positive haplotypes cannot complement dm2 in graft rejection; (4) strong CML cross reactivity (37–44% lysis) occurred against DBA/1 and B10.AKM targets. Because these targets have *q/q* and *k/q* alleles at the *K /D* regions and because AKR, B10.BR and C3H (k/k) targets were not killed as strongly (18–20% lysis) the strong CML cross reaction against H-2^q maps in the *D*-end; (5) lysis of C3H.OH (d/k) targets was less (7%) than that of k/k targets (see 4). Therefore the cross reactivity CML target of H-2^k is coded for by K^k; (6) strong CML cross reactivity (41% lysis) also occurred against H-2^{dm1} target cells. Hence this complex *D*-end mutant still expresses CML antigens specified by H-2L, although in altered form; (7) C57BL/6 (b/b) and D2.GD (d/b) targets were lysed similarly (20–27% lysis). Because C3H.OH (d/k) targets were not lysed as strongly (see 5), the CML cross reaction against H-2^b is specified by the *D*-end of this haplotype; (8) Cross reactions were seen against B10.R III (H-2^r, 20% lysis), C3H.NB (H-2^p, 16% lysis), B10.M (H-2^f, 14% lysis) and B10.S (H-2^s, 9%) target cells. Due to lack of *H-2* recombinants, we have not mapped these cross-reactions within the *H-2* complex.

In summary, targets of all *H-2* haplotypes tested, except that of the MLR responder were killed by H-2^{dm2} anti-H-2^d effector cells, although to a widely varying extent (table 1).

Table 1

CML H-2^{dm2} anti-H-2^d Intensity of kill	Target
	H-2
strong	L^d
↓	D^qL^q, dm1
↓	K^k, D^b, r
↓	p
↓	f
↓	s
weak	C3H.OH (d/k)
negative	dm2

H-$2L^d$ expresses a unique CML target structure not expressed in other haplotypes. Cross reactions are directed against both K-end and D-end antigens. The strong CML cross reaction against D^qL^q targets corresponds with the observation that these targets share serologically defined specificity H-2.65 with D^dL^d. Cross reactions against other haplotypes do not show apparent specificity for any known serologically defined antigen or family of antigens. (The investigations were supported in part by the Foundation for Medical Research FUNGO which is subsidized by the Netherlands Organization for the Advance of Pure Research (ZWO).

Are the Polymorphic Epitopes of Class I Alloantigens Clustered in Structurally Independent Domains? J. C. Howard., A. R. C., Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, England.

Our studies with monoclonal alloantibodies raised against the products of the rat MHC have led to the definition of two topographically separated polymorphic sites P and S on the Class I alloantigenic molecules of the $RT1^a$ haplotype (Howard et al.: *Immunol. Rev.*, in press 1979). The two sites have been defined in reciprocal competitive inhibition of binding assays using internally labelled antibodies. Sequential precipitation of surface radio-iodinated material by monoclonal antibodies directed at P and S site determinants demonstrated clearly that the P and S sites were carried on the same molecule or molecules. It is not known whether the specificities identified by the monoclonal antibodies that we used are carried on both the Class I alloantigenic molecules of the rat as defined by Sporer and co-workers (*Immunogenetics* 7:507, 1978). However, it is clear from the sequential precipitation data that all molecules carrying P-site determinants also carry S-site determinants and vice versa.

An analysis of the strain distribution of the specificities characterizing the P and S sites of $RT1^a$ showed that the two sites were found together only on products of the $RT1^a$ haplotype, its known

recombinant derivatives $RT1^{r1}$ (Butcher and Howard,: *Nature 266*:362, 1977), and $RT1^{r2}$ (Stark et al.: *Immunogenetics 5*:183, 1977), and the $RT1^{e}$ haplotype of BDVII. This last haplotype is now thought to carry the *K/D*-like *A* region from $RT1^{a}$ (Kunz et al.: *Transplant. Proc.*, in press, 1979). Thus the conjunction of the P and S sites may be said to be 'private' to $RT1A^{a}$. However, reactivities corresponding to each $RT1A^{a}$ site alone were found independently in other haplotypes. $RT1^{k}$ of the SHR strain appears to specify Class I alloantigens carrying the $RT1A^{a}$ P site alone, while a haplotype designated $RT1^{o?}$ (pending comparison with the type $RT1^{o}$ of MR) appears to specify the $RT1A^{a}$ S site alone. In both cases, quantitative aspects of monoclonal antibody binding differed between the isolated site and its type expression in $RT1^{a}$. In the case of the P site of $RT1^{k}$, there was evidence for diminished expression of the site on the target cell, while in the case of the S site of $RT1^{o?}$ there was evidence for a qualitative modification of the site reflected in low avidity binding of one S-site-related monoclonal antibody.

The physical separation and genetic independence of the P and S sites strongly suggests a domain-like organization of the polymorphic regions of the Class I alloantigenic molecules.

A similar interpretation may be used to resolve the apparent paradox of the mouse mutant haplotype $H\text{-}2^{bm}$. This gain-and-loss mutation maps to K^{b} or $I\text{-}A^{b}$ and affects the apparent expression of H-2.33 the K^{b} private specificity (page 308, abstract McKenzie et al., 1979). Confusingly, however, the $H\text{-}2^{bm}$ mutant also complements with $H\text{-}2^{ba}$ for acceptance of $H\text{-}2^{b}$ skin and has consequently been considered not to represent a K^{b} structural gene mutation (page 308, abstract McKenzie et al., 1979), despite the mapping and the clear evidence for alteration of the expression of H-2.33. The independent behaviour of the P and S sites of the rat Class I alloantigens suggests a clear analogy with $H\text{-}2^{bm}$ and $H\text{-}2^{ba}$. It seems probable that the two mutations bring about antigenic alterations at structurally independent sites which will, of course, fully complement each other in the F_1 hybrid. When the sequence change resulting from the $H\text{-}2^{bm}$ mutation is known, comparison with the position of the $H\text{-}2^{ba}$ mutation should give information about the specification of two structurally independent polymorphic epitopes of the H-2K molecule. (This work was supported in part by U.S.P.H.S. grant No. AI.13162.)